
**WHO FOOD
ADDITIVES
SERIES: 55**

Safety evaluation of certain contaminants in food

**Prepared by the Sixty-fourth meeting
of the Joint FAO/WHO Expert
Committee on Food Additives
(JECFA)**

**FAO
FOOD AND
NUTRITION
PAPER
82**

World Health Organization, Geneva, 2006

IPCS—International Programme on Chemical Safety

**Food and
Agriculture
Organization
of the
United Nations
2006**

WHO Library Cataloguing-in-Publication Data

Safety evaluation of certain contaminants in food / prepared by the sixty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

(WHO food additive series ; 55)

(FAO food and nutrition paper ; 82)

1. Food contamination. 2. Risk assessment. 3. Maximum allowable concentration. 4. Acrylamide - adverse effects. 5. Cadmium - adverse effects. 6. Tin - adverse effects. 7. Hydrocarbons, Brominated - adverse effects. 8. Polycyclic hydrocarbons, Aromatic - adverse effects. I. Joint FAO/WHO Expert Committee on Food Additives. Meeting (64th : 2005: Rome, Italy) II. Series.

ISBN 92 4 166055 4 (WHO)

(NLM classification: WA 701)

ISSN 0300-0923 (WHO)

ISBN 92-5-105426-6 (FAO)

© WHO and FAO 2006

All rights reserved. Publications of the World Health Organization can be obtained from WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel: +41 22 791 3264; fax: +41 22 791 4857; email: bookorders@who.int). Requests for permission to reproduce or translate WHO publications – whether for sale or for noncommercial distribution – should be addressed to WHO Press, at the above address (fax: +41 22 791 4806; email: permissions@who.int). Applications for permission to reproduce material in this FAO information product for resale or other commercial purposes should be addressed to the Chief, Publishing Management Service, Information Division, FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy, or by e-mail to copyright@fao.org.

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 World Health Organization or the Food and Agriculture Organization of the United Nations concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization or the Food and Agriculture Organization of the United Nations in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by WHO and FAO to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either express or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization or the Food and Agriculture Organization of the United Nations be liable for damages arising from its use.

This publication contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization or the Food and Agriculture Organization of the United Nations.

CONTENTS

Preface	v
Acrylamide.....	1
Cadmium — Impact assessment of different maximum limits	157
Ethyl carbamate.....	205
Inorganic tin (addendum).....	317
Polybrominated diphenyl ethers	351
Polycyclic aromatic hydrocarbons	563
Annexes	
Annex 1	Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives 745
Annex 2	Abbreviations used in the monographs..... 755
Annex 3	Participants in the sixty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives..... 761
Annex 4	The formulation of advice on compounds that are both genotoxic and carcinogenic 765
Annex 5	Approach to dose–response modelling..... 769

This publication is a contribution to the **International Programme on Chemical Safety (IPCS)**.

The IPCS, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO) and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessing the risk to human health and the environment to exposure from chemicals, through international peer review processes as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

PREFACE

The monographs contained in this volume were prepared at the sixty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met at FAO headquarters in Rome, Italy, 8–17 February 2005. These monographs summarize the data on selected food contaminants reviewed by the Committee.

The sixty-fourth report of JECFA has been published by the World Health Organization as WHO Technical Report No. 930. 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 and Contaminants 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 the 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 preparation and editing of the monographs included in this volume were made possible through the technical and financial contributions of the Participating Organizations of the International Programme on Chemical Safety (IPCS), which supports the activities of JECFA.

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 the IPCS 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, International Programme on Chemical Safety, World Health Organization, Avenue Appia, 1211 Geneva 27, Switzerland.

ACRYLAMIDE

First draft prepared by

**S.H. Henry,¹ A. Agudo,² S. Barlow,³ L. Castle,⁴ J. Garey,⁵ K.-E. Hellenas,⁶
K. Koehler,¹ J.C. Larsen,⁷ J.-C. LeBlanc,⁸ M. Paule,⁵ J. Schlatter,⁹ W. Slob¹⁰
and D.R. Doerge⁵**

¹ **Food and Drug Administration, College Park, Maryland, USA**

² **Catalan Institute of Oncology, Hospitalet del Llobregat, Barcelona, Spain**

³ **Harrington Road, Brighton, East Sussex, United Kingdom**

⁴ **Central Science Laboratory, Sand Hutton, York, United Kingdom**

⁵ **National Center for Toxicological Research, Little Rock, Arkansas, USA**

⁶ **National Food Administration, Uppsala, Sweden**

⁷ **Division of Toxicology and Risk Assessment, Danish Institute of Food and
Veterinary Research, Søborg, Denmark**

⁸ **Institut Nationale de la Recherche Agronomique/Institut Nationale
Agronomique Paris-Grignon, Paris, France**

⁹ **Food Toxicology Section, Swiss Federal Office of Public Health, Zurich,
Switzerland**

¹⁰ **National Institute of Public Health and the Environment (RIVM), Bilthoven,
The Netherlands**

Explanation	4
Biological data	5
Biochemical aspects	5
Absorption, distribution and excretion	5
Biotransformation	9
Effects on enzymes and other biochemical parameters.....	11
Physiologically based pharmacokinetic (PBPK) modelling....	11
Toxicological studies	12
Acute toxicity	12
Short-term studies of toxicity	12
Long-term studies of toxicity and carcinogenicity	22
Genotoxicity	25
Reproductive toxicity	39
Special studies	50
Observations in domestic animals.....	60
Observations in humans.....	60
Biomarkers of exposure	60
Biomarkers of effects	64
Clinical observations	64
Epidemiological studies	64

Analytical methods.....	68
Chemistry	68
Description of analytical methods.....	68
Introduction	68
Screening tests	69
Quantitative methods	69
Sampling.....	76
Effects of processing	77
Heat-induced formation of acrylamide in foods	77
Chemical mechanisms	78
Formation from amino acid and sugar by Maillard reactions	78
Formation via acrylic acid.....	79
Formation from 3-aminopropionamide	79
Elimination of acrylamide	79
Analogues: formation of other compounds.....	80
Formation factors	80
Heating temperature and time.....	80
Precursors.....	81
Acidity	82
The food matrix	82
Water activity	82
Levels and patterns of contamination of food commodities	83
Surveillance data.....	83
National occurrence	85
Australia	85
Austria.....	85
Belgium	85
Canada	86
China	86
China, Hong Kong Special Administrative Region	86
Denmark	87
France.....	87
Germany	87
Japan	87
The Netherlands	88
Norway.....	88
Sweden	88
Switzerland	89
Syria.....	89
United Arab Emirates.....	89
United Kingdom	89
United States	90
Summary of national occurrence data	90
International occurrence.....	90
Dietary intake assessment.....	91
Exposure to acrylamide from non-food sources	91
National assessments of intake from diet.....	95

Australia	96
Belgium	96
Canada	96
China	96
China, Hong Kong Special Administrative Region	97
Czech Republic	97
Denmark	97
France	97
Germany	98
The Netherlands	98
New Zealand	98
Norway	99
Sweden	99
Switzerland	100
United Arab Emirates	100
United Kingdom	100
United States	100
Summary of national intake estimates	101
Regional estimates of intake from GEMS/Food diet	107
Prevention and control	108
Mitigation achievements	110
Mitigation experiments in food models	110
Lowering the amount of precursors	110
Chemical interference with formation or elimination	111
Optimized time/temperature regimen	111
Drying	111
Dose–response analysis and estimation of carcinogenic/toxic risk	112
Contribution of above data to assessment of risk	112
Pivotal data from biochemical and toxicological studies	112
Pivotal data from human clinical/epidemiological studies	117
General modelling considerations	117
Selection of models	118
Selection of data	119
Measure of intake	120
Measure of response	120
Selection of mathematical model	121
Estimates of BMDs and BMDLs	121
Mammary tumours	121
Testis tumours	123
Central nervous system tumours	125
Thyroid tumours	125
Potency estimates in humans	126
Comments	126
Absorption, distribution, metabolism and excretion	126
Toxicological data	128
Genotoxicity	129
Carcinogenicity	130
Observations in humans	130

Analytical methods	131
Formation of acrylamide during cooking and heat processing	131
Prevention and control	132
Levels and pattern of food contamination.....	132
Dietary intake assessment.....	133
Dose–response analysis	134
Evaluation.....	136
Recommendations	136
References	137

1. **EXPLANATION**

Acrylamide ($\text{CH}_2=\text{CHCONH}_2$, CAS Registry Number 79-06-1) is an important industrial chemical that has been used since the mid-1950s as a chemical intermediate in the production of polyacrylamides, which are used as flocculants for clarifying drinking-water and in other industrial applications. It is well established that acrylamide is neurotoxic in humans, as revealed by the consequences of occupational and accidental exposures. In addition, experimental studies in animals have shown that acrylamide has reproductive toxicity and is genotoxic and carcinogenic.

Studies conducted in Sweden in 2002 showed that high concentrations of acrylamide are formed during the frying or baking of a variety of foods. Owing to concerns about the possible public health risks associated with dietary exposure to acrylamide, a consultation was held by FAO/WHO in June 2002 (FAO/WHO, 2002). On the basis of the recommendations arising from this consultation, numerous studies of metabolism, bioavailability, toxicokinetics, DNA adduct formation and mutagenicity in vitro and in vivo have been performed. Concurrently, a major worldwide effort has produced extensive survey data that can be used to estimate the extent and levels of contamination in food and to estimate national intakes.

At its present meeting, the Committee responded to a request from the Codex Committee on Food Additives and Contaminants (CCFAC) at its Thirty-sixth Session (CAC, 2004) to:

- comment on the extent to which acrylamide is bioavailable in food and on the safety implications;
- consider the threshold-based end-points of concern, such as neurotoxicity and reproductive toxicity, and eventually derive a tolerable dietary intake;
- evaluate the degree of uncertainty related to the assessments made;
- provide estimates of dietary intake for various population groups, including susceptible groups such as young children and regional populations, and to identify and quantify as far as possible the major sources of dietary intake;
- provide estimates and margins of exposure (MOEs), safety and intake for various end-points of concern (non-cancer and cancer). These estimates

should contain comparisons between the levels of exposure shown to produce effects in animal studies and demonstrated no-effect levels versus estimates of dietary intake for humans;

- provide quantitative estimates of risk for various end-points, including cancer, for varying degrees of dietary exposure to acrylamide; and
- provide comments on the toxicological significance of the main metabolite, glycidamide, and whether this may be more genotoxic than the parent compound.

Acrylamide has not been evaluated previously by the Committee.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Absorption

Studies in male F344 rats indicate that orally administered [2,3-¹⁴C]acrylamide (AA) is rapidly and extensively absorbed from the gastrointestinal tract (Dixit et al., 1982; Miller et al., 1982). Groups of three male F344 rats received a single oral application of [2,3-¹⁴C]acrylamide at 1, 10 or 100 mg/kg bw. Urine and faeces were collected daily for up to 7 days. Independent of the dose levels, 53–67% of the radioactivity was excreted within 24 h; by 7 days, 65–82% had been eliminated. Approximately 74% was recovered in urine and 8% in faeces. Total radioactivity recovered in fluids, tissues and excreta after 7 days was $90 \pm 13\%$ of the administered dose (Miller et al., 1982). Rapid and extensive absorption of [1-¹⁴C]acrylamide was reported in male Sprague-Dawley rats administered a single oral dose of 50 mg/kg bw. Radioactivity was detected in blood 5 min after administration, and peak plasma levels occurred at 38 min (Kadry et al., 1999). Following administration of a single oral gavage dose of unlabelled acrylamide at 50 mg/kg bw to male and female B6C3F1 mice, peak serum concentrations of acrylamide were observed at 0.5 h, the earliest time point assayed (Twaddle et al., 2004a).

Measurements of acrylamide and its metabolite, glycidamide (GA), in serum using liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) have been made after intravenous, gavage and dietary administration of unlabelled acrylamide at 0.1 mg/kg bw to male and female B6C3F1 mice (Doerge et al., 2005a) and F344 rats (Doerge et al., 2005b). In these studies, acrylamide was rapidly absorbed after gavage administration, because maximum serum concentrations were observed in mice at 0.5 h, and the half-time for absorption in rats was 0.34 ± 0.04 h (females) and 0.63 ± 0.25 h (males). Absorption of acrylamide was extensive in both mice and rats, consistent with essentially complete oral absorption. After dietary administration of an acrylamide dose of 0.1 mg/kg bw to male and female B6C3F1 mice and F344 rats added to a basal diet containing low levels of acrylamide (approximately 0.01 mg/kg), the absolute bioavailability of dietary acrylamide, defined as area under the curve (AUC)-oral/AUC-intravenous,

was found to be 23% in both male and female mice, 28% in female rats and 47% in male rats. This was lower than the corresponding bioavailabilities from aqueous gavage, which were 32% for female mice, 52% for male mice, 60% for male rats and 98% for female rats. These calculations do not consider the increased first-pass metabolism of acrylamide to glycidamide that occurred subsequent to oral administration relative to intravenous dosing. These differences in metabolism and internal exposures are reflected by increased AUC ratios for glycidamide/acrylamide for oral versus intravenous administration (see Table 1).

Table 1. Toxicokinetic parameters from administration of acrylamide (0.1 mg/kg bw) to B6C3F1 mice and F344 rats

Sex / species / route	GA AUC ($\mu\text{mol/l}$ per hour)	GA/AA AUC	Absolute bioavailability	V_d (l/kg bw)
Male / mouse / intravenous	1.8	1.1	1	0.67
Male / mouse / gavage	2.6	2.9	0.52	—
Male / mouse / diet	1.0	2.7	0.23	—
Female / mouse / intravenous	1.1	0.42	1	0.59
Female / mouse / gavage	2.0	2.4	0.32	—
Female / mouse / diet	1.0	1.7	0.23	—
Male / rat / intravenous	0.58	0.14	1	0.77
Male / rat / gavage	1.3	0.57	0.60	—
Male / rat / diet	0.60	1.0	0.47	—
Female / rat / intravenous	4.4	0.13	1	0.87
Female / rat / gavage	1.5	0.96	0.98	—
Female / rat / diet	—	1.0	0.28	—

From Doerge et al. (2005a, 2005b)

AA, acrylamide; AUC, area under the curve; GA, glycidamide; V_d , volume of distribution

(b) Distribution

Groups of male Swiss-Webster mice received a single oral dose of [2,3- ^{14}C]acrylamide at 116–121 mg/kg bw and were sacrificed at 0.33, 1, 3 and 9 h and 1, 3 and 9 days post-administration. In addition, pregnant females received acrylamide on days 13.5 and 17.5 of gestation and were sacrificed at 3 and 24 h post-administration. Sections for whole-body autoradiography were taken at each of the sacrifice times; quantification of radioactivity intensity was by visual inspection (Marlowe et al., 1986).

In the males, radioactivity was highest in stomach and intestinal contents after 0.33 and 1 h post-administration. Radioactivity was also observed in the epithelia of the oral cavity and oesophagus, the liver and gall-bladder; to a lesser extent,

labelling also occurred in bronchial epithelium, testis and brain. At 3 h, very little radioactivity was seen in the stomach. At this stage, radioactivity was still seen in intestinal contents. Also, high concentrations were still present in kidneys, testis, pancreas and the lens of the eye. Within the brain, radioactivity appeared to be highest in the region of the cerebellar cortex. At 9 h, the pattern of distribution was essentially similar. At 24 h, radioactivity levels had declined substantially in the liver, kidneys, pancreas and most other organs, with the exception of testis and intestinal contents. Three days post-administration, distribution of radioactivity was uniform and low, with the exception of the epididymis (lumen and wall of epididymal ducts). At 9 days, the only significant levels of radioactivity were seen in the reproductive tract (epithelium of glans penis) (Marlowe et al., 1986).

On the 13.5th day of gestation, radioactivity was uniformly distributed in both dams and fetuses at 3 and 24 h. For 17.5th-day pregnant mice, radioactivity in the fetus was concentrated in the kidney, bladder, liver and intestinal contents. In addition, high levels of radioactivity were seen in fetal skin. No notable accumulation of radioactivity was seen in peripheral nerves of adults or fetuses at any time point (Marlowe et al., 1986).

Following intravenous administration of [2,3- 14 C]-labelled acrylamide at 10 mg/kg bw to male F344 rats, total radioactivity was rapidly distributed to all tissues examined (brain, sciatic nerve, spinal cord, fat, liver, kidney, testes, lung, small intestine, skin, muscle). Peak concentrations of radiolabel were observed by 1 h after dose administration in liver, fat, kidney, nervous tissues and testes (Miller et al., 1982). Several studies have indicated a propensity for radiolabel accumulation in red blood cells (Hashimoto & Aldridge, 1970; Miller et al., 1982) following administration of [14 C]-labelled acrylamide, presumably because of covalent binding to haemoglobin by acrylamide and/or glycidamide. However, the Committee noted that the utility of such studies is limited because the chemical composition of radioactivity in tissues was not determined.

Direct measurements of acrylamide and glycidamide were made in several tissues (liver, lung, muscle, brain in mice at 1 and 2 h; liver, muscle, brain, testes, mammary gland in rats at 2 and 4 h) using LC-electrospray ionization (ESI)/MS/MS following gavage administration of acrylamide at 0.1 mg/kg bw to male and female B6C3F1 mice (Doerge et al., 2005a) and male and female F344 rats (Doerge et al., 2005b). The concentrations of acrylamide in mouse tissues were similar to each other and to the serum concentrations, but the glycidamide concentrations varied among tissue types. The apparent rates of acrylamide and glycidamide elimination from the selected mouse tissues were similar to those observed for serum (Doerge et al., 2005a). In general, the concentrations of acrylamide and glycidamide in rat tissues were slightly less than those in serum, particularly in the liver, testes and mammary gland. The apparent rates of acrylamide elimination in the selected rat tissues were also similar to the rate observed for the serum (Doerge et al., 2005b).

Acrylamide concentrations were comparable in serum and rat sciatic nerves 0.5 h after the final intraperitoneal dose from either a daily repeated exposure for 10, 30 and 90 days at dose levels between 3.3 and 30 mg/kg bw or in serum after

a single exposure to the same dose (Crofton et al., 1996). The serum and sciatic nerve concentrations of acrylamide were linearly dependent on dose in the range of 3.3–30 mg/kg bw after a single dose and also after 10, 30 and 90 days of administration.

The volume of distribution, V_d , was determined in male and female B6C3F1 mice (Doerge et al., 2005a) and male and female F344 rats (Doerge et al., 2005b; Table 1) following intravenous administration of acrylamide at 0.1 mg/kg bw. In mice, V_d was 0.67 and 0.59 l/kg bw in males and females, respectively; in rats, V_d was 0.77 and 0.87 l/kg bw in males and females, respectively. The V_d in mice and rats following intravenous administration of an equimolar dose of glycidamide was 0.68–0.77 l/kg bw (Doerge et al., 2005a, 2005b). These findings are consistent with extensive distribution of acrylamide and glycidamide in total cellular water.

Groups of 3–8 male dogs and 3–6 male miniature pigs received acrylamide at 1 mg/kg bw per day administered in the diet for a period of 3–4 weeks followed by a single oral dose of aqueous [$1\text{-}^{14}\text{C}$]acrylamide at 1 mg/kg bw. Dietary administration of non-radiolabelled acrylamide continued until sacrifice. Animals were sacrificed 6 h or 1, 2, 4 and 14 days after administration of radiolabelled acrylamide. Urine and faeces samples were collected and a wide range of tissues analysed for the presence of radioactive material. The tissues analysed were blood, heart, lung, liver, spleen, gastrointestinal tract, kidney, testes, skeletal muscle, bile and gall-bladder, brain and fat. At the 6-h time point only, the amount of radiolabel in a series of brain and spinal cord sections was determined in both pigs and dogs. In addition, evolved carbon dioxide was collected over a 2-day period from just one dog. In this study, no attempt was made to identify potential metabolites (Ikeda et al., 1987).

For both dogs and pigs, and in all tissues examined, the recovery of radiolabel was greatest 6 h after administration and declined gradually over the 14-day observation period. In dogs, the greatest amount of radiolabel was recovered from skeletal muscle; approximately 35% of the administered dose was found in this tissue at 6 h. Smaller amounts were found mainly in the liver, blood and gastrointestinal tract (14%, 5% and 5%, respectively), and the total amount accounted for after 6 h was about 64% of the administered dose. On day 2, 17% of the administered dose was found in muscle, with little (approximately 1%) being found in the gastrointestinal tract. Acrylamide was found in all tissues sampled at all observation points (indicating wide distribution) except at 14 days, when none was detected in the bile and gall-bladder. Only small amounts (<1%) were found in brain or fat (measured at 6 h only). On completion of 14 days, <1% of the administered dose was found in individual tissues, except muscle, which still contained about 5% (Ikeda et al., 1987).

In pigs, again the greatest amount of radiolabel was recovered from skeletal muscle; approximately 32% of the administered dose was found in this tissue at 6 h. At the same time point, recovery was 20% from the gastrointestinal tract and 5% from each of liver, fat and blood, with smaller amounts being found in other tissues. The total amount accounted for at 6 h was about 71% of the administered dose. On day 2, a large amount of radiolabel (17%) was still found in the

gastrointestinal tract, indicating that absorption was slower in pigs than in dogs. Acrylamide was found in all tissues sampled at all observation points (indicating wide distribution) except at 4 and 14 days, when none was detected in the bile and gall-bladder. Only small amounts (<1%) were found in brain, whereas fat was one of the major sites of distribution. On completion of 14 days, <1% of the administered dose was found in individual tissues, except muscle, which still contained approximately 7% (Ikeda et al., 1987).

Placental transfer of total radioactivity has been demonstrated in studies on pregnant beagle dogs and mini-pigs administered [$1\text{-}^{14}\text{C}$]-labelled acrylamide at 5 mg/kg bw, where comparable levels of radioactivity were observed in maternal tissues and in fetal tissues (Ikeda et al., 1985). Acrylamide transfer was also measured in an *in vitro* model using postpartum human placenta, and acrylamide has been measured in human milk from lactating women after consumption of potato chips¹ (Sörgel et al., 2002).

(c) *Excretion*

In the Ikeda et al. (1987) study on dogs and pigs (see above), the urine was the major route of excretion of radiolabel, accounting for approximately 60% of the administered dose for both species after 14 days, and the faeces accounted for a further 7–27%. Most of the radiolabel was recovered from the urine during the first 2 days, with only very little extra being excreted over the next 12 days.

In male F344 rats given doses of ^{14}C -labelled acrylamide by intravenous (10 mg/kg bw) or oral routes (1, 10 or 100 mg/kg bw), 71% of the administered radioactivity was excreted in urine over 7 days (Miller et al., 1982). Little radioactivity was found in the faeces (6%), and approximately 10% of the administered radioactivity was bound to red blood cells. No exhalation of radiolabelled carbon dioxide was observed. Essentially identical urinary total radioactivity elimination profiles were observed following intravenous and oral dosing (Miller et al., 1982).

In a pilot study, food with an estimated amount of about 800 µg of acrylamide was administered to 11 healthy volunteers (9 males, 2 females: 1 female pregnant, 1 female lactating), and levels of acrylamide were determined in several body fluids (Sörgel et al., 2002). This study showed that acrylamide in food given to humans is absorbed in the gut; from the urinary excretion in two males, half-lives of 2.2 and 7 h were estimated. Acrylamide was also shown to be excreted into human milk and to penetrate the human placenta.

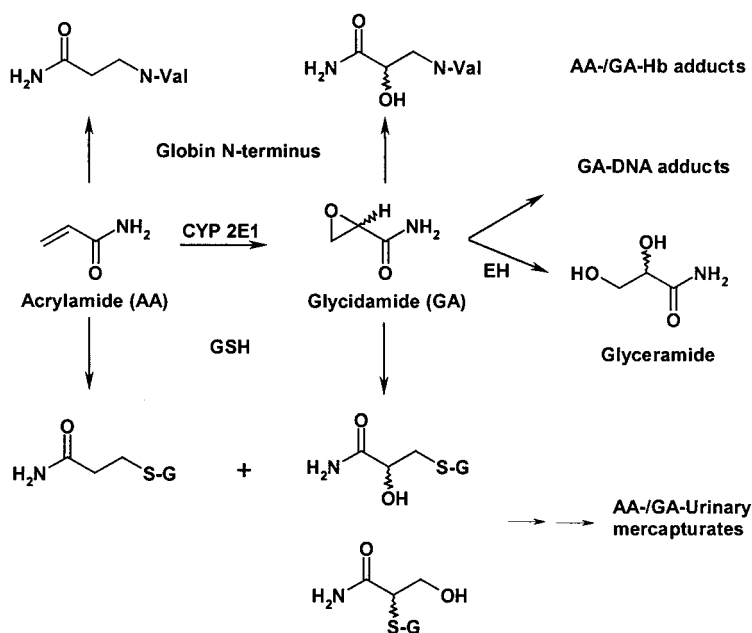
2.1.2 *Biotransformation*

Results from rodent studies indicate that acrylamide is extensively converted to metabolites that are excreted predominantly in the urine (Dixit et al., 1982; Miller et al., 1982; Sumner et al., 1992, 1999, 2003). A metabolic scheme for acrylamide is shown in Figure 1. The prominent role for cytochrome P450 2E1 (CYP2E1) in

¹ In most cases, terminology is as given in the original report. In general, the Committee uses the terms potato chips (french fries in the USA) and potato crisps (potato chips in the USA).

conversion of acrylamide to glycidamide was demonstrated by dosing wild-type and CYP2E1 knockout mice with acrylamide (50 mg/kg bw by gavage) to compare the urinary metabolites. All glycidamide-derived metabolites were absent in the urine of CYP2E1 knockout mice (Sumner et al., 1999). Acrylamide and glycidamide also react readily with glutathione (Dixit et al., 1982) to form conjugates that are processed to mercapturic acid derivatives that appear in the urine (Sumner et al., 1992). Evidence has been presented that suggests no added role for catalysis by glutathione *S*-transferase (Paulsson et al., 2005). In addition, glycidamide is converted to glyceramide by epoxide hydrolase (Sumner et al., 1992). Metabolism of acrylamide in men was similar to that in rodents, except that glyceramide was the predominant glycidamide metabolite found in the urine of men who consumed an acrylamide dose of 3 mg/kg bw (Fennell et al., 2005).

Figure 1. Metabolism of acrylamide



AA, acrylamide; EH, epoxide hydrolase; GA, glycidamide; GSH, reduced glutathione; Hb, haemoglobin; Val, valine

Several studies have identified and quantified urinary acrylamide metabolites in rodents (Sumner et al., 1992, 1999, 2003) and in humans (Fennell et al., 2005). The major metabolite in both male F344 rats and male B6C3F1 mice dosed orally with ¹³C-labelled acrylamide at 50 mg/kg bw was the *N*-acetyl-S-(2-carbamoyl-ethyl)cysteine. Small amounts of acrylamide were observed but were too low to

quantify. Other urinary metabolites, which were derived from glycidamide, included unchanged glycidamide, glyceramide, *N*-acetyl-S-(2-hydroxy-2-carbamoylethyl)-cysteine and *N*-acetyl-S-(1-carbamoyl-2-hydroxyethyl)cysteine (Figure 1). The amount of metabolites derived from glycidamide was higher in mice (59%) than in rats (33%). Approximately 50–60% of the administered dose was detected in urine. At a lower acrylamide dose of 3 mg/kg bw administered to male F344 rats, glycidamide-derived metabolites accounted for 42% of total urinary metabolites (Fennell et al., 2005), which is consistent with observations that as the acrylamide dose is decreased, the fraction of acrylamide metabolized to glycidamide increases (Bergmark et al., 1993). In a study of men administered ¹³C-labelled acrylamide at 3 mg/kg bw, *N*-acetyl-S-(2-carbamoylethyl)cysteine was also the major metabolite (64%), with smaller amounts attributable to glycidamide (2%) and glyceramide (10%) and no detectable mercapturates derived from glycidamide; however, only 34% of the administered dose was recovered (Fennell et al., 2005). In recent studies, mercapturic acid metabolites of acrylamide and glycidamide have been quantified in urine from people exposed to acrylamide only through the diet (Boettcher et al., 2005). These studies show that despite common metabolic pathways between species, the relative flux through conjugation versus oxidation pathways can vary considerably, particularly when a high dose of acrylamide was used.

2.1.3 Effects on enzymes and other biochemical parameters

The α,β -unsaturated double bond of acrylamide reacts with sulfhydryl, α - and ϵ -amino and imidazole groups in proteins to form covalent adducts that can lead to irreversible loss of enzymatic activity (Friedman, 2003). Kinetic studies showed that sulfhydryl groups were 100–300 times more reactive than amino groups (Friedman, 2003). Some examples of proteins that undergo alkylation of specific cysteine residues by acrylamide and/or loss of enzymatic activity are creatine kinase, aldolase, β -lactoglobulin, bovine serum albumin, fucosidase and glycer-aldehyde dehydrogenase (Friedman, 2003).

2.1.4 Physiologically based pharmacokinetic (PBPK) modelling

Kirman et al. (2003) published a physiologically based pharmacokinetic (PBPK) model for acrylamide based on three rat data sets (Miller et al., 1982; Sumner et al., 1992; Raymer et al., 1993). The model simulates the distribution of acrylamide in five compartments (arterial blood, venous blood, liver, lung and all other tissues combined). The model fit kinetic parameters using a single set of parameters, including those for saturable enzymatic oxidation to glycidamide in the liver and first-order formation of glutathione conjugates of acrylamide and glycidamide. Saturable metabolism of glycidamide to glyceramide by epoxide hydrolase was also included. It should be noted that the kinetic data from Miller et al. (1982) used by Kirman et al. (2003) were derived from administration of radio-labelled acrylamide, so that no measurements of glycidamide or tissue partition coefficients were available. Subsequently, additional kinetic studies of acrylamide and glycidamide in rodents and in humans have become available that should

significantly assist in future model development (Barber et al., 2001; Twaddle et al., 2004a, 2004b; Doerge et al., 2005a, 2005b; Fennell et al., 2005).

2.2 Toxicological studies

2.2.1 Acute toxicity

Acute toxic effects from oral dosing (Table 2) were seen only above acrylamide doses of 100 mg/kg bw, and reported median lethal doses (LD₅₀s) are generally above 150 mg/kg bw (Dearfield et al., 1995).

2.2.2 Short-term studies of toxicity

Studies involving dermal, eye or inhalation exposure have not been included, since the Committee has been asked to consider primarily dietary exposure to acrylamide and its primary metabolite glycidamide.

(a) Mice

In a lung adenoma bioassay, groups of 16–40 male and female A/J mice received aqueous acrylamide at doses up to 25 mg/kg bw per day by oral gavage or up to 60 mg/kg bw per day by the intraperitoneal route 3 days/week for 8 weeks. These animals were sacrificed after 8–9 months. There was an exposure-related increase in the formation of lung tumours (Bull et al., 1984a). The Committee noted the high background incidence of lung tumours in this strain of mice.

In a skin initiation/promotion assay, groups of 16–40 female SENCAR and ICR mice received acrylamide at 0–50 mg/kg bw in water or in ethanol (for dermal studies) 3 days/week for 2 weeks by oral gavage, intraperitoneal injection or topically. Then, most groups of animals, except some non-tetradecanoyl-phorbol acetate (TPA) controls, received TPA dermally 3 days/week for 20 weeks; animals were sacrificed at 52 weeks. An acrylamide dose-related increase in tumour formation was noted for all routes of acrylamide administration when TPA was administered subsequently, but not when mice were treated with acrylamide without TPA. These results suggest that acrylamide was “initiating” tumour formation (Bull et al., 1984a, 1984b).

Hashimoto et al. (1981) focused on potential neurotoxic and testicular effects in a study with six male mice per group (strain not stated). Mice received acrylamide (>95% purity) at 0 or 36 mg/kg bw in saline by oral gavage twice weekly for 8 weeks. In the test group, clinical signs of toxicity were weakness and ataxia of the hindlimbs and in some cases aggressiveness and increased “alertness.” Rota-rod performance was assessed twice weekly and showed a clear and progressive decrease from week 3 onwards in the length of time that acrylamide-exposed animals were able to stay on the rod. Relative testicular weight was reduced to 83% of control value. No abnormalities were seen in the terminal haematology examination (red and white blood cells, haemoglobin concentration and haematocrit), but light microscopy of the testes revealed some “degeneration of epithelia in spermatids and spermatocytes” (presumably meaning that reduced

Table 2. Acute oral toxicity of acrylamide

Species, strain, age, sex	Number	Route	Dose (mg/kg bw)	Duration (days)	NOEL	Effects	Reference
Rats, F344, male	Groups of 10	Single oral	50, 100, 125, 200, 250	7 days post-dose	LD ₅₀ at 7 days 175 mg/kg bw, no NOEL estimated	At 24 h: 3/10 and 7/10 dead at 200 and 250 mg/kg bw, respectively At 7 days: 8/10 and 10/10 dead at same doses At 12 h, postural and motor incoordination, hindlimb muscular dysfunction, hyper-reflexia, recurrent episodes of tonic-clonic convulsions and tremor (especially at 250 mg/kg bw)	Tilson & Cabe (1979)
Rats, Porton, female	Not given	Not given	Not given	Not given	LD ₅₀ 203 mg/kg bw	Fine tremor at 203 mg/kg bw, which lasted approximately 48 h, animals either recovered completely or died within 3 days; fatty accumulation in liver; no other abnormalities reported	Fullerton & Barnes (1966)
Mice, unspecified sex and strain	Groups of 4	Saline solution, oral	Not given	Not given	LD ₅₀ 107 mg/kg bw	No details	Hashimoto et al. (1981)
Rats, unspecified strain and sex	Groups of 5	Single oral	126 or 252	Not given	LD ₅₀ 150–180 mg/kg bw	Weight loss (not quantified), all animals receiving 252 mg/kg bw died within 24 h, lethargy	McCollister et al. (1964)
Rabbits, unspecified strain and sex	Groups of 4	Single oral	126 or 63, 126 or 252	Not given	LD ₅₀ 150–180 mg/kg bw	Tremors, pupil dilation; at 63 mg/kg bw, rabbits lost weight, 1 rabbit died at 126 mg/kg bw	McCollister, et al. (1964)
Guinea-pigs, unspecified strain and sex	Groups of 4	Single oral	126 or 252	Not given	LD ₅₀ 150–180 mg/kg bw	Weight loss	McCollister et al. (1964)

Table 2. (contd)

Species, strain, age, sex	Number	Route	Dose (mg/kg bw)	Duration (days)	NOEL	Effects	Reference
Mice, adult male (60 days) or prepubertal (30 days), strain unspecified	Not given	Single oral	0, 100 or 150	Observed 1–10 days, with sacrifice	None specified	All animals at 100 mg/kg bw survived, 50% of prepubertal and 65% of adult mice at 150 mg/kg bw died during 10-day period; testicular weight unaffected, severe lesions reported 1 day after administration of 100 or 150 mg/kg bw; early and mid-phase spermatids more sensitive than later spermatids	McCollister et al. (1964)

From European Commission (2002a)

numbers of spermatids and spermatocytes were observed in the epithelium when compared with controls), reduction of spermatozoa and the presence of multinucleate giant cells. Sertoli cells, interstitial cells and the epididymides were apparently unaffected. No other histopathological investigations were performed.

Groups of five female BALB/c mice received acrylamide (99% pure) at 0 or 26 mg/kg bw per day in drinking-water for 12 days (Gilbert & Maurissen, 1982). After a recovery period of 44 days, treated animals then received 20 mg/kg bw per day for 19 days. An additional control group received 4–6% saccharin in order to mimic the reduction in water consumption in acrylamide-exposed animals, which may have affected performance in some tests conducted. Another control group was given a restricted amount of food each day. Rotarod tests were conducted twice per week and were repeated 3 times on each of those occasions, and a landing foot-spread test was conducted once per week and repeated 5 times. Some body weight loss and reduction in water consumption, perhaps related to unpalatability, were noted. Hindlimb foot splay was increased from 6 days onward, and rotarod retention time decreased from 8 days after initial acrylamide exposure. After the 44-day recovery period, hindlimb foot splay and rotarod retention were both apparently restored to control values, as were body weight and water consumption. A similar pattern of effects and time taken to the onset of effects was noted for the second exposure period. Body weight, water consumption and rotarod retention values were restored by day 31 of the recovery period following the second acrylamide exposure. The hindlimb foot splay effects were still unresolved after the 31st day of recovery. Animals receiving distilled water, saccharin or restricted food intake showed no obvious changes in rotarod performance or hindlimb foot splay, demonstrating that the impairment in performance in test animals was due to acrylamide.

(b) Rats

Burek et al. (1980) performed a 90-day study in which F344 rats received acrylamide in drinking-water. This study was primarily a neurotoxicity study and is discussed in section 2.2.6.

Tilson & Cabe (1979) administered acrylamide in water to groups of 10 male F344 rats at doses of 0, 5, 10 or 20 mg/kg bw by gavage 3 days/week for 13 weeks. Several behavioural tests (hindlimb extensor response, spontaneous motor activity, forelimb grip strength) were performed pre-dose and in weeks 1, 4, 7, 10 and 13 of acrylamide exposure. After 13 weeks, neuropathological examination (medulla oblongata, sciatic nerve at mid-thigh, branches of tibial nerve supplying calf muscles) was performed on 5 controls, all animals at 10 mg/kg bw and 5 of 10 animals at 20 mg/kg bw. The remaining animals in the control and 20 mg/kg bw dose groups were retained for further behavioural tests at weeks 1 and 5 of the recovery period followed by a neuropathological examination. No observations were mentioned at 5 mg/kg bw. Reduced body weight gain (about 15%) was noted among animals receiving 10 (only up to week 7) or 20 mg/kg bw. Hindlimb extensor response was reduced only at 20 mg/kg bw in weeks 7, 10 and 143 and in week 1 of recovery. No abnormality in hindlimb response was seen

after 5 weeks of recovery. Reduced spontaneous locomotor activity was noted only at 20 mg/kg bw in weeks 10 and 13. Recovery was complete after 5 weeks post-exposure. Forelimb grip strength was reduced at 20 mg/kg bw at weeks 4 and 7 and in week 1 of recovery, but not at any other time point. After 13 weeks of exposure, slight neuropathology (distal nerve fibre degeneration) was seen in 9 of 10 animals and moderate neuropathology (formation of Schwann cell columns) in 1 of 10 at 10 mg/kg bw. At 20 mg/kg bw, all 5 animals examined showed moderate damage (fibre degeneration and Schwann cell column formation with regenerating or remyelinating fibres). After 5 weeks of recovery, animals at 20 mg/kg bw still showed moderate neuropathology (distal regeneration of large-diameter myelinated fibres with clusters of small regenerating myelinated fibres in peripheral nerves). In summary, peripheral neuropathy (as seen in histopathology and in behavioural tests) was observed at acrylamide doses of 10 and 20 mg/kg bw when administered 3 days/week for 13 weeks by the oral route. Recovery was seen by 5 weeks at 10 mg/kg bw, but not at 20 mg/kg bw.

Groups of four male Wistar rats received acrylamide at 0, 52, 80, 125 or 200 mg/l in drinking-water for 90 days (Tanii & Hashimoto, 1983). The published report did not state actual daily dosages; however, assuming a mean body weight of 200 g and daily water consumption of 30 ml, these concentrations would approximate 0, 7.5, 12, 19 and 30 mg/kg bw per day. All treated animals demonstrated a slight reduction in body weight gain. Rotarod performance was recorded weekly; the results at day 90 showed impairment only at the two highest exposure levels. No other rotarod results were available. Other clinical signs of toxicity apparently included weakness, tendency towards spreading and dragging hindlimbs and occasionally, among more severely affected animals, urinary incontinence. When light microscopy examination was performed on posterior tibial nerves and sural nerves from the lower calf muscle region, moderate to severe changes were shown: shrinkage and loss of myelinated fibres, myelin retraction and corrugation of myelin sheaths at about 30 mg/kg bw per day. A no-observed-adverse-effect level (NOAEL) was not identifiable from this study, as the incidence and severity of findings at other exposure levels were not reported.

Groups of 10 male and female Sprague-Dawley rats received aqueous acrylamide (99% pure) at 0, 10 or 30 mg/kg bw per day by oral gavage 7 days/week for 3 weeks (Schulze & Boysen, 1991). The 3-week exposure period was followed by a 10-day recovery before readministration of acrylamide at 0, 10 or 20 mg/kg bw per day for 1 week. The high dose level was reduced due to four male and two female mortalities. A functional observational battery (FOB) conducted according to United States Environmental Protection Agency (US EPA) guidelines was conducted pre-exposure, 1, 6 and 24 h after the first administration and once per week thereafter. Parameters recorded in the FOB included assessment of movement, response to stimuli such as sound or tactile response, measurement of food consumption and body weight gain and terminal histopathology on all major organs including eyes, sciatic, tibial and sural nerves, lumbar and cervical dorsal and ventral roots, dorsal root ganglion, trigeminal ganglia and sections from different regions of the brain and spinal cord. Body weight gain and food consumption were statistically significantly reduced in

animals at 30/20 mg/kg bw per day. The onset of alterations in FOB parameters was about 2 weeks after the beginning of acrylamide exposure. At 30/20 mg/kg bw per day, the following changes were noted in the FOB: an increased incidence of rigid/difficult handling, slight ptosis, slight to moderately impaired respiration, soiled fur, hunched posture/prostration, slight to severely impaired gait, abnormal behaviour, reduced tactile response, impaired righting reflex (also seen at 10 mg/kg bw per day), decreased rearing counts (also seen at 10 mg/kg bw per day), reduced forelimb and hindlimb grip strength, reduced response to bright light and reduced activity. Histopathological examination of white matter from cervical and lumbar spinal cord sections, trigeminal and dorsal root ganglia and sciatic, tibial and sural nerves revealed altered diameter of axons (increased or decreased diameter), disruption, fragmentation and distortion of axons and/or dilation and fragmentation of myelin sheaths, and occasionally an increased number of macrophages. Findings were more prevalent and more severe in animals at 30/20 mg/kg bw per day than at 10 mg/kg bw per day. Brain regions were not significantly affected. There was also an increased incidence of splenic pigment observed in males and females at 30/20 mg/kg bw per day and in females at 10 mg/kg bw per day, increased incidence of granulomatous inflammation in lungs of animals at 20/30 mg/kg bw per day and haemorrhage of the urinary bladder in several males at 30/20 mg/kg bw per day.

Groups of 10 male and female Sprague-Dawley rats received aqueous acrylamide at 0, 12.5, 25 or 50 mg/kg bw per day by gavage for 7 days followed by a 7-day observation period (Newton et al., 1992; Hughes et al., 1994). Examinations were limited to a FOB and histopathological examination of nervous tissue. The parameters recorded in the FOB were scored pre-exposure and on days 7 and 14, according to US EPA guidelines. Histopathological examination was performed on five males and females on day 15 and included forebrain, mid-brain, cerebellum and pons, medulla oblongata, spinal cord, trigeminal ganglia, dorsal root ganglia and fibres, ventral root fibres and sciatic, sural and tibial nerves. Reduced activity was noted in all acrylamide-exposed groups; higher prevalence was seen at 50 mg/kg bw per day on day 7. Body weight gain was reduced among all acrylamide-exposed groups on days 7 and 14, but was statistically significant only at 50 mg/kg bw per day. At this dose, but not at the lower doses, hindlimbs were splayed with a corresponding impairment of mobility; a reduced number of rearing counts was observed. Mean forelimb and hindlimb grip strength were reduced among males and females at 50 mg/kg bw per day on days 7 and 14. Landing foot splay was increased among all acrylamide-treated animals on days 7 and 14, although to a lesser degree at 12.5 and 25 mg/kg bw per day. Histopathologically, axonal degeneration (minimal to marked) was seen in all animals at 50 mg/kg bw per day, especially in the sural and tibial nerves, and to a lesser degree (trace) in a small number of animals at 25 mg/kg bw per day. No effects were seen in the nerves of animals in the 12.5 mg/kg bw per day dose group.

Studies have also been done investigating changes in brain biogenic amine levels and the possible relationship with acrylamide neurotoxicity (Dixit et al., 1982; Aldous et al., 1983; Husain et al., 1987). Some changes were observed

(inconsistent between the various studies); the workers concluded that biogenic amine levels were directly related to the neurotoxic effects induced by acrylamide. Neurotoxicity has been suggested, on the basis of some evidence, to arise as a result of changes in microtubule formation in the nerve fibres themselves. The alterations in brain biogenic amines may be a secondary consequence of systemic toxicity.

(c) *Cats*

Groups of 17–23 cats of unknown breed received acrylamide (>98% purity) at 0–15 mg/kg bw per day by dietary administration 7 days/week for up to 16 weeks (Post & McLeod, 1977). Abnormal gait (hindlimbs only) was noted within 4–6 weeks. From 12 to 16 weeks, animals were unable to walk and showed weight loss (not quantified) and diarrhoea. Motor conduction velocity in the posterior tibia nerve and greater splanchnic nerve (a branch of the sciatic nerve) was significantly reduced from week 12; the amplitude of externally recorded muscle action potential (from muscles in the foot) and action potential in the greater splanchnic nerve were reduced from weeks 4 to 6 and markedly so from week 12. Fibre density of large-diameter nerve fibres in the region of the left gastrocnemius muscle and small fibres in the vagus nerve and greater splanchnic nerve were reduced from weeks 4 to 6 onwards and were slightly more reduced from week 12. Histopathology of nerve fibres supplying the left gastrocnemius muscle, greater splanchnic nerve and left cervical vagus nerve showed only a reduced number of myelinated fibres, which was more pronounced in the gastrocnemius muscle. Electron microscopy showed an increased density of neurofilaments and abnormal membranous configurations between the axolemma and Schwann cell membrane. Degenerating fibres of the gastrocnemius muscle and splanchnic nerve showed loss of myelin, and unmyelinated fibres also showed signs of degeneration.

Groups of 1–3 cats of unknown origin received acrylamide at 0, 0.03, 0.1, 1, 3 or 10 mg/kg bw per day by dietary administration 5 days/week for up to 1 year (McCollister et al., 1964). Two control animals died and one was killed due to intercurrent infection after less than 6 months. Signs of peripheral neuropathy (loss of use of hindlimbs, abnormal gait) were observed at 1 mg/kg bw per day and above. All animals at 0.03 and 0.1 mg/kg bw and 1 of 2 cats at 3 mg/kg bw per day died, apparently from intercurrent infection. It was reported that there were no pathological abnormalities attributable to acrylamide at any exposure level. However, the extent of examination was unclear, and it was difficult to draw firm conclusions due to the generally poor condition of animals used in this study.

(d) *Dogs*

In a study by Satchell & McLeod (1981), 14 dogs received acrylamide at 7 mg/kg bw per day by dietary admixture for about 10 weeks. No control animals were used. Clinical signs of toxicity included severe impairment of hindlimb function, "toe-folding" being observed from about day 30, ataxia from about day 40, clear signs of muscle weakness from around day 50 and regurgitation from

around day 60. In 3 of 14 dogs, expansion of the oesophagus (megaoesophagus) was noted radiologically. Since only three animals were examined, the significance of the findings is uncertain; there were no controls, and megaoesophagus was reported to occur spontaneously in the dog with unknown etiology.

In a study focusing on respiratory effects, four dogs received acrylamide (99% pure) at 6 mg/kg bw per day in gelatin capsules for 6–7 weeks, with up to 8 weeks' recovery (Hersch et al., 1989). Resting respiration was measured using an intratracheal technique, and electrocardiography, electroencephalography and heart rates were recorded. In two animals, blood levels of carbon dioxide and transcutaneous oxyhaemoglobin were also recorded. The Hering-Breuer lung inflation reflex was quantified by measuring the duration of apnoea produced during lung inflation and was used as an indicator of the function of the vagus nerve. Parameters for each animal were recorded pre-exposure and served as controls for this study. Loss of use of hindlimbs and "toe-folding" were observed from about week 3 and resolved during the 5th week of recovery. One animal was killed due to pneumonia at about week 10. Decreased respiratory frequency and slightly increased tidal volume were observed during the acrylamide exposure period, but were restored during the recovery period. The Hering-Breuer lung inflation reflex was impaired (as indicated by increased tidal volume and decreased respiratory frequency). Other parameters were not adversely affected. The Hering-Breuer reflex changes could be indicative of damage to the vagus nerve; the toxicological significance of these respiratory effects is unclear.

(e) *Non-human primates*

In an extensive study, four feral-born macaque monkeys received acrylamide (>99% purity) at 10 mg/kg bw per day in fruit juice for 5 days/week for 44–61 days until the time of onset of clinical signs of toxicity (Maurissen et al., 1983). Animals were allowed to recover, and examinations were performed for up to 146 days. Two control animals received tap water only for about 13 weeks, using a similar dosing regimen. Investigation included recording body weight; clinical signs of toxicity; a visuomotor task (time taken to pick up a food reward) performed twice per week; sensitivity to an electrical or a vibration stimulus, also performed twice per week; and sural nerve histopathology, performed first when vibration thresholds were elevated (about days 51–58 of acrylamide exposure) and then during the recovery phase (up to 146 days after the last acrylamide exposure). In treated animals, clinical signs of toxicity included loss of balance, decreased activity, hindlimb weakness and forelimb tremor in the final week of acrylamide treatment for one particular animal. With the exception of forelimb tremor, which persisted for up to 4 weeks, these clinical signs of toxicity resolved within 2 weeks post-treatment. Body weight loss was noted in 3 of 4 animals during treatment. One control animal also showed body weight loss. In treated animals, response to a 60-Hz electrical stimulus was not apparently affected during or after treatment. There was a decreased sensitivity (as measured by an increased time to key-pressing) towards a vibration stimulus (40 Hz and 150 Hz) during the treatment phase, with effects being even more pronounced in the first 10 weeks post-treatment. An increased time taken to pick up a food reward was noted in test

animals towards the end of the treatment period and was also more pronounced in the first 3 weeks post-treatment. Sural nerve biopsies were prepared from two acrylamide-exposed animals during the elevated vibration thresholds (days 51 and 58 of treatment) and then during the recovery phase (days 146 and 136). The first examination revealed no visible axons in some areas; myelin had formed balls. Under light microscopy, most nerve fibres appeared to be normal. Electron microscopy showed that most myelinated nerve fibres were normal, but others showed axolemma invagination, disruption of myelin, other undescribed "severe axonal alterations" or a loss of axons. Some Schwann cells lacked an axon and contained disintegrating or contorted myelin. In one animal, about 25% of nerve fibres were affected, but in the other, only "occasional" fibres were affected. There were no abnormalities observed in unmyelinated nerve fibres. When the second biopsy was performed during the recovery phase, when no abnormalities were seen in vibration sensitivity, degenerative changes were less frequent than during the treatment period; regenerative fibres were also seen. Loss of vibration sensitivity did not appear to be associated with the neuropathological findings.

Adult macaque monkeys (three) received acrylamide at 10 mg/kg bw in fruit juice 5 days/week for 6–9 weeks (Maurissen et al., 1990). Two additional monkeys were used as controls. There was a second treatment period after a 30-week recovery. The investigators focused on body weight changes, time taken to pick up a food reward, response to electrical stimulus and response to a vibration stimulus (40 and 150 Hz). Results were essentially similar to those obtained by the earlier study by the same investigators.

A group of seven macaque monkeys received acrylamide at 10 mg/kg bw per day in fruit juice for 5 days/week for up to 13 weeks with approximately 20–30 weeks' recovery (Eskin et al., 1985). There were two control animals. Brain, optic nerve and eyes were removed for histopathological (light and electron microscopy) examination. For acrylamide-treated animals sacrificed immediately after 9–13 weeks, distal axonal swelling was most prominent in distal optic tract fibres, particularly within the lateral geniculate nucleus. Myelin sheaths were disproportionately thin, and degenerating myelin and occasional shrunken axons were observed. Degenerating myelin and degenerating/atrophic axons were seen in the optic nerve and the proximal optic tract. Axonal swellings were seen in the lateral geniculate nucleus of the brain, and occasional alterations in the retinal axon terminals and synapses were observed by light and electron microscopy. Dilation of the axonal terminals, degeneration of myelin, degenerating/atrophic axons and an increased number of astroglial processes were also seen in the lateral geniculate nucleus. No abnormalities were seen in controls. The optic nerves of acrylamide-exposed monkeys showed a loss of axons and diminished numbers of fibres in the optic nerve. Electron microscopy showed disproportionately thin myelin sheaths, densely packed astroglial processes, lipid vacuolation and degenerating myelin fragments in the phagocytes and astrocytes.

Three adult macaque monkeys received acrylamide at 10 mg/kg bw in fruit juice 5 days/week for 33–47 doses (6–10 weeks, after which pronounced ataxia was observed) (Merigan et al., 1982). After the administration period, one animal was sacrificed for histopathological examination, and the other two were observed

for a further 90 days. A fourth animal served as control. Observations for visual acuity and flicker-fusion were performed 5 mornings per week, and cortical evoked potentials were recorded on 2–3 afternoons per week. Visuomotor coordination was monitored daily by measuring the time taken to pick up a food reward. Body weights were measured, but no results were presented. No histopathological information was presented. After 4 weeks of acrylamide exposure, a marked increase in cortical evoked potential was observed. This change preceded a decrease in visual acuity and flicker-fusion frequency apparent 2 weeks later. Towards the end of the treatment period, a marked increase in the time taken for a pick-up test was apparent; this time was still markedly increased for about 2 weeks after cessation of dosing. After 3 weeks, flicker-fusion frequency was restored; cortical evoked potential values were restored within 7 weeks. Within 3 weeks, visual acuity stabilized, but was still at a level below that recorded pre-exposure for the rest of the 90-day post-exposure observation period. Among acrylamide-exposed animals, weight loss, hindlimb weakness, gait disturbances and tremors were observed. No abnormalities were seen in papillary or eye movement or other ophthalmological characteristics. In the control animal, there were no significant changes observed.

A companion study of two investigations of potential effects on the visual system was performed by Merigan et al. (1985). (Histopathological details are described in Eskin et al. [1985].) Three macaque monkeys received acrylamide at 10 mg/kg bw per day in fruit juice 5 days/week for about 6–10 weeks, with one animal used as control. One acrylamide-exposed monkey was sacrificed at 10 weeks for reasons of animal welfare; the other two monkeys had a 140-day recovery period. A number of tests for visual capacity were conducted. Reduced contrast sensitivity was noted at the end of the exposure period. Visual acuity was also impaired in all acrylamide-exposed animals, with a slight recovery within 5 weeks post-administration. Flicker-fusion frequency was reduced from about week 2 onwards and recovered within 5 weeks post-administration. In acrylamide-exposed animals, visual evoked potentials were impaired. The authors suggested that these changes in latency and increase in amplitude correlated with a conduction block in large-diameter optic nerve fibres.

In the only non-human primate study that investigated a range of different exposure levels, one female monkey (unspecified species) per dose level received aqueous acrylamide at 0, 0.03, 0.1, 0.3 (two animals at this exposure level), 1, 3 or 10 mg/kg bw per day by oral gavage or dietary administration 5 days/week for up to 1 year (McCollister et al., 1964). Blood cholinesterase measurements and macroscopic and microscopic pathology were conducted after acrylamide exposure, but no details were available regarding these examinations. At 10 mg/kg bw per day, there were clear and severe clinical signs of neuropathy. At 3 mg/kg bw per day, occasional abnormalities were observed, such as reduced knee jerk reaction, reduced pupillary reflexes (response to bright light) and lethargic behaviour. There were no apparent effects on body weight, no clinical signs of toxicity, no changes in haematology (the parameters measured were not clearly reported), liver and kidney weight and no macroscopic or microscopic pathology abnormalities (extent of examination unclear, probably at least the brain and spinal cord) at

0.1, 0.3 and 1 mg/kg bw per day exposure for 1 year. Conclusions cannot be easily drawn from this study due to limited reporting and the use of only one animal per dose level.

2.2.3 Long-term studies of toxicity and carcinogenicity

Groups of 60 male and female F344 rats received acrylamide at 0, 0.01, 0.1, 0.5 or 2.0 mg/kg bw per day in drinking-water for 2 years (Johnson et al., 1986). Increased cumulative mortality was observed in both groups of rats treated with the high dose after 21 months. Decreased mean body weights were observed for the high-dose males only. Significant increases were reported in tibial nerve degeneration observed microscopically (severe in males; moderate in females). Significant increases in the incidences of tumours were observed in the thyroid gland of males and females (high dose only), peritoneal mesotheliomas in the region of the testis in males (0.5 and 2.0 mg/kg bw per day doses), mammary gland (high-dose females), central nervous system (high-dose males), adrenal gland (high-dose males), pituitary gland (high-dose females), oral cavity (high-dose males and two highest doses in females), uterus (high-dose females) and clitoral gland (high-dose females) (Table 3).

In a second study also using F344 rats, acrylamide was administered to males (groups of 75–102) at 0, 0.1, 0.5 or 2.0 mg/kg bw per day and to females (groups of 50–100) at 0, 1.0 or 3.0 mg/kg bw per day for 2 years in drinking-water (Friedman et al., 1995). Increased cumulative mortality was observed only in males treated with the high dose during weeks 68–72. Decreased mean body weights were observed for the high-dose males and females. Significant increases were reported in sciatic nerve degeneration observed microscopically in high-dose males and females. Significantly increased incidences of thyroid follicular cell tumours in males and females (high doses), peritesticular mesotheliomas in males (high dose) and mammary tumours in females (both doses) were also observed in this study (Table 4).

The Committee noted that oral cavity papillomas, clitoral gland adenomas and uterine adenocarcinomas reported as increased in incidence in female rats in the Johnson et al. (1986) study were reported as not increased by Friedman et al. (1995). Glial tumours of the brain and spinal cord were also reported as not increased by Friedman et al. (1995); however, some primary brain tumours diagnosed as “malignant reticulosis” were not included in the analysis (Rice, 2005).

The consistent findings from chronic acrylamide bioassays of increased tumour incidences in endocrine-responsive tissues from male and female F344 rats, including thyroid, mammary gland and peritesticular mesothelium, have led to some investigation of non-genotoxic mechanisms for acrylamide carcinogenicity (Park et al., 2002; Lafferty et al., 2004). The reactivity of acrylamide and glycidamide with thiol groups in numerous cellular proteins makes enzymes, receptors and cytoskeletal elements susceptible to modifications in structure and function that could disrupt hormonal and cellular redox regulation processes and lead to transformation by epigenetic mechanisms (Park et al., 2002; Lafferty et al., 2004). However, the wide body of evidence supporting genotoxicity associated with

glycidamide exposure is not incompatible with hormonal dysregulation by acrylamide, because it is clear that other factors beyond DNA damage are probably required for the observed target tissue specificity of acrylamide tumorigenesis in rats (Lafferty et al., 2004; Doerge et al., 2005c; Manière et al., 2005). Moreover, it should be noted that the pattern of acrylamide-induced tumour organs in F344 rats resembles that observed for other genotoxic carcinogens — e.g. ethylene oxide (IARC, 1994a) and acrylonitrile (IARC, 1999). Findings of increased incidences of central nervous system tumours for acrylamide are particularly significant, because all known carcinogens for the nervous system are genotoxic or are converted to genotoxic metabolites (Rice & Wilbourn, 2000; Rice, 2005). The Committee noted that the evidence currently available was insufficient to support non-genotoxic mechanisms of acrylamide-induced cancer, particularly in light of the consistent evidence for a genotoxic mechanism (see also section 2.2.4).

Table 3. Numbers of Fischer 344 rats with tumours at various organ sites after receiving drinking-water containing acrylamide for 2 years

Type of tumour	Sex	Dose ^a (mg/kg bw per day)				
		0	0.01	0.1	0.5	2.0
Thyroid gland, follicular adenomas	M	1/60	0/58	2/59	1/59	7/59*
Peritesticular mesotheliomas	M	3/60	0/60	7/60	11/60*	10/60*
Adrenal gland, ^b pheochromocytomas	M	3/60	7/59	7/60	5/60	10/60*
Mammary tumours	F	10/60	11/60	9/60	19/58	23/61*
Central nervous system, glial tumours	F	1/60	2/59	1/60	1/60	9/61*
Thyroid gland, follicular adenomas or adenocarcinomas	F	1/58	0/59	1/59	1/58	5/60*
Oral cavity, squamous papillomas	F	0/60	3/60	2/60	1/60	7/61*
Uterus, adenocarcinomas	F	1/60	2/60	1/60	0/59	5/60*
Clitoral gland, adenomas ^c	F	0/2	1/3	3/4	2/4	5/5*
Pituitary adenomas ^b	F	25/59	30/60	32/60	27/60	32/60*

Data from Johnson et al. (1986), as compiled by Rice (2005)

F, female; M, male

^a Asterisk (*) indicates $P = 0.05$; pair-wise Mantel-Haenszel comparison with the control group adjusted for mortality.

^b The historical incidence of adrenal gland pheochromocytomas in males was 8.7% (range, 1.2–14.0%); that of pituitary adenomas in females was 38.1% (range, 28.2–46.9%).

^c Only clitoral glands with gross lesions were examined histologically.

Table 4. Numbers of Fischer 344 rats with tumours at various organ sites after receiving drinking-water containing acrylamide for 2 years^a

Type of tumour	Sex	Dose ^b (mg/kg bw per day)						
		0	0	0.1	0.5	1.0	2.0	3.0
Peritesticular mesotheliomas	M	4/102	4/102	9/204	8/102	—	13/75*	—
Brain and spinal cord, glial neoplasms ^c	M	1/102 ^d	1/102 ^d	2/204 ^e	1.102 ^f	—	3/75 ^d	—
	F	0/50 ^g	0/50 ^g	—	—	2/100 ^g	—	2/100 ^g
Thyroid gland, follicular adenomas	M	2/100	1/102	9/203	5/101	—	15/75* ^h	—
	F	0/50	0/50	—	—	7/100	—	16/100* ^h
Thyroid gland, follicular cell carcinomas	M	1/100	2/102	3/203	0/101	—	3/75	—
	F	1/50	1/50	—	—	3/100	—	7/100
All follicular cell neoplasms	M	3/100	3/100	12/203	5/101	—	17/75	—
	F	1/50	1/50	—	—	10/100	—	23/100*
Mammary gland, fibroadenomas and adenocarcinomas	F	7/46	4/50	—	—	21/94 ⁺	—	30/95*

Data from Friedman et al. (1995), as compiled by Rice (2005)

^a Certain tumours that occurred at increased incidence in treated rats in the previous study (Johnson et al., 1986) were not reported as occurring at increased incidences in this study. These included papillomas of the oral cavity in females, adenomas of the clitoral gland and uterine adenocarcinomas. Numbers of these neoplasms were not given.

^b Asterisk (*) indicates statistical significance, $P < 0.001$.

^c Does not include seven rats with "malignant reticulosis" of the brain, including five dosed females, one dosed male and one control male.

^d All brains of high-dose rats and all control brains (both subgroups) were examined, but only 82/102 and 90/102 control spinal cords and 51–75 high-dose spinal cords were examined.

^e Only 98/204 brains and 68/204 spinal cords were examined.

^f Only 50/102 brains and 37/102 spinal cords were examined.

^g All brains were examined, but only 45/50, 44/50, 21/100 and 90/100 spinal cords in control, control, low- and high-dose females, respectively, were examined. The study used two groups of control animals in an effort to increase the statistical power of the study and to obtain a better description of the dose–response curve.

^h Includes three male rats and one female rat with multiple tumours in the highest dose groups.

Long-term carcinogenicity bioassays of acrylamide and glycidamide are under way at the United States Food and Drug Administration's National Center for Toxicological Research under the auspices of the National Toxicology Program. Concentrations of acrylamide used in 14-day dose range-finding studies in male and female B6C3F1 mice and male and female F344 rats were 1–50 mg/kg bw per day in drinking-water and dosed feed, and molar equivalent doses of glycidamide were administered in drinking-water. Subchronic studies (90-day) were also conducted using doses of 1–25 mg/kg bw per day in drinking-water and dosed feed for acrylamide, and the molar equivalent doses of glycidamide were administered in drinking-water. Doses for the 2-year studies were chosen after reviewing the pathological data from the subchronic study. Studies began in May 2005. For rats, doses of acrylamide are 0.6–5 mg/kg bw per day in drinking-water; for mice, doses are 1.8–14 mg/kg bw per day in drinking-water. Corresponding equimolar doses of glycidamide in drinking-water are being administered to rats and mice.

2.2.4 Genotoxicity

The results of genotoxicity studies with acrylamide are summarized in Table 5. Acrylamide is mainly negative in prokaryotic in vitro test systems but predominantly positive in mammalian test systems and in vivo tests of mutagenicity. Furthermore, exposure to acrylamide leads to DNA adduct formation (see Figure 2). However, most of the genotoxicity of acrylamide seems to be mediated by glycidamide:

- The reactivity of glycidamide with DNA bases is greater than that of acrylamide (Solomon et al., 1985; Gamboa da Costa et al., 2003).
- Treatment of rodents with glycidamide produces higher levels of DNA adducts than does treatment with acrylamide (Gamboa da Costa et al., 2003; Doerge et al., 2005c).
- Micronuclei induction is caused by glycidamide (Paulsson et al., 2003a).
- Glycidamide is the active metabolite responsible for germ cell mutations and dominant lethality in male mouse spermatids (Ghanayem et al., 2005a; reviewed by Favor & Shelby, 2005).
- Glycidamide, but not acrylamide, is mutagenic in *Salmonella* (Hashimoto & Tanii, 1985).
- The mutational spectrum produced by glycidamide in transgenic Big Blue mouse embryonic fibroblasts in vitro (Besaratina & Pfeifer, 2004) corresponds with the DNA adducts observed in vivo (Gamboa da Costa et al., 2003).

An assessment of the mutagenic potential for glycidamide–DNA adducts can be made based on the in vitro mutagenicity studies of Besaratina & Pfeifer (2004), even though an indirect method was used to measure DNA adducts (i.e. polymerase termination position). Treatment of embryonic fibroblasts from transgenic (Big Blue) mice with glycidamide in vitro (0.05–5000 $\mu\text{mol/l}$) increased the mutant frequency in the *cII* transgene relative to control cells. In addition, glycidamide-

Table 5. Results of acrylamide genotoxicity testing

Assay	Test system	Dose/concentration	HID or LED	Result	Reference
<i>Bacterial gene mutation assays</i>					
Reverse mutation	<i>Salmonella typhimurium</i> TA1535, TA1537, TA98, TA100	10–10 000 µg/plate ± S9 activation	100	Weakly positive in TA98, TA100 only with activation; others negative	Zeiger et al. (1987)
	<i>S. typhimurium</i> TA1535, TA97, TA98, TA100	100–10 000 µg/plate ± S9 activation	10 000	Negative	
	<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100, TA102	1–100 mg/plate ± S9 activation	100	Negative	Knaap et al. (1988)
	<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100	0.5–50 mg/plate ± S9 activation	50	Negative in both systems	Tsuda et al. (1993)
	<i>Escherichia coli</i> WP2 <i>uvrA</i> [−]				
	<i>S. typhimurium</i> TA1535	Up to 5 mg/plate ± S9 activation	5	Negative	Jung et al. (1992); Müller et al. (1993)
	<i>S. typhimurium</i> TA1535, TA1537, TA1538, TA98, TA100	Up to 1 mg/plate ± S9 activation	1	Negative	Lijinsky & Andrews (1980)
Fluctuation test	<i>S. typhimurium</i> TA1535, TA1537, TA1538, TA98, TA100	0.5–5000 µg/plate ± S9 activation	5000	Negative	Hashimoto & Tanii (1985)
	<i>Klebsiella pneumoniae</i> <i>ur</i> [−] <i>pro</i> [−]	2–10 mg/ml	10	Negative	Knaap et al. (1988)

Table 5. (contd)

Assay	Test system	Dose/concentration	HID or LED	Result	Reference
<i>Non-mammalian gene mutation assays in vivo</i>					
Sex-linked recessive lethal	<i>Drosophila melanogaster</i>	40–50 mmol/l, abdominal injection	50	Negative	Knaap et al. (1988)
	<i>D. melanogaster</i>	0.24–5 mmol/l, larvae feeding	1.0	Positive	Tripathy et al. (1991)
Somatic mutation, recombination	<i>D. melanogaster</i>	1–1.5 larvae feeding (unit unspecified, but probably mmol/l)	1	Weakly positive	Knaap et al. (1988)
	<i>D. melanogaster</i>	1–1.5 mmol/l, larvae feeding	1	Positive	Batiste-Alentorn et al. (1991)
	<i>D. melanogaster</i>	0.25–5 mmol/l, larvae feeding	1.0	Positive	Tripathy et al. (1991)
<i>Mammalian gene mutation assays in vitro</i>					
	Mouse lymphoma L5178Y Tk ^{+/−} , tk locus	10 mmol/l	10	Positive (more pronounced without activation)	Barfknecht et al. (1988)
	Mouse lymphoma L5178Y Tk ^{+/−} , tk locus	0–0.85 mg/ml, without activation	0.5	Positive	Moore et al. (1987)
	Mouse lymphoma L5178Y Tk ^{+/−} , tk and hprt loci	0.5–7.5 mg/ml with or without metabolic activation		Equivocal, increases only at cytotoxic concentrations	Knaap et al. (1988)
	Mouse lymphoma L5178Y Tk ^{+/−} , hprt locus	0.1–0.5 mg/ml with co-cultivated mammalian cells	0.3	Positive	Knaap et al. (1988)
	Chinese hamster V79H3 cells, hprt locus	1–7 mmol/l, with no activation	7	Negative	Tsuda et al. (1993)

Table 5. (contd)

Assay	Test system	Dose/concentration	HID or LED	Result	Reference
	Mouse embryonic fibroblasts, Big Blue mouse	0.52–16 000 $\mu\text{mol/l}$	3.2–320	Positive	Besaratinia & Pfeifer (2003)
	Mouse embryonic fibroblasts, Big Blue mouse	0.5–5000 $\mu\text{mol GA/l}$	0.5–5000	Positive	Besaratinia & Pfeifer (2004)
<i>Mammalian gene mutation assays in vivo</i>					
Transgenic mouse <i>lacZ</i>	Muta™ Mouse	5 × 50 mg/kg bw per day, intraperitoneal injection	5 × 50	Weakly positive, no statistical analysis	Hoorn et al. (1993)
	Muta™ Mouse	50–100 mg/kg bw, intraperitoneal injection	100	Negative	Krebs & Favor (1997)
Mouse spot test	Mouse embryos (T × HT)F1	1 × 50 or 75 mg/kg bw	50	Positive	Neuhäuser-Klaus & Schmahl (1989)
		3 × 50 or 75 mg/kg bw intraperitoneal injection	3 × 50	Positive	
Transgenic mouse <i>cII</i>	Big Blue mouse (M, F)	10 mg/kg bw drinking-water (1 month)	50 AA 62 GA	Positive: AA and GA	Manjanatha et al. (in press)
		50 mg AA/kg bw (and equimolar GA)			
Transgenic mouse <i>hprt</i>	Big Blue mouse (M, F)	10 mg/kg bw drinking-water (1 month)	10 AA 12 GA	Positive: AA and GA Positive: AA and GA	Manjanatha et al. (in press)
		50 mg AA/kg bw (and equimolar GA)			
Transgenic mouse <i>Tk</i> ^{+/-}	B6C3F1 neonatal mice (<i>Tk</i> ^{+/-} and wild-type)	10 and 50 mg AA/kg bw (and equimolar GA), intraperitoneal injection	50 AA 12 GA	Negative: AA Positive: GA	Von Tungeln et al. (2005)

Table 5. (contd)

Assay	Test system	Dose/concentration	HID or LED	Result	Reference
Transgenic mouse <i>hprt</i>	B6C3F1 neonatal mice (<i>Tk</i> ^{+/+} and wild-type)	10 and 50 mg AA/kg bw (and equimolar GA), intraperitoneal injection	50 AA 62 GA	Negative: AA Positive: GA	Von Tungeln et al. (2005)
Morphological specific locus	Mouse (C3H/R1 × 101/R1)F1 (M)	5 × 50 mg/kg bw, intraperitoneal injection	50	Positive (post-spermatogonia)	Russell et al. (1991)
	Mouse (102/E1 × C3H/E1)F1 (M)	100–125 mg/kg bw, intraperitoneal injection	100	Positive (post-spermatogonia; spermatogonia)	Ehling & Neuhäuser-Klaus (1992)
<i>Chromosomal alterations in mammalian cells in vitro</i>					
Chromosomal aberrations	Chinese hamster cells	0.5–5 mmol/l, no activation used	2	Positive	Tsuda et al. (1993)
	Chinese hamster cell line (V79)	0.1–3 mg/ml ± S9 activation	1	Positive, with or without metabolic activation	Knaap et al. (1988)
	Mouse lymphoma L5178Y <i>Tk</i> ^{+/+} –3.7.2 cells	0.65–0.85 mg/ml, without activation	0.75	Positive	Moore et al. (1987)
Cell division aberration	Chinese hamster lung cell line DON:Wg3h	0.2–1 mg/ml	0.2	Positive	Warr et al. (1990)
	Chinese hamster lung fibroblast LUC2 p5	0.01–1 mg/ml	0.01	Positive	Warr et al. (1990)
Polyploidy	Chinese hamster cell line (V79)	0.5–5 mmol/l	1	Positive	Tsuda et al. (1993)
Spindle disturbances	Chinese hamster cell line (V79)	0.01–1 mg/ml	0.01	Positive	Adler et al. (1993)

Table 5. (contd)

Assay	Test system	Dose/concentration	HID or LED	Result	Reference
Micronucleus	Seminiferous tubular segments (spermatids from SD rats)	5–50 µg/ml	50	Negative	Lähdetie et al. (1994)
<i>Chromosomal alterations in mammalian cells in vivo</i>					
Chromosomal aberrations	Mouse (101/E1 × C3H/E1)F1 bone marrow cells	50–150 mg/kg bw, intraperitoneal injection	50	Positive	Adler et al. (1988)
	Mouse (ICE-SPF) bone marrow cells	100 mg/kg bw, intraperitoneal injection	100	Positive	Cihák & Vontorková (1988)
	Mouse (ddY) bone marrow cells	100–200 mg/kg bw, intraperitoneal injection	200	Negative	Shiraishi (1978)
	Mouse (ddY) bone marrow cells	500 mg/kg in diet for 7–21 days (78 mg/kg bw per day)	78	Negative	Shiraishi (1978)
	Rat bone marrow cells	100 mg/kg bw, intraperitoneal injection	100	Negative	Krishna & Theiss (1995)
	Mouse (C57BL/6J) spleen lymphocytes	50–125 mg/kg bw, intraperitoneal injection	125	Negative	Backer et al. (1989)
	Mouse (C57BL/6) splenocytes	100 mg/kg bw, intraperitoneal injection	100	Negative	Kligerman et al. (1991)
	Mouse (101/E1 × C3H/E1)F1 spermatogonia	50–150 mg/kg bw, intraperitoneal injection	150	Negative	Adler et al. (1988)
	Mouse (C57BL/6J) spermatogonia	50–125 mg/kg bw, intraperitoneal injection	125	Negative	Backer et al. (1989)

Table 5. (contd)

Assay	Test system	Dose/concentration	HID or LED	Result	Reference
Chromosomal aberrations (contd)	Mouse (102/E1 × C3H/E1)F1 spermatogonia	5 × 50 mg/kg bw per day, intraperitoneal injection	5 × 50	Negative	Adler (1990)
	Mouse (102/E1 × C3H/E1)F1 spermatocytes	100 mg/kg bw, intraperitoneal injection	100	Positive	Adler (1990)
	Mouse (102/E1 × C3H/E1)F1 spermatocytes	100 mg/kg bw, intraperitoneal injection	100	Positive	Adler (1990)
	Mouse (CF1) first-cleavage embryos	150 mg/kg bw, intraperitoneal injection	150	Positive in embryos from which the males had mated 6–8 days following treatment (early spermatozoa stage)	Valdivia et al. (1989)
	Mouse (B6C3F1) first-cleavage one-cell zygotes, examined after mating (M)	75 and 125 mg/kg bw or 5 × 50 mg/kg bw per day, intraperitoneal injection	75	Positive	Pacchierotti et al. (1994)
Polyploidy or aneuploidy	Mouse bone marrow cells	100–200 mg/kg bw, intraperitoneal injection	100	Positive	Shiraishi (1978)
	Mouse bone marrow cells	500 mg/kg in the diet for 7–21 days (78 mg/kg bw per day)	78	Positive	Shiraishi (1978)
Spindle disturbances	Mouse (102/E1 × C3H/E1) bone marrow cells	120 mg/kg bw, intraperitoneal injection	120	Negative	Adler et al. (1993)
Micronucleus	Mouse (101/E1 × C3H/E1)F1 bone marrow cells (M, F)	50–125 mg/kg bw, intraperitoneal injection	50	Positive	Adler et al. (1988)
	Mouse (ICR-SPF) bone marrow cells (M)	100 mg/kg bw, intraperitoneal injection	100	Positive	Cihák & Vontorková (1988)

Table 5. (contd)

Assay	Test system	Dose/concentration	HID or LED	Result	Reference
Micronucleus (contd)	Mouse (ICR-SPF) bone marrow cells (M)	25–100 mg/kg bw per day for 2 days, intraperitoneal injection	25	Positive	Cihák & Vontorková (1988)
	Mouse (Swiss NIH) bone marrow cells (M, F)	136 mg/kg bw, intraperitoneal injection	136	Positive	Knaap et al. (1988)
	Mouse (ICR-SPF) bone marrow cells (M, F)	42.5–100 mg/kg bw per day (1, 2 or 3 days), intraperitoneal injection	M: 42.5 F: 55	Positive	Cihák & Vontorková (1988)
	Rat (Sprague-Dawley) bone marrow cells (M)	100 mg/kg bw, intraperitoneal injection	100	Negative	Paulsson et al. (2002)
	Rat bone marrow cells	100 mg/kg bw, intraperitoneal injection	100	Negative	Krishna & Theiss (1995)
	Mouse (BALB/c) reticulocytes	50–100 mg/kg bw, intraperitoneal injection	50	Positive	Russo et al. (1994)
	Mouse (CBA) reticulocytes	25–50 mg/kg bw, intraperitoneal injection	25	Positive	Paulsson et al. (2002)
	Mouse (C57BL/6J) spleen lymphocytes (M)	50–125 mg/kg bw, intraperitoneal injection	50	Positive	Backer et al. (1989)
	Mouse (C57BL/6) splenocytes (M)	100 mg/kg bw, intraperitoneal injection	100	Positive	Kligerman et al. (1991)
	Mouse (C57BL/6J) spermatids	10–100 mg/kg bw, intraperitoneal injection	50	Positive	Collins et al. (1992)
	Mouse (BALB/c) spermatids	50–100 mg/kg bw or 4 × 50 mg/kg bw per day, intraperitoneal injection	50	Positive	Russo et al. (1994)

Table 5. (contd)

Assay	Test system	Dose/concentration	HID or LED	Result	Reference
Micronucleus (contd)	Rat (Lewis) spermatids	50–100 mg/kg bw or 4 × 50 mg/kg bw per day, intraperitoneal injection	100	Positive	Xiao & Tate (1994)
	Rat (Sprague-Dawley) spermatids	50–100 mg/kg bw or 4 × 50 mg/kg bw per day, intraperitoneal injection	4 × 50	Positive	Lähdetie et al. (1994)
Synaptonemal complex aberrations	Mouse (C57BL/6J) germ cells (M)	50–150 mg/kg bw, intraperitoneal injection	150	Negative	Backer et al. (1989)
Synaptonemal complex irregularities	Mouse (C57BL/6J) germ cells (M)	50–150 mg/kg bw, intraperitoneal injection	50	Weakly positive, asynapsis in meiotic prophase	Backer et al. (1989)
Heritable translocations	Mouse (C3H × 101)F1 (M)	5 × 40–50 mg/kg bw per day, intraperitoneal injection	40	Positive	Shelby et al. (1987)
	Mouse (C3H/E1) (M)	50–100 mg/kg bw, intraperitoneal injection	50	Positive	Adler et al. (1994)
Reciprocal translocations	Mouse (C3H/E1) (M)	5 × 50 mg/kg bw per day, intraperitoneal injection	50	Positive	Adler (1990)
<i>DNA damage and repair and DNA adduct formation</i>					
Spore rec assay	<i>Bacillus subtilis</i> H17 (rec+) and M45 (rec-)	1–50 mg/disc	10	Positive	Tsuda et al. (1993)
DNA breakage	Mouse (C3H × C57BL/10)F1 (M)	25–125 mg/kg bw, intraperitoneal injection	25	Positive	Sega & Generoso (1990)

Table 5. (contd)

Assay	Test system	Dose/concentration	HID or LED	Result	Reference
In vitro UDS	Rat primary hepatocytes	5–20 mmol/l	17.5	Weakly positive	Barfknecht et al. (1988)
	Rat (Fischer 344) primary hepatocytes (M)	0.01–1 mmol/l	1	Negative	Butterworth et al. (1992)
	Human mammary epithelial cells	1–10 mmol/l	1	Positive	Butterworth et al. (1992)
In vivo/in vitro UDS	Rat (Fischer 344) hepatocytes (M)	1 × 100 mg/kg bw, 5 × 30 mg/kg bw per day, gavage	1 × 100 5 × 30	Negative	Butterworth et al. (1992)
	Rat (Fischer 344) spermatocytes (M)	1 × 100 mg/kg bw, 5 × 30 mg/kg bw per day, gavage	5 × 30	Positive	Butterworth et al. (1992)
In vivo UDS	Mouse (C3H × 101)F1 and (C3H × BL10)F1 germ cells (M)	7.8–125 mg/kg bw, intraperitoneal injection	7.8	Positive	Sega et al. (1990)
DNA adducts	Mouse (C3H × BL10)F1 testis	46 mg/kg bw, intraperitoneal injection	46	Positive	Sega et al. (1990)
	Mouse (C3H × BL10)F1 liver (M)	46 mg/kg bw, intraperitoneal injection	46	Positive	Sega et al. (1990)
	Rat (Sprague-Dawley) liver, lung, kidney, brain, testis	46 mg/kg bw, intraperitoneal injection	46	Positive	Segeberäck et al. (1995)
	Mouse (BALB/c) liver, kidney, brain	53 mg/kg bw, intraperitoneal injection	53	Positive	Segeberäck et al. (1995)

Table 5. (contd)

Assay	Test system	Dose/concentration	HID or LED	Result	Reference
DNA adducts (contd)	Mouse (B6C3F1) liver, kidney, lung (M, F)	50 mg AA/kg bw (or equimolar GA), intraperitoneal injection	50 AA 62 GA	Positive	Gamboa da Costa et al. (2003)
	Mouse (B6C3F1) liver (M, F)	Untreated (AA-containing basal diet)	approximately 10 µg/kg bw per day	Positive	Twaddle et al. (2004a)
	Mouse (B6C3F1) liver (M, F)	50 mg/kg bw, gavage	50	Positive	Twaddle et al. (2004b)
	Mouse (B6C3F1) liver (M, F)	0.1 mg AA/kg bw (or equimolar GA), gavage	0.1 AA 0.12 GA	Positive	Doerge et al. (2005a)
	Rat (F344) liver, brain, thyroid, mammary, testis, leukocytes (M, F)	50 mg AA/kg bw (or equimolar GA), intraperitoneal injection	50 AA 62 GA	Positive	Doerge et al. (2005b)
	Mouse (B6C3F1) liver, lung, kidney, testis, leukocytes (M, F)	50 mg AA/kg bw (or equimolar GA), intraperitoneal injection	50 AA 62 GA	Positive	Doerge et al. (2005b)
	Mouse (B6C3F1) liver (M, F)	1 mg/kg bw per day in drinking-water (42 days)	1	Positive	Doerge et al. (2005b)
	Rat (F344), liver (M, F)	1 mg/kg bw per day in drinking-water (28 days)	1	Positive	Doerge et al. (2005b)
	Rat (F344), liver (M, F)	0.1 mg AA/kg bw (or equimolar GA), gavage	0.1 AA 0.12 GA	Positive	Doerge et al. (2005c)

Table 5. (contd)

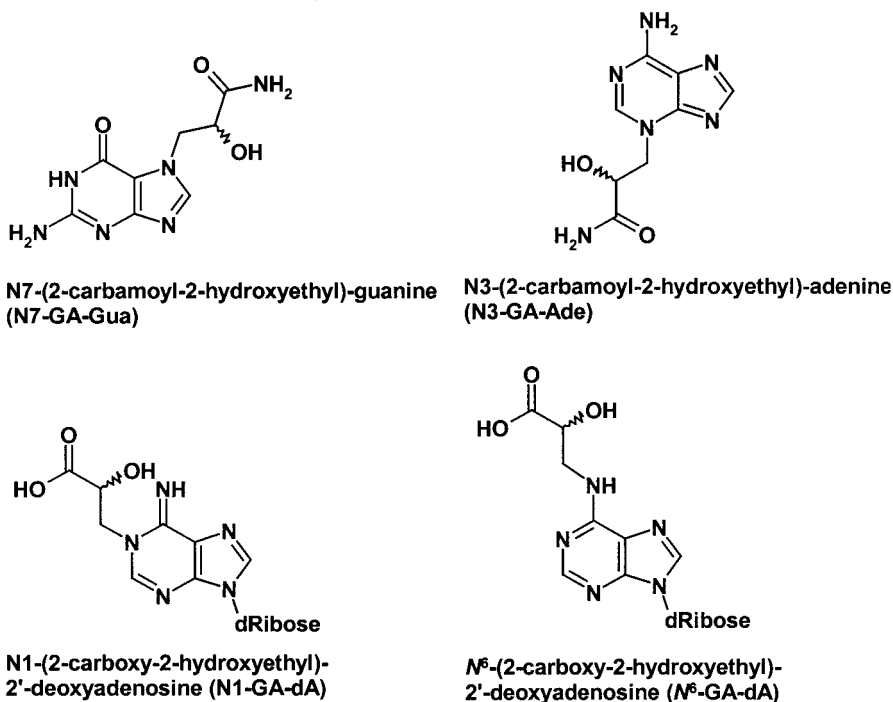
Assay	Test system	Dose/concentration	HID or LED	Result	Reference
DNA adducts (contd)	Rat (Sprague-Dawley), liver, brain, testis	18, 54 mg/kg bw, gavage	18	Positive	Manière et al. (2005)
	Rat (Sprague-Dawley) liver, brain, testis, adrenal, bone marrow, leukocytes	18, 36, 54 mg/kg bw, gavage	54	Positive comet assay in brain, leukocytes, testis; weakly positive and transient in liver, bone marrow, adrenal	Manière et al. (2005)
<i>Sister chromatid exchange</i>					
In vitro	Chinese hamster V79 cells	0.1–1 mg/ml \pm S9 activation	0.3	Positive at 0.3 mg/ml without S9 and 1.0 mg/ml with S9	Knaap et al. (1988)
	Chinese hamster V79 cells	0.5–2.5 mmol/l, no activation used	1	Positive	Tsuda et al. (1993)
In vivo	Mouse (C57BL/6J) spleen lymphocytes (M)	50–125 mg/kg bw, intraperitoneal injection	50	Positive	Backer et al. (1989)
	Mouse (C57BL/6) splenocytes (M)	100 mg/kg bw, intraperitoneal injection	100	Positive	Kligerman et al. (1991)
	Mouse (BALB/c) differentiating spermatogonia	50–100 mg/kg bw	50	Positive	Russo et al. (1994)
<i>Cell transformation</i>					
	Mouse C3H/10T1/2 clone 8 cells	25–200 μ g/ml	50	Positive	Banerjee & Segal (1986)

Table 5. (contd)

Assay	Test system	Dose/concentration	HID or LED	Result	Reference
	Mouse NIH/3T3 cells	2–200 µg/ml	0.0125	Positive	Banerjee & Segal (1986)
	Mouse C3H/10T1/2 cells	0.01–0.3 mg/ml	0.3	Negative	Abernethy & Boreiko (1987)
	Mouse BALB/c 3T3 cells	0.5–2 mmol/l	1	Positive	Tsuda et al. (1993)
	Syrian hamster embryo cells	0.1–0.7 mmol/l	0.5	Positive	Park et al. (2002)
	Syrian hamster embryo cells	0.001–10 mmol/l	10	Negative	Kaster et al. (1998)
<i>Germ cell effects</i>					
Sperm head DNA alkylation	Mouse (C3H × 101)F1	125 mg/kg bw, intraperitoneal injection	125	Weakly positive	Sega et al. (1989)
Sperm head protamine alkylation	Mouse (C3H × 101)F1	125 mg/kg bw, intraperitoneal injection	125	Positive	Sega et al. (1989)
Sperm head abnormalities	Mouse (ddY)	0.3–1.2 mmol/l in drinking-water for 4 weeks	1.2	Positive	Sakamoto & Hashimoto (1986)

AA, acrylamide; F, female; GA, glycidamide; HID, highest ineffective dose/concentration for negative tests; LED, lowest effective dose/concentration for positive tests; M, male; UDS, unscheduled DNA synthesis

Figure 2. DNA adducts from glycidamide



induced mutants had a different mutation spectrum from spontaneous mutations in control cells (i.e. increased G→T transversions, G→C transversions and A→G transitions). This mutation spectrum is consistent with miscoding potential for all glycidamide–DNA adducts identified *in vitro* (Gamboa da Costa et al., 2003; Figure 2); however, it was not possible from this study to determine whether the observed mutagenicity is secondary to formation of abasic sites, because during the 8-day duration of the study, extensive amounts of adduct depurination would occur (Besaratina & Pfeifer, 2004). These findings of mutagenicity were recently confirmed *in vivo* using transgenic mice. Increased mutation frequencies were observed in Big Blue mice from short-term exposures to acrylamide and glycidamide through the drinking-water (acrylamide at 10 and 50 mg/kg bw per day and equimolar glycidamide for 1 month) (Manjanatha et al., *in press*) and in *Tk*^{+/−} neonatal mice (three intraperitoneal acrylamide doses of either 10 or 50 mg/kg bw per day and equimolar doses of glycidamide) (Von Tungeln et al., 2005).

Single oral doses of acrylamide (18, 36 or 54 mg/kg bw) induced DNA damage as measured by the Comet assay in rat leukocytes, brain and testes. Despite similar levels of DNA adducts formed in all tissues examined, some other tissues showed no DNA damage, as measured by the Comet assay (liver, adrenals, bone marrow). These results suggest that additional cellular factors

beyond adduct formation may affect the DNA damage caused by acrylamide in vivo (Manière et al., 2005).

2.2.5 Reproductive toxicity

(a) Fertility and multigenerational toxicity

(i) Mice

Intraperitoneal route

After a single intraperitoneal acrylamide dose of 125 mg/kg bw in mice, females were paired with untreated males for approximately 1 year and litters removed when they were born. This single dose of acrylamide had no effect on reproductive capacity. This study suggests that there is little evidence of direct toxicity of acrylamide to oocytes (Bishop et al., 1997).

Oral route

Testicular damage was induced by acrylamide in male mice of the ddY strain dosed orally by gavage with acrylamide at 0.5 mmol/kg bw (35.5 mg/kg bw), twice weekly for 8 weeks (Hashimoto et al., 1981).

In a fertility study in ddY mice using acrylamide, groups of 9 or 14 males were given acrylamide at concentrations of 0, 0.3, 0.6, 0.9 or 1.2 mmol/l in the drinking-water for 4 weeks prior to mating. The highest acrylamide dose of 1.2 mmol/l (85.2 mg/l) gave an approximate intake of 15 mg/kg bw per day for the males, based on mean body weight and water intake. Half the males were mated with untreated females (one male per three females), and the females, except for half of those in the group mated to the 1.2 mmol/l males, were killed on day 13 of gestation and the contents of the uterus examined. The remaining females mated to the 1.2 mmol/l males were allowed to deliver and raise their litters up to 4 weeks postnatally. Half of the males were killed immediately following the end of dosing for examination of the liver, testes, seminal vesicles, sperm count and morphology. At the top dose, sperm counts were reduced by 35%, the percentage of abnormal sperm was significantly increased and male fertility was significantly reduced (only 5 out of 27 females to which they were mated were pregnant). In those pregnant, the number of fetuses per dam was significantly reduced at both 0.9 and 1.2 mmol/l in a dose-related manner. The number of resorptions per dam increased in a dose-related manner across all acrylamide treatment groups but attained statistical significance only at 1.2 mmol/l. Similarly, in the few fertile matings from the 1.2 mmol/l dose group in those allowed to litter out, the number of live offspring per dam was significantly reduced compared with controls. In a second experiment to investigate effects on females, 24 females were given drinking-water containing acrylamide at 1.2 mmol/l for 4 weeks, then mated to untreated males and killed on day 13 of gestation or allowed to litter out. There were no effects on reproduction, litter growth or survival to 4 weeks, apart from a small but statistically significant increase in resorptions per dam (Sakamoto & Hashimoto, 1986).

In a follow-up study to investigate which aspect of testicular pathology might be responsible for the effects observed on male reproduction, single oral doses of 100 or 150 mg/kg bw were given to prepubertal (30-day-old) or adult (60-day-old) ddY male mice, and testicular histopathology was followed for the next 10 days. Early-phase round spermatids showed marked degeneration, but other spermatogenic cell types were relatively unaffected (Sakamoto et al., 1988).

Acrylamide was given via the drinking-water to Swiss mice in a study using a continuous breeding protocol (3, 10 and 30 mg/l, equivalent to 0.8, 3.2 and 7.2 mg/kg bw per day in females; doses for males could not be calculated due to sipper-tube manipulation). Males and females were housed separately for the first 7 days, followed by 98 days of cohabitation with continued dosing. Controls comprised 40 mating pairs and treated groups 20 mating pairs per dose. Pups were removed from their dams after birth. At the end of the 98 days, males and females were separated and continued on treatment for a further 6-week holding period, during which the females were allowed to deliver and rear their last litter to weaning. After weaning, the litters were culled to two per sex per litter and maintained on the same treatment as their parents until 74 ± 10 days of age, when they were assessed for fertility by pairing with non-sibling animals from the same treatment group. After the 6-week holding period, some high-dose and control males and females were mated in a crossover trial to identify which gender might be affected. Some control and high-dose males at the end of the 98-day cohabitation period were also mated with untreated females to assess dominant lethal effects. There was no effect of treatment on food consumption or female water consumption, and there were no treatment-related effects on the proportion of fertile pairs, average number of litters delivered, proportion of pups born alive or pup weight. Considering all the litters born in each dose group, there was a slight but significant reduction in the aggregate mean number of live pups per female at 30 mg/l. In the crossover mating, there were no statistically significant differences between groups, but the high-dose male \times control female matings produced fewer pups per litter compared with control male \times control female and control male \times high-dose female matings. The dominant lethal study showed significant increases in early resorptions and total post-implantation losses and a significant reduction in live fetuses at 30 mg/l. There were no effects on estrous cycles or gross or histopathological effects of treatment on the reproductive tracts of adults, apart from a significant 10–12% reduction in spermatids per gram of testis in 10 and 30 mg/l males. No other sperm parameters were altered. When litters were reared to around 74 days of age and mated, there was no effect on pup survival or weight gain to day 21, but F1 female body weight was significantly reduced at the time they were mated and postpartum. Exposure of the F1 animals, based on their water consumption as adults, was calculated to be 0.86, 2.9 and 7.7 mg/kg bw per day. From the matings of F1 animals, the only treatment-related effect on reproductive parameters was a significant decrease of about 45% in the number of live pups in the 30 mg/l group. Again, there were no effects on estrous cycles or reproductive tracts of the F1 animals, apart from a significant reduction in absolute, but not relative, prostate weight at 30 mg/l and testicular degeneration in 1 of 10 males in each of the 10 and 30 mg/l groups. The authors concluded that the effects on reproduction were consistent with a dominant lethal effect in the male

and established a no-observed-effect level (NOEL) of 3 mg/kg bw per day (Chapin et al., 1995).

(ii) *Rats*

Oral route

Male Long-Evans rats aged 10 weeks were monitored for their "baseline" mating behaviour and then assigned to dose groups (15 per group), matched for body weight, sperm count and ejaculation latency. Acrylamide was administered for 10 consecutive weeks via the drinking-water at concentrations of 0, 50, 100 or 200 mg/l, equivalent to 0, 5, 7 and 12 mg/kg bw per day at the beginning of exposure, to 5 and 8 mg/kg bw per day at week 10 in the 50 and 100 mg/l groups and to about 12 mg/kg bw per day at week 6 in the 200 mg/l group. During this time, males were mated weekly with ovariectomized, estrogen-primed, non-acrylamide-exposed females. Mating behaviour was monitored on alternate weeks; during week 9, the females were killed to recover the ejaculate. During week 10, each male in the control and high-dose groups was housed individually overnight with an intact, untreated female in estrus to assess fertility. Water was withdrawn to ensure that females were not exposed. These females were killed on day 17 of gestation and the contents of the uterus examined. At the end of treatment, the males were killed, and one testis and epididymis from each male were fixed in Bouin's fluid for histological examination; the other testis was homogenized for spermatid count, and the other epididymis was minced for sperm count. The 200 mg/l group showed significant decreases in body weight and water intake compared with controls. Body weight and water intake were also decreased in the 100 mg/l group, but the differences from controls were not significant. Males in the 200 mg/l group showed neurotoxicity (hindlimb splaying) by week 4; one died and two others were moribund in week 5, so all were sacrificed in week 6. Some 100 mg/l males showed hindlimb splaying by week 8, but there was no mortality in this or any other group. Preceding the appearance of ataxia, significant disruptions in copulatory behaviour (more mounts and intromissions) were evident from week 2 and week 6 in the 100 and 200 mg/l groups, respectively. These disruptions interfered with the ejaculatory process and subsequent transport of sperm, since semen was found in the uterus in only 1 of the 15 females that mated with the 100 mg/l males at week 9, and only 5 of the 15 females mated to 100 mg/l males in week 10 were pregnant, compared with 11 of 14 in controls ($P < 0.01$). Post-implantation losses were also significantly increased in the five pregnant females mated to 100 mg/l males. Ejaculated sperm counts, evaluated in week 9, were significantly reduced in the 100 mg/l dose group. However, there were no effects on reproductive organ weights, sperm parameters or testis histology in any of the treatment groups, including the 200 mg/l group sacrificed at week 6. The authors concluded that the apparent effects on fertility at 100 and 200 mg/l may be secondary to the neuromotor toxicity, that the disturbances in intromission may have hindered proper sperm deposition and that the females may have been insufficiently stimulated to trigger sperm transport. A NOEL of around 5 mg/kg bw per day for neurotoxicity and for effects on copulatory behaviour and reproductive capacity can be taken from this study (Zenick et al., 1986).

Groups of 15 female Long-Evans rats were exposed to acrylamide in the drinking-water at concentrations of 0, 25, 50 or 100 mg/l for 2 weeks prior to mating and throughout pregnancy and lactation. The lowest dose tested (25 mg/l) was equivalent to a dose range of about 2.5–10 mg/kg bw per day over the time course of the study. Females were mated with untreated males overnight during week 3, and water bottles were removed overnight to ensure that the males were not exposed. Pregnant females were allowed to litter out, and litters were culled to four pups per sex on postnatal day (PND) 4 and reared to weaning. At weaning, litters were culled again to two per sex and maintained until sacrifice at 42 days of age. There were no deaths in any group. At 25 mg/l, only female pup body weight was significantly reduced at 7 and 14 days of age. Females in the 50 mg/l group showed significantly reduced weight gain during lactation only. In the 100 mg/l group, hindlimb splaying appeared during weeks 1–2 of gestation, and weight gain was significantly reduced from the second week of treatment — i.e. before mating and throughout gestation and lactation. Similar time trends were seen in reductions in water intake in the 50 and 100 mg/l groups. There were no significant effects of treatment on pregnancy rates, litter size at birth or pup survival to weaning. Birth weight and all subsequent weekly offspring body weights were significantly reduced in both sexes in the 100 mg/l group. In the 50 mg/l group, body weights from 7 days of age onwards were slightly but significantly reduced in both sexes. A regression analysis, performed without the 100 mg/l group because of incapacity of the dams, showed that litter weight at weaning was significantly related to cumulative acrylamide intake ($P \leq 0.01$) and that maternal body weight and fluid intake did not contribute to reduced litter weight at weaning. As would be expected from the reduced body weights, the time of vaginal opening was delayed, by about 3 days, in the females from the 100 mg/l group compared with controls. The authors concluded that the effects on pup growth may have been due to direct exposure to acrylamide via the milk or could have been secondary to maternal toxicity. A clear NOEL for effects on pup weight gain could not be established (Zenick et al., 1986).

In two dominant lethal studies, groups of five male Long-Evans rats were exposed to acrylamide via oral gavage at doses of 0, 5, 15, 45 or 60 mg/kg bw per day for 5 consecutive days. Following treatment, they were mated overnight to untreated females in estrus at weekly intervals for 4 weeks and, in the three highest dose groups, also on weeks 7 and 10 after treatment. The females were killed on gestation day (GD) 15 and examined for corpora lutea, implantation sites and fetuses. The mating index (number of sperm positive per number mated) was unaffected by treatment. Fertility was significantly reduced and pre- and post-implantation losses were significantly increased in a dose-related manner at all doses except 5 mg/kg bw per day. A second series of experiments was carried out using oral gavage doses of 0, 15 or 45 mg/kg bw per day for 5 days, with 10 or 15 males per group. Mating behaviour, ejaculated sperm and fertilization rates were evaluated over 4 subsequent weeks. Copulatory behaviour was unaffected, but the effects observed in week 1 in the preceding dominant lethal studies appeared to be due to lack of sperm transport into the uterus in those mated during week 1, the effect disappearing from week 2 onwards. The only other significant difference was reduced sperm motility at week 3. Fertilization of oocytes was evaluated in

females killed on the morning following the overnight mating and was significantly reduced in a dose-related manner at 15 and 45 mg/kg bw per day week 1 and at 45 mg/kg bw per day at week 3. These findings appear to confirm that the immediate effects of acrylamide in males, also seen in their earlier study (Zenick et al., 1986), are probably attributable to neurotoxicity interfering with mating; in addition, however, the findings show delayed effects consistent with an impairment in fertilizing ability of sperm. Both of these mechanisms may contribute to the overall reduced fertility and reproductive performance of acrylamide-treated males (Sublet et al., 1989).

Groups of 25 male Long-Evans rats aged approximately 11 weeks were gavaged with acrylamide at 0, 5, 15, 30, 45 or 60 mg/kg bw per day for 5 consecutive days. On day 8, males were paired overnight with untreated females in proestrus/estrus. On day 9, males were evaluated for forelimb and hindlimb grip strength and then killed and autopsied. Five males per group were perfusion fixed before autopsy and the sciatic nerves examined. In the remaining 20 males per group, in addition to the autopsy, they were investigated for cauda epididymis sperm number and motility. Mated females were killed on GD 15 and examined for corpora lutea and implantation sites. There were no deaths in any group. By the 5th day of dosing, mean male body weights were significantly reduced compared with controls at 30, 45 and 60 mg/kg bw per day, and male body weight changes were significantly reduced at all doses except 5 mg/kg bw per day. Post-dosing, body weight gain showed some recovery in all dose groups except the 5 mg/kg bw per day group, but overall weight gain over the 8 days was still reduced in a clear dose-related manner in all treated groups compared with controls. Piloerection, rough coat, lethargy and unsteady movement were observed at 45 and 60 mg/kg bw per day in dose-related patterns of incidence and severity, beginning on day 4. There were no effects on forelimb grip strength, but hindlimb grip strength was significantly reduced at 60 mg/kg bw per day. There were no histopathological abnormalities in the sciatic nerves in any group. The mating index was significantly reduced at 60 mg/kg bw per day, and, considering sperm-positive females, the fertility and pregnancy indices were both reduced at 60 mg/kg bw per day and showed a significant linear trend for depression at 15, 45 and 60 mg/kg bw per day. The number of implantation sites and number of live implants per female were also significantly reduced at 45 and 60 mg/kg bw per day. Considering only those pregnant, there were no treatment-related effects on corpora lutea per dam, implantation sites per litter or pre-implantation losses per litter, but post-implantation losses (resorptions) showed a clear dose-related increase, and the increases were statistically significant at 45 and 60 mg/kg bw per day, indicating a clear dominant lethal effect. In the males, there were no effects on the reproductive tract, epididymal sperm concentrations or percent motile sperm, but sperm beat cross-frequency (frequency of side-to-side movement) was significantly increased at 60 mg/kg bw per day. In this study, effects on body weight from 15 mg/kg bw per day upwards were the most sensitive indicator of acrylamide toxicity. There were clear effects on male reproduction at 45 and 60 mg/kg bw per day and arguable effects, based on trend testing, at 15 and 30 mg/kg bw per day. The authors concluded that the data were consistent with systemic toxicity, specifically

neurotoxicity, being causative of (or at least contributory to) the observed reproductive toxicity (Tyl et al., 2000a).

From this study, a review by CERHR (2004) calculated a benchmark dose (BMD) associated with a relative response increase of 10% (BMD₁₀) of 8 mg/kg bw for effects on live implants and the dose associated with the lower 95% confidence interval around the BMD (BMDL) of 6 mg/kg bw.

In a two-generation study in Fischer 344 rats, groups of 30 F0 male and 30 F0 female rats were given acrylamide in the drinking-water for 10 weeks prior to mating at concentrations giving intakes of 0.0, 0.5, 2.0 or 5.0 mg/kg bw per day. Exposure of F0 females continued throughout gestation and lactation of the F1 litters. After mating, F0 males were removed from exposure and mated again, one male to two untreated females, for the dominant lethal study. Thirty-five F1 weanlings per sex per group were exposed for 11 weeks to the same dose as their parents and then mated to produce the F2 generation. In F0 and F1 animals prior to breeding, significant reductions in body weight and body weight gain were observed at 2 and 5 mg/kg bw per day, and increases in head tilt and foot splay were seen at all doses in F0 males. There were no reproductive effects in the F0 and F1 animals, except for a significant 35% reduction in implantations per dam and 65% reduction in live pups per litter at 5 mg/kg bw per day. F1 and F2 pups in the 5 mg/kg bw per day group showed significantly reduced survival from birth through to PND 4 (Tyl et al., 2000b).

In a dominant lethal assay in Fischer 344 rats in which acrylamide was administered in the drinking-water at 0.0, 0.5, 2.0 or 5.0 mg/kg bw per day, a significant reduction in live implants and increases in pre- and post-implantation losses were observed at 5 mg/kg bw per day. Peripheral neuropathy was present at 5 mg/kg bw per day in adult F1 males (axonal fragmentation and/or swelling). The authors concluded that adult systemic toxicity, including neurotoxicity, was present at lower doses (NOEL \leq 0.5 mg/kg bw per day) than dominant lethality (NOEL 2 mg/kg bw per day). The NOEL for developmental toxicity in this study was 2 mg/kg bw per day (Tyl et al., 2000b).

(b) Developmental toxicity

(i) Mice

Intraperitoneal route

T-stock female mice received acrylamide by intraperitoneal injection once on GD 12 (75 mg/kg bw) or three daily injections on GD 10, 11 and 12 (50 or 75 mg/kg bw). Controls were injected with vehicle only. The females were killed on GD 18 and the contents of the uterus examined. In those given 75 mg/kg bw for 3 days, litter size was reduced, the proportion of growth-retarded fetuses was increased and mean fetal weight was reduced. The incidence of kinked tail was also increased after one or three doses at 75 mg/kg bw. There were no effects at 50 mg/kg bw (Neuhäuser-Klaus & Schmahl, 1989).

Groups of 20–57 female mice (strain not specified) received intraperitoneal acrylamide injections of 125 mg/kg bw at time intervals of 1, 6, 9 or 25 h after a 30-min mating period. These intervals correspond to exposure of the sperm and egg at approximately the time of fertilization, early pronuclear zygote stage, pronuclear DNA synthesis stage and two-cell embryo stage, respectively. Controls were injected with vehicle only. Uterine contents were examined on GD 17. There was no effect of treatment on the number of implantations per female, but early resorptions were increased and live fetuses decreased at all four time points compared with controls. The percentage of live abnormal fetuses was said to be significantly increased in the 6-, 9- and 25-h groups compared with controls, with the highest incidence seen in the 6-h group (16.5% compared with 5.6% in controls). In this group, from 203 live fetuses examined, the abnormalities included 8 with bent limbs, 10 with bent tail, 5 with eye defects, 6 with oedema, 3 with abdominal wall defects, 5 with cleft palate and 2 with exencephaly (Rutledge et al., 1992). The Committee noted that given the stage of treatment, the mechanism of production of these abnormalities is more likely to be mutagenic rather than teratogenic.

Groups of 9–20 ICR female mice received acrylamide by a single intraperitoneal injection of 125 mg/kg bw on one of days 0, 1, 2 or 3 of gestation. Controls were untreated. The animals were killed on GD 18 and the contents of the uterus examined. Live fetuses were examined for external abnormalities only. It should be noted that litter data were analysed on a per implant/fetus basis, which is not the correct statistical unit. The number of implantations per dam was significantly decreased after treatment on GD 0 or 1. The percentage of resorptions per dam was significantly increased in all treated groups. Fetal weight was significantly decreased after treatment on day 1 or 3. Fetal malformations were increased only after treatment on day 0; polydactyly was the most common abnormality, followed by kinked tail, oedema, exencephaly, open eyelid, cleft palate, ectopia cordis and omphalocele (Nagao, 1994). As in the previous experiment, the cause of these abnormalities is likely to be mutagenic rather than teratogenic.

ICR mice were given acrylamide as three daily intraperitoneal injections of 50 or 100 mg/kg bw per day on GD 6–8 or 9–11 (nine females per dose group). Thirteen controls were injected with vehicle only on GD 8–13. Despite some maternal mortality and signs of weakness and severe ataxia in those given 100 mg/kg bw per day, there were few statistically significant effects. Fetal weight was significantly reduced in those given 100 mg/kg bw on days 6–8. There was no significant treatment-related effect on external malformations (Nagao, 1994).

Male C57BL/6J mice were given acrylamide at daily intraperitoneal doses of 10–50 mg/kg bw for 5 days and then serially mated to untreated C3H/J females for up to 5 weeks after treatment. The females were killed about 3.5 days after confirmed mating and the embryos flushed out of the uterus and examined. High percentages of abnormal embryos were found in pregnancies sired by treated males in all dose groups except 10 mg/kg bw per day. The greatest effect was in the first week after treatment, with abnormalities declining over time in subsequent weeks. Some females in the 50 mg/kg bw per day group were kept until GD 15 or 16, then killed and examined for pre-implantation losses. In percentage terms,

these were comparable to the incidence of abnormal embryos and are indicative of a dominant lethal effect (Holland et al., 1999).

Oral route

Acrylamide was given daily by oral gavage at doses of 0, 3, 15 or 45 mg/kg bw to groups of 30 Swiss CD-1 mice on GD 6–17. The animals were killed on GD 17 and the contents of the uterus examined. There were no maternal deaths, but hindlimb splaying was observed in almost half of the 45 mg/kg bw per day dams between GD 15 and 17. Maternal weight gain during gestation was significantly reduced at 45 mg/kg bw per day but not after correction for gravid uterine weight, which was significantly reduced at 15 and 45 mg/kg bw per day. There were no statistically significant effects on the numbers of pregnancies at termination, implantations, resorptions, live fetuses, malformations or variations. Fetal body weight was significantly reduced in both sexes at 45 mg/kg bw per day. There was a significant dose-related trend towards increases in extra ribs across all doses. The effects observed were small in magnitude and could have been secondary to maternal toxicity. The NOEL for maternal and developmental toxicity in this study was 15 mg/kg bw per day (Field et al., 1990).

(ii) Rats

Oral route

Porton strain rats were given acrylamide at dietary concentrations of 0, 200 or 400 mg/kg from the day of mating throughout gestation. The 200 mg/kg group comprised eight rats and was allowed to give birth and raise litters to weaning. Acrylamide was not given during the lactation period. The offspring were kept until 6 weeks of age. No adverse effects were seen, apart from slight abnormalities of gait in the mothers at the time the litters were born. The 400 mg/kg group comprised six rats, which were killed on GD 20, and the contents of the uterus were examined and compared with eight controls. The dams showed moderate to severe ataxia by GD 20. A significant reduction of about 50% in maternal food intake during the final week of gestation was accompanied by a reduction in mean fetal weight at 400 mg/kg. No treatment-related increases in external, soft tissue or skeletal abnormalities were found. Maternal body weights were not stated, but the review by CERHR (2004) estimated that maternal acrylamide intake in the 400 mg/kg group was around 16 mg/kg bw per day, assuming a body weight of 300 g. This study indicated that 16 mg/kg bw per day via the diet was an effect level for maternal toxicity, including neurotoxicity, but did not cause developmental toxicity (Edwards, 1976).

Acrylamide was given to groups of 20 Sprague-Dawley rats at concentrations of 0, 25 or 50 mg/kg in the diet for 2 weeks prior to mating. Immediately after mating, the treated group resumed on acrylamide diet for the first 19 days of gestation. The dams were not treated during lactation. Acrylamide intakes were estimated by the authors to be 1.75–1.90 and 3.45–3.82 mg/kg bw per day in the 25 and 50 mg/kg groups, respectively. The animals were allowed to litter out, and

litters were culled to three males and three females on PND 4. After weaning, offspring were kept until they reached 6 weeks of age, and then two per sex of the controls and four per sex of the high-dose group were killed for histopathological examination of the brain, spinal cord and sciatic/tibial/plantar nerve complex. There was no effect on maternal mortality or maternal food intake. Maternal body weight gain was slightly but significantly reduced during the pre-mating phase at 50 mg/kg. There were no effects on reproductive parameters, including mating, pregnancy or pup survival and growth to PND 21. There was no evidence of a major teratogenic effect on the brain in the offspring examined at 6 weeks of age. There were some fine structural differences between treated and control groups, with some treated animals showing scattered nerve fibre degeneration in sciatic and optic nerves, and the study pathologists noted that fibres from treated animals were more susceptible to preparation artefacts. The incidence and severity of the lesions were not reported (Bio/dynamics, Inc., 1979).

Acrylamide was given by oral gavage at doses of 0, 2.5, 7.5 or 15 mg/kg bw per day to groups of 29–30 Sprague-Dawley rats on GD 6–20. The animals were killed on GD 20 and the contents of the uterus examined. There were no maternal deaths and no evidence of maternal neurotoxicity. Maternal weight gain during gestation was significantly reduced at 15 mg/kg bw per day and at 7.5 and 15 mg/kg bw per day after correction for gravid uterine weight, which was not significantly affected at any dose. There were no statistically significant effects on the numbers of pregnancies at termination, implantations, resorptions, live fetuses, fetal body weight or malformations. There was a significant dose-related trend towards increases in variations, the most common variation being extra rib; on pairwise comparisons, however, there were no significant differences between treated and control groups, and the analysis of extra rib alone did not show significant differences. In this study, the NOEL for maternal toxicity was 2.5 mg/kg bw per day and for developmental toxicity was ≥ 15 mg/kg bw per day.

Groups of 17 Fischer 344 rats were given water vehicle or acrylamide at 20 mg/kg bw per day by oral gavage from days 6 to 17 of gestation and allowed to litter out and rear offspring to weaning at 24 days of age. On the day of birth, pups were pooled within each group and fostered or cross-fostered in a randomized manner to form four groups: control pups with control dams, control pups with treated dams, treated pups with treated dams and treated pups with control dams. At PND 14, 21 and 60, four pups per litter were killed and the intestinal tissue was removed for enzyme assays (acid phosphatase, alkaline phosphatase, β -glucuronidase, citrate synthase and lactate dehydrogenase). There were no effects on parturition or litter parameters (pup numbers, sex ratio or body weights). Maternal intestinal enzymes on day 24 after parturition were unaffected by treatment. According to the authors, intestinal enzyme activities at the various time points sampled in the offspring showed a number of significant differences indicative of effects on all but citrate synthase (Walden et al., 1981).

The review by CERHR (2004) pointed out that the authors did not address the issue of multiple comparisons and that 60 such comparisons would give a 95% likelihood of identifying a significant difference at a nominal *P* value of 0.05. In their analysis of the data, CERHR (2004) concluded that “the findings are

suggestive of changes in alkaline phosphatase and perhaps a 'developmental' effect (delay or acceleration in normal pattern of enzyme changes) on postnatal day 21 for β -glucuronidase." The implications of such changes for development are unclear.

Wistar rats were given acrylamide by oral gavage at a daily dose of 25 mg/kg bw during lactation only. Offspring were weaned at PND 21 and the male offspring kept until 90 days of age, while the females were discarded at weaning. Effects on maternal mortality, food intake and body weight or on pup mortality were not mentioned, and no data on these parameters were tabulated. There were no significant effects on body or brain weight of the offspring. Some offspring from each group were killed for neurochemical assays (noradrenaline, dopamine and 5-hydroxytryptamine, and the activities of monoamine oxidase and acetylcholinesterase) at 2, 4, 8, 15, 30, 60 and 90 days of age. In the treated group, there were significant reductions in whole brain noradrenaline and dopamine levels up to 15 days of age and significant reductions in 5-hydroxytryptamine levels up to 30 days of age compared with controls; monoamine oxidase activity was also significantly increased and acetylcholinesterase activity significantly decreased at all ages up to and including 30 days of age. The decrease in catecholamines was said to be associated with progressive behavioural changes leading to complete hindlimb paralysis, but the data were not shown (Husain et al., 1987).

In a second experiment, groups of 15 normal Wistar rats starting at 12, 15, 21 or 60 days of age were given vehicle or acrylamide at 25 mg/kg bw orally for 5 consecutive days and then killed for neurochemical assays on discrete brain regions. Treated offspring showed the following significant effects: noradrenaline was reduced in the basal ganglia, pons medulla and midbrain after treatment at 12, 15 and 21 days and in the midbrain after treatment at 12, 15, 30 and 60 days of age; dopamine was significantly reduced in the cerebellum, pons medulla and midbrain after treatment at 12, 15, 21 and 60 days of age; and 5-hydroxytryptamine was reduced in the pons medulla, hypothalamus and cerebral cortex after treatment at 12, 15, 21 and 60 days of age (Husain et al., 1987).

Wistar rats (15 per group) were given acrylamide at 25 mg/kg bw per day and were dosed throughout the lactation period. Maternal toxicity was assessed twice daily by clinical observations and a FOB. Grip strength was assessed in the offspring postnatally, but no neurochemical measurements were carried out. The treated dams showed toxicity, including mortality (two animals), severely reduced food and water intake, reduced body weight and body weight gain and progressive signs of neurotoxicity from lactational day (LD) 13 onwards (hindlimb splaying, ataxia, tremors, eye squinting/partial closure, hunched position, hindlimb foot splay prone positioning and morbidity). Control and treated pup body weights on PND 0 were comparable, but they were significantly reduced in the treated group from PND 4 onwards. Post-weaning, in the retained males, body weight gain in the treated group was similar to controls, although the absolute body weights in the treated group remained lower. The proportion of pups in the treated group surviving to PND 21 was significantly reduced, and others were sacrificed moribund on PND 22–24. The morbidity and mortality in pre-weaning animals were associated with little or no milk in the stomachs. Male offspring forelimb and

hindlimb grip strength were significantly reduced in the treated group at PND 30, and hindlimb grip strength was also reduced, but not significantly so, on PND 60 and 90. The authors concluded that their results did not support the findings of Husain et al. (1987) and that their findings were consistent with inanition in the pups secondary to maternal toxicity, with recovery of animals post-weaning (Friedman et al., 1999).

Groups of 12 Sprague-Dawley rats were exposed to acrylamide by oral gavage at doses of 0, 5, 10, 15 or 20 mg/kg bw per day from GD 6 through to LD 10. The animals were allowed to deliver, and the offspring were kept up to postnatal week 11 and evaluated for survival, growth, development, behaviour and histological changes to brain, spinal cord and peripheral nerve. Maternal weight gain during gestation was significantly reduced by 14% and 26% in the 15 and 20 mg/kg bw per day groups, respectively. Maternal weight gain during lactation was also significantly reduced by 45% and 90% in the 10 and 15 mg/kg bw per day groups. All females in the 20 mg/kg bw per day group showed hindlimb splaying from LD 1 to LD 4, and all were sacrificed between presumed GD 24 and LD 4 because of excessive pup mortality. There was also a significant reduction in litter size at birth at 20 mg/kg bw per day. The only other reproductive effect was a significant increase in pup deaths between PND 4 and 21 at 15 mg/kg bw per day. In this group, the dams also showed hindlimb splaying from LD 4 onwards. Significant, dose-related decreases in pre-weaning body weight of male and female pups were observed in all dose groups at all time points, except the 5 mg/kg bw per day group, in which pup body weight was significantly reduced during the first week only, and only in females. Post-weaning, body weight remained significantly reduced only in the 15 mg/kg bw per day group. The only significant effects on motor behaviour were an increase in horizontal motor activity in the open-field female pups from the 15 mg/kg bw per day group on PND 21, which may reflect their developmental delay (exploratory motor activity in rats normally peaks around PND 17). A similar trend was seen in females at PND 17 and in males at PND 17 and PND 21, but not at PND 59. In auditory startle habituation tests, offspring in the 15 mg/kg bw per day group showed a significant reduction in peak amplitude on PND 22 (both sexes) and on PND 59 (females only). There were no treatment-related effects in passive avoidance tests performed on PND 24, 31, 59 and 66. Absolute brain weight was reduced in offspring in the 10 and 15 mg/kg bw groups on PND 11 and at postnatal week 11, but this was secondary to their reduced body weights; relative brain weight in these groups was slightly increased compared with controls (no statistical evaluation was carried out). There were no treatment-related effects on neural histology. In this study, the NOEL for maternal toxicity was 5 mg/kg bw per day, and the NOEL for maternal neurotoxicity was 10 mg/kg bw per day. The overall NOEL for developmental toxicity was ≤ 5 mg/kg bw per day, based on effects on transient female pup body weight at 5 mg/kg bw per day, and the NOEL for developmental neurotoxicity was 10 mg/kg bw per day. The authors concluded that acrylamide is not a selective developmental neurotoxicant, as effects were seen on offspring body weight at a dose lower than that affecting behaviour (Wise et al., 1995).

2.2.6 Special studies

(a) Covalent binding to nucleic acids and proteins

(ii) DNA adducts

In vitro

Prolonged incubation of calf thymus DNA with acrylamide at physiological pH and temperature for approximately 40 days produced several reaction products with DNA bases. However, reaction of glycidamide with DNA was much faster (Segerbäck et al., 1995; Gamboa da Costa et al., 2003). Several DNA adducts from reaction of glycidamide with salmon testes DNA *in vitro* have been characterized (see Figure 2 in section 2.2.4). These included two base adducts, N7-(2-carbamoyl-2-hydroxyethyl)guanine (N7-GA-Gua; Segerbäck et al., 1995) and N3-(2-carbamoyl-2-hydroxyethyl)adenine (N3-GA-Ade; Gamboa da Costa et al., 2003), that arise from thermal hydrolysis of the labile *N*-glycosidic bond of the adducted purine nucleosides. In addition, two stable nucleoside adducts were detected: N1-(2-carboxy-2-hydroxyethyl)-2'-deoxyadenosine (N1-GA-dA) and its Dimroth rearrangement product, *N*⁶-(2-carboxy-2-hydroxyethyl)-2'-deoxyadenosine (*N*⁶-GA-dA). Reaction of glycidamide with DNA *in vitro* produced N7-GA-Gua, N1-GA-dA and N3-GA-Ade in a ratio of approximately 100:19:1.7 (Gamboa da Costa et al., 2003).

In vivo

When either acrylamide or glycidamide was administered to F344 rats and B6C3F1 mice, N7-GA-Gua and N3-GA-Ade were observed in all organs examined in a ratio of approximately 100:1, similar to the ratio observed *in vitro*; however, methodological limitations precluded observation *in vivo* of N1-GA-dA (or after conversion to *N*⁶-GA-dA), even though it is formed *in vitro* at levels 11-fold higher than that of N3-GA-Ade. The levels of DNA adducts in adult mice treated with glycidamide were 1.2- to 1.5-fold higher than in those treated with acrylamide; however, in neonatal mice, glycidamide produced 5- to 7-fold higher adduct levels than acrylamide, presumably reflecting the deficiency of CYP2E1 in neonates (Gamboa da Costa et al., 2003).

Administration of acrylamide to rats in a single intraperitoneal injection of 50 mg/kg bw produced levels of DNA adducts in tissues that were generally lower than those observed in mice (Doerge et al., 2005c), which is consistent with previous observations in mice of more extensive formation of glycidamide-derived urinary metabolites (Sumner et al., 1992) and haemoglobin adducts (Paulsson et al., 2002; Sumner et al., 2003). Administration of glycidamide to rats produced a much larger incremental increase in DNA adduct formation, relative to acrylamide dosing, than was observed in mice. The adduct levels produced by glycidamide were greater in rat tissues than in the respective mouse tissue.

Modest differences in DNA adduct levels were observed between the different rodent tissues examined (Doerge et al., 2005c). These findings are consistent with the large volumes of distribution determined for acrylamide and glycidamide (see

Table 1 in section 2.1.1) and the wide tissue distributions observed in rodents (Doerge et al., 2005a, 2005b). In addition to liver, the rat tissues examined were those in which chronic administration of acrylamide at doses between 0.5 and 3 mg/kg bw per day resulted in elevated tumour incidences in F344 rats (i.e. thyroid, testes, mammary gland and brain). While the mammary gland and testes showed the highest DNA adduct levels of all tissues tested, the thyroid was lowest; therefore, it seems unlikely that differences in DNA adduct formation alone can account for the tumour tissue specificity observed in chronic carcinogenicity bioassays.

The role of CYP2E1-mediated oxidation of acrylamide (El-Hadri & Ghanayem, 2004) in the formation of glycidamide-derived DNA adducts was studied using wild-type and CYP2E1 knockout mice strains (Ghanayem et al., 2005b). Administration of a single intraperitoneal dose of acrylamide (50 mg/kg bw) to male CYP2E1 knockout and wild-type mice produced glycidamide concentrations in serum at 6 h that were 20-fold higher in wild-type mice and acrylamide concentrations at 6 h that were 140-fold lower in wild-type mice. In addition, N7-GA-Gua and N3-GA-Ade levels in liver, lung and testes were significantly lower in knockout mice (52- to 66-fold). Similarly, glycidamide-haemoglobin adducts were 33-fold lower and acrylamide-haemoglobin adducts were 2-fold higher in knockout mice.

The kinetics of glycidamide-DNA adduct loss were measured in rats following a single gavage dose of acrylamide at either 18 or 54 mg/kg bw. Half-lives for loss of N7-GA-Gua from liver, testes, brain, leukocytes and adrenals were in the range of 53–89 h. For N3-GA-Ade, the range of half-lives was 19–33 h for liver, testes and brain. The authors concluded that because the kinetics of adduct loss observed in these tissues were similar to those observed *in vitro* at 37 °C, it is unlikely that any active DNA repair processes are operative in rats for either adduct. This conclusion appears valid for rat tissues in which elevated tumour incidences were observed following chronic exposure to acrylamide (brain, testes), as well as in non-target tissues (liver, leukocytes) (Manière et al., 2005).

Repeated dosing of rats and mice with acrylamide administered in the drinking-water resulted in production of steady-state serum levels of glycidamide (0.4–0.6 µmol/l in rats) and in accumulation of N7-GA-Gua adducts in liver. In mice, a steady state level of N7-GA-Gua was attained in approximately 14 days with a half-life of about 4 days (Doerge et al., 2005c). Female rats also appeared to reach a steady-state level of adducts in approximately 14 days, with a formation half-life of about 4 days. Adduct levels in males appeared to reach a maximum at approximately 14 days, with a formation half-life of about 3 days. N3-GA-Ade was not detected in any rat liver sample and only in the terminal mouse samples assessed after 42 days of dosing. The authors speculated that this finding likely reflects the lower steady-state levels reached by this adduct, relative to N7-GA-Gua, because of its 3-fold faster removal kinetics and the inherently lower amounts formed in DNA (approximately 1%) (Doerge et al., 2005c).

Recent studies of DNA adduct formation in B6C3F1 mice and F344 rats have shown that at low oral doses of acrylamide (0.1 mg/kg bw), the level of N7-GA-Gua is linearly correlated with AUC for glycidamide (Doerge et al., 2005b). These

findings show that internal exposure to glycidamide is the principal determinant of DNA adduct formation *in vivo*.

(ii) *Haemoglobin adduct formation*

Adducts of reactive electrophiles with proteins provide complementary information to the corresponding DNA adducts, primarily in estimates of internal exposures (see also section 2.4.1 and Figure 1) (reviewed by Törnqvist et al., 2002). *N*-Terminal valine haemoglobin adducts from acrylamide and glycidamide are detected and quantified through use of the Edman reagents, as the substituted phenylthiohydantoin of *N*-(2-carbamoyl-ethyl)valine and *N*-(2-carbamoyl-2-hydroxyethyl)valine, respectively, using gas chromatography (GC)/MS/MS (Paulsson et al., 2003b) and LC/MS/MS (Fennell et al., 2003; Vesper et al., 2004).

In order to use haemoglobin adduct data for AUC estimation, the rate constant for reaction between acrylamide or glycidamide and *N*-terminal valine residues of haemoglobin is required, along with measurements of erythrocyte turnover (Törnqvist et al., 2002). These rate constants have been determined, and the reactivity of either rat or human haemoglobin with acrylamide is lower than the corresponding reaction with glycidamide (2.5-fold, Bergmark et al., 1993; 1.3- to 1.6-fold, Fennell et al., 2005). The acrylamide- and glycidamide-haemoglobin adduct levels in rats and mice exposed to acrylamide and *N*-methylolacrylamide (NMA) have been measured in several studies (Paulsson et al., 2002, 2003a; Fennell et al., 2003), and the correlation between glycidamide-haemoglobin adduct levels and micronucleus formation has been determined (Paulsson et al., 2002, 2003a). In general, the haemoglobin adduct data are consistent with toxicokinetic studies that show greater conversion of acrylamide to glycidamide in the mouse than in the rat, specifically at higher acrylamide doses (25–100 mg/kg bw). However, observations that rats produce higher relative amounts of glycidamide-haemoglobin adducts as the administered dose of acrylamide is decreased (Calleman, 1996) have been extended by recent toxicokinetic determinations that show similar glycidamide AUCs in rats and mice administered the same low acrylamide dose (0.1 mg/kg bw), particularly by the oral route (Doerge et al., 2005a, 2005b).

(b) *Neurotoxicity*

A number of reviews have been published on the subject of acrylamide neurotoxicity (Spencer & Schaumburg, 1974a, 1974b; Tilson, 1981; Hattis & Shapiro, 1990; Gold & Schaumburg, 2000; LoPachin et al., 2003; Tyl & Friedman, 2003; LoPachin, 2004). The cumulative evidence described in these reviews indicates that sufficient, repeated exposure to acrylamide by any route (dermal, oral, intraperitoneal, etc.) eventually results in peripheral neuropathy. Crofton et al. (1996) suggested that the observed neurotoxicity after acrylamide exposure results from an accumulation of toxic damage from repeated exposures, since acrylamide has not been shown to accumulate at the sites of toxicity.

The neurobehavioural deficits associated with acrylamide peripheral neuropathy (hindlimb weakness, foot splay and gait abnormalities) occur relatively early

during exposure in rats and in the absence of detectable axonal degeneration (LoPachin et al., 2000). Acrylamide-induced nerve terminal degeneration in the cerebellum likely contributes to these characteristic gait abnormalities (Lehning et al., 2002). Nerve terminal damage in the brain stem and spinal cord may develop prior to axonopathy and the appearance of significant gait disturbances. These observations and observations in the forebrain — where there is a clear absence of axonal degeneration in the presence of significant terminal degeneration — led to the suggestion that nerve terminals, rather than axons, may be the primary site of acrylamide intoxication (Lehning et al., 2002; LoPachin et al., 2002). These studies also demonstrated that with continued dosing, terminal degeneration emerged in brain areas critical for learning, memory and other cognitive functions (i.e. cerebral cortex, thalamus and hippocampus). Subsequent studies led to the hypothesis that acrylamide reacts with specific target thiol-containing presynaptic proteins (e.g. synaptosomal-associated protein of 25 kDa; *N*-ethylmaleimide-sensitive factor) to disrupt normal neurotransmission and presynaptic membrane turnover (LoPachin et al., 2002, 2003). Others have suggested that inhibition of fast, bidirectional axonal transport by acrylamide might serve to cause or contribute to the noted terminal degeneration (Sickles et al., 2002). Regardless of mechanism, degeneration of nerve terminals appears to precede the observation of axonopathy originally reported by Spencer and colleagues (Spencer & Schaumburg, 1975, 1977), and it is now thought that the axonopathy is secondary to nerve terminal degeneration.

The key neurotoxicity study considered by the Committee was that conducted by Burek et al. (1980). Groups of 10 male and female F344 rats received acrylamide at doses of 0, 0.05, 0.2, 1, 5 or 20 mg/kg bw per day in drinking-water for 90 days. An additional 10 males per group were used for a 14-day recovery period and a further 6–9 males for interim sacrifices and electron microscopy during the 90-day period. Investigations conducted weekly included recording of body weight, clinical signs of toxicity, test for peripheral neuropathy (measuring foot splay after being dropped onto a horizontal surface from a low height) and water consumption. Haematology was performed on day 76, at termination and on day 60 of the recovery period for those not sacrificed after 90 days of acrylamide exposure. Urinalysis was performed on day 76 and on completion of the exposure period. Extensive macroscopic and microscopic pathology examinations were performed on 59 males and 60 females after 92–93 days of acrylamide exposure and on 4 males per exposure group after 144 days of recovery. Electron microscopy was performed on males during the 90 days of acrylamide exposure (days 7, 33 and 90) and during the 144-day recovery period (days 25, 11 and 144).

Reduced body weight gain was noted only at 20 mg/kg bw per day. Among those animals examined (males only), body weight was restored by day 141 of the recovery period. Significantly reduced water consumption was noted among females at 20 mg/kg bw per day.

Blood biochemistry was not remarkable. Haematology examinations on day 75 and at termination showed decreased packed cell volume, red blood cells and haemoglobin values among male and females at 20 mg/kg bw per day. Significant decreases in these parameters were also observed at termination among females

at 5 mg/kg bw per day. Haematology performed on day 4 of the recovery period still showed a reduction in packed cell volume, red blood cells and haemoglobin values among males that had received 20 mg/kg bw per day. By day 60 of the recovery period, a slight, but statistically significant, reduction in red blood cells was still apparent, but other values had returned to normal. There were no further haematology examinations, and, as with blood biochemistry examinations, the magnitude of effects was not reported.

Statistically significant increases in landing foot splay were observed among males and females at 20 mg/kg bw per day on day 22 and were more pronounced at day 29, so this test was discontinued to prevent injury. Other clinical signs of toxicity included curling of toes, splayed hindlimbs, incoordination and hindlimb weakness. At the end of 90 days, there was a loss of use of hindlimbs. On males and females at 5 mg/kg bw per day, there were no abnormalities on the landing foot spread test at day 29. On day 12 of the recovery period, control males and males at 5 mg/kg bw per day were tested on the landing foot splay test. There were no abnormalities seen in this test and no clinical signs of toxicity at this or lower exposure levels.

Histopathology of peripheral nerves after 90 days showed axon and myelin degeneration; both enlarged and unusually small axons were observed, whereas others were fragmented, broken or absent. Myelin degeneration was prominent and observed as clumping of myelin, vacuolization, myelin debris or absence of myelin. Interstitial space between individual nerve fibres was increased. These peripheral nerve lesions were marked in all animals at 20 mg/kg bw per day. Peripheral nerve lesions were also observed in most animals at 5 mg/kg bw per day, but varied in severity from equivocal to very slight (focal or multifocal changes in individual nerves) in 9 of 10 males and 6 of 10 females. Spinal cord sections, taken from the cervical, thoracic and lumbrosacral regions, showed equivocal to slight degenerative myelopathy (demyelination, swollen astrocytes and swollen axons) in the dorsomedial funiculi of one or all spinal cord sections in 5 of 10 males and 9 of 10 females at 20 mg/kg bw per day only. Transverse sections through the cerebrum, cerebellum and midbrain did not reveal any abnormalities among those animals examined (control and high dose levels).

The other major pathology findings, after 90 days at 20 mg/kg bw per day, were atrophy of skeletal muscle (2/10 males and 8/10 females); ulcerative gastritis or hyperkeratosis of the non-glandular stomach (4/10 males); testicular atrophy (10/10 males); mineralization of focal or multifocal seminiferous tubules of the testes (5/10 males); increased cellular debris and/or decreased spermatogenic elements in the tubular lumina of epididymides (9/10 males); vacuolization of the smooth muscle of the bladder (1/10 males and 2/9 females); and suppurative, chronic-active or granulomatous inflammation in the lungs (3/10 males and 5/10 females).

Portions of perfused sciatic and brachial nerves from males after 25, 111 and 144 days of recovery were also examined. Nerve damage similar to that seen during the treatment phase was seen in males that had received 20 and 5 mg/kg bw per day only. Findings after 25 days of recovery were apparently more severe

than those observed during the 90-day exposure period, but gradual recovery of the nerve damage was observed subsequently such that at 144 days of recovery, only very slight to slight alterations were seen in sciatic nerves of males that had received 20 mg/kg bw per day. However, peripheral nerve lesions (altered tinctorial properties and/or vacuolization of fibres) were still present at this dose in sciatic and brachial nerves, although findings were less severe than after 90 days of treatment. There was evidence that some regeneration had occurred. There were no signs of nerve damage at this time point in other groups.

At the end of the recovery period, all four males that had received 20 mg/kg bw per day still had testicular lesions (slight focal or multifocal atrophy of seminiferous tubules and mineralization and cellular debris in focal or multifocal tubules). Lesions in the urinary bladder had essentially recovered by this time. Some inflammatory lesions were observed in the liver and lungs of males that had received 20 mg/kg bw per day, but the significance of these was uncertain.

Electron microscopy of nerve tissue provided additional evidence of substantial neuropathy, with some post-exposure recovery, at 20 and 5 mg/kg bw per day. There were also some axolemmal invagination at 1 mg/kg bw per day at 90 days. No ultrastructural changes were observed at lower doses.

In summary, this important study demonstrated that oral administration of acrylamide to rats for 90 days principally resulted in several lesions of peripheral nerves and spinal cord at 20 mg/kg bw per day (with associated clinical signs of toxicity); atrophy of skeletal muscle; testicular atrophy (although all the stages of spermatogenesis were still apparent); and decreased red blood cell parameters. Peripheral nerve lesions were also observed at 5 mg/kg bw per day, and slight changes in nerve tissue (electron microscopy) were seen at 1 mg/kg bw per day.

Male and female Fischer 344 rats were treated with acrylamide via the drinking-water at doses of 0, 0.01, 0.1, 0.5 or 2.0 mg/kg bw per day for 2 years. Degeneration of peripheral nerves was observed in rats receiving 2.0 mg/kg bw per day (Johnson et al., 1986).

In a lifetime (106 weeks) carcinogenicity study conducted in Fischer 344 rats, acrylamide was administered in drinking-water at doses of 0, 0.1, 0.5 or 2.0 mg/kg bw per day to males or 0, 1.0 or 3.0 mg/kg bw per day to females (Friedman et al., 1995). Significant increases were reported in sciatic nerve degeneration observed microscopically in high-dose males and females.

Acrylamide doses of 0, 5, 10, 15 or 20 mg/kg bw per day were provided to maternal Sprague-Dawley rats by oral gavage from GD 6 to LD 10. The animals were allowed to deliver, and offspring were evaluated for behavioural effects, as well as histological changes to brain, spinal cord and peripheral nerve. Behavioural assessments were conducted during both the pre-weaning and adult periods and included open-field motor activity, auditory startle habituation and passive avoidance tests. All F0 and F1 animals in the 20 mg/kg bw per day group were euthanized early in the lactation period due to high pup mortality. Hindlimb splaying was observed in dams in the two highest dose groups. Significant decreases in average horizontal motor activity and auditory startle response were

observed only in weanlings of the 15 mg/kg bw per day group. In F1 adult animals, a decrease in auditory startle responses in females of the 15 mg/kg bw per day group was observed. No effects were observed in the passive avoidance test or in the histological examination of the nervous system of pre-weaning pup or adult animals. Based on the authors' results, the NOEL for developmental neurotoxicity was determined to be 10 mg/kg bw per day. The authors indicated that because behavioural changes in the offspring were observed only at doses that were also maternally toxic (i.e. pup body weight was affected at a dose lower than that which produced maternal effects or offspring behavioural effects), acrylamide may be a selective developmental toxicant but not a selective developmental neurotoxicant (Wise et al., 1995).

Fischer 344 F0 weanling rats were exposed to acrylamide in drinking-water at 0.0, 0.5, 2.0 or 5.0 mg/kg bw per day for 10 weeks and then mated. Exposure of F0 females continued through gestation and lactation of F1 litters. F0 males, after F0 mating, were removed from exposure and mated (one male to two untreated females). F1 weanlings were exposed for 11 weeks to the same dose levels as their parents and then mated to produce F2 offspring. F0 and F1 parents and F1 and F2 weanlings were necropsied. Pre-breeding exposure of F0 and F1 animals resulted in increased head tilt and/or foot splay at 0.5–5.0 mg/kg bw per day. At 5.0 mg/kg bw per day, adult F1 male peripheral nerves exhibited axonal fragmentation and/or swelling, while F1 female spinal cord sections were unremarkable. The NOEL for adult systemic toxicity, including neurotoxicity, was ≤ 0.5 mg/kg bw per day (Tyl et al., 2000b).

Male Sprague-Dawley rats were injected intraperitoneally once a day with either acrylamide (8–14 days, 25 or 50 mg/kg bw per day) or glycidamide (8–14 days, 50 or 100 mg/kg bw per day). Both compounds affected rotarod performance, but only acrylamide had a significant effect in the hindlimb splay test. Morphological abnormalities were observed in sciatic nerves and dorsal root ganglion cells of rats treated with acrylamide at 50 mg/kg bw per day for 12 days, but not in rats exposed to glycidamide at 100 mg/kg bw per day for 11 days. Despite evidence of an effect of glycidamide on motor skills, the authors concluded, based on the hindlimb test results as well as the morphological studies, that acrylamide was the compound primarily responsible for neurotoxicity (Costa et al., 1992, 1995).

Male Sprague-Dawley rats were given acrylamide or glycidamide intraperitoneally at an acrylamide-equivalent dose of 50 mg/kg bw. Both treatment groups demonstrated similar neuropathological changes in the central and peripheral nervous system, with glycidamide producing the more severe lesions. The authors concluded that glycidamide is an active neurotoxic metabolite (Abou-Donia et al., 1993).

Male rats injected intraperitoneally with acrylamide (25 or 50 mg/kg bw) or glycidamide (50 or 100 mg/kg bw) once daily for eight doses demonstrated greater toxic effects of acrylamide than glycidamide in a wheel rotation test, hindlimb splay test and activities of glyceraldehyde-3-phosphate dehydrogenase in peripheral nerves of rats. Evidence of peripheral nerve damage was observed only in

acrylamide-treated animals. The authors concluded that nerve tissue was more vulnerable to acrylamide than to glycidamide (Deng et al., 1997).

In a reproductive study discussed in more detail in section 2.2.5, neurotoxicity was assessed in treated F1 animals via grip strength tests; the authors concluded that there were only minor effects on grip strength at 10 and 30 mg/l and no neural histopathology (Chapin et al., 1995).

Neurotoxicity studies in animals administered acrylamide in drinking-water or by gavage are summarized in Table 6.

(c) Hormonal activity/effects

Acrylamide administration (10–30 mg/kg bw per day) elevated binding of agonists to dopaminergic and serotonergic receptors in several rat brain regions (Agrawal et al., 1981).

Acrylamide administration (10–20 mg/kg bw per day) increased serotonin metabolism in rat brain regions (frontal cortex, striatum, hypothalamus, hippocampus and brain stem) and increased dopamine release from caudate nucleus, but not hypothalamus or frontal cortex (Ali, 1983).

Acrylamide administration (single doses of 50–100 mg/kg bw or 10–20 mg/kg bw per day for 10–20 days) increased Met-enkephalin in frontal cortex, but not other brain regions, and had no effects on neurotensin, β -endorphin or substance P. Acrylamide administration to male rats reduced testosterone and prolactin, but not growth hormone (Ali et al., 1983).

(d) Thyroid function

A study on thyroid effects from short-term (2 or 7 days) dosing with acrylamide in female F344 rats with 2 or 15 mg/kg bw per day showed no significant changes in triiodothyronine (T3)/thyroxine (T4), thyroid-stimulating hormone (TSH) or prolactin (Khan et al., 1999); however, changes in thyroid follicle colloid areas and cell heights were interpreted as evidence for endocrine disruption that could produce a tumorigenic response under chronic high dosing rates, as in rodent cancer bioassays. While there is little precedent for production of rat thyroid follicular tumours in the absence of increases in TSH, it is possible that alterations in the responsiveness of the pituitary or hypothalamus from hypothetical increases in dopaminergic tone could actually reduce serum TSH, because dopamine is an inhibitory factor in release of TSH from the thyrotrophs (Scanlon & Toft, 1996).

(e) Related contaminants

NMA is a structural analogue of acrylamide that is used similarly in a number of commercial products requiring polymeric properties, whose presence in foods has not been demonstrated. The United States National Toxicology Program reported results from toxicity (16 days and 13 weeks) and carcinogenicity (2 years) studies of NMA administered by gavage to male and female B6C3F1 mice and F344 rats (Bucher et al., 1990). Evidence for neurotoxicity was more severe in

rats, but was also apparent in mice and during the 13-week studies. Increased incidences of tumours in liver, lung and Harderian gland were related to NMA administration in both sexes of mice, and ovarian tumours were also observed in females; no evidence for tumorigenicity was observed in rats.

Table 6. Neurotoxicity in animals repeatedly exposed to acrylamide by the oral route

Species, sex	Exposure conditions (mg/kg bw per day)	NOEL (mg/kg bw per day)	LOEL (mg/kg bw per day)	Effect	Reference
Fischer 344 rat, M and F	0, 0.05, 0.2, 1, 5, 20	0.2	1	Morphological changes in nerves (EM)	Burek et al. (1980)
	90 days in drinking-water	1	5	Degenerative changes in nerves (LM)	
		5	20	Hindlimb foot splay	
		5	20	Decreased body weight (8–20%)	
		5	20	Atrophy of testes and skeletal muscle	
Fischer 344 rat, M and F	0, 0.01, 0.1, 0.5 or 2.0	0.5	2.0	Degenerative changes in nerves (LM)	Johnson et al. (1986)
	2 years in drinking-water	2.0	ND	Hindlimb foot splay	
		0.5	2.0	Decreased body weight (<5%, M only)	
		0.5	2.0	Early mortality after 24 weeks	
		2.0	ND	Other non-neoplastic lesions	
Fischer 344 rat, M and F	0, 0.1, 0.5, 2.0 (M)	0.5 M	2.0 M	Degenerative changes in nerves (LM)	Friedman et al. (1995)
	0, 1.0, 3.0 (F)	1.0 F	3.0 F		
	2 years in drinking-water	2.0 M	ND	Hindlimb foot splay	
		3.0 F	ND		

Table 6. (contd)

Species, sex	Exposure conditions (mg/kg bw per day)	NOEL (mg/kg bw per day)	LOEL (mg/kg bw per day)	Effect	Reference
Fischer 344 rat, M and F (contd)		0.5 M	2.0 M	Decreased body weight (8–9%)	Friedman et al. (1995)
		1.0 F	3.0 F		
		0.5	2.0	Early mortality after 60 weeks	
		2.0 M	ND	Other non-neoplastic lesions	
		3.0 F	ND		
Fischer 344 rat, M and F	0, 0.5, 2.0, 5.0 2 generations in drinking-water	2.0	5.0	MM implantation losses (F0 & F1)	Tyl et al. (2000a)
		2.0	5.0 M	Degenerative changes in nerves (LM)	
		ND	0.5 M	Hindlimb foot splay and head tilt (F0 M only)	
		0.5	2.0 M	Decreased body weight (4–6%)	
CD-1 mouse, M and F	0, 0.8, 3.1, 7.5 2 generations in drinking-water	3.1	7.5	MM implantation losses (F0 and F1)	Chapin et al. (1995)
		7.5	ND	Degenerative changes in nerves (F1; LM)	
		3.1	7.5	Mild deficits in grip strength (F1 and F2)	
		7.5	ND	Hindlimb foot splay	
		3.1 F	7.5 F	Decreased body weight (8%, F1 only)	
Sprague-Dawley rat, F	0, 5, 10, 15, 20	10	15	Decreased maternal weight gain	Wise et al. (1995)
	GD 6–10 by gavage	10	15	Hindlimb splay, maternal	

Table 6. (contd)

Species, sex	Exposure conditions (mg/kg bw per day)	NOEL (mg/kg bw per day)	LOEL (mg/kg bw per day)	Effect	Reference
Sprague-Dawley rat, F (contd)		ND	5	Decreased body weight in offspring	Wise et al. (1995)
		10	15	Increased overall horizontal activity and decreased auditory startle response in offspring	

EM, electron microscopy; F, female; GD, gestation days; LM, light microscopy; LOEL, lowest-observed-effect level; M, male; MM, male-mediated; ND, not determined; NOEL, no-observed-effect level

The neurotoxic potency of NMA appears to be significantly lower than that of acrylamide (Bucher et al., 1990), and both acrylamide and glycidamide adducts with haemoglobin were detected in mice and rats following dosing with either acrylamide or NMA (Paulsson et al., 2002; Fennell et al., 2003); however, the uncertainties about metabolic and non-enzymatic conversion of NMA to acrylamide make quantitative comparisons of potency based on relative adduct levels difficult.

2.3 Observations in domestic animals

Seven Charolais cattle from a herd accidentally exposed, while grazing, to acrylamide and NMA that were released from grouting activities associated with tunnel construction in Sweden showed clinical signs of neurotoxicity over an 8-month period, including hindlimb paralysis. The severity of the neurological symptoms correlated directly with the circulating levels of acrylamide–haemoglobin adducts. The cattle recovered over the course of the study, and the gestation of four pregnant cows proceeded normally (Godin et al., 2002).

2.4 Observations in humans

2.4.1 Biomarkers of exposure

The internal dose of acrylamide may be estimated from determinations of concentrations of acrylamide or its metabolite glycidamide in bodily fluids or alternatively by quantifying the extent of adduct formation of acrylamide or glycidamide with proteins or DNA. Acrylamide itself is a potential biomarker that has been detected at low levels in human urine. Glycidamide itself has not been detected in human urine to date. Haemoglobin adducts of acrylamide and glycidamide are

currently used as the main biomarkers of acrylamide exposure (Tareke et al., 2002). The background level of haemoglobin determined in human blood has been estimated to correspond to a daily intake of approximately 100 µg of acrylamide per day, corresponding to 1.7 µg/kg bw per day for a 60-kg person.

In a study carried out in a Chinese factory manufacturing acrylamide and polyacrylamide from acrylonitrile, blood samples and 24-h urine were collected from 41 heavily exposed workers and 10 non-exposed controls from the same city. Among workers, air sample data over the few months preceding the sampling as well as an index of neurological symptoms were also obtained. Free acrylamide in plasma and acrylamide–haemoglobin (Cys) adducts were measured, and the mercapturic acid conjugates of acrylamide were measured in urine. All categories of exposed workers had values of acrylamide–mercapturic acid conjugates in urine higher than controls; its correlation coefficient with the neurological index was $r = 0.42$ among workers. High values of AA–Cys adducts ranging from 0.3 to 34 nmol/g globin were observed among exposed workers, and a clear distinction was evident between adduct levels in exposed and unexposed subjects. Unexposed control subjects had levels below 0.01 nmol/g. Furthermore, there was a close relationship ($r = 0.67$) between adducts and the index of neurological symptoms among workers. The GA–Cys adduct level was 1.6–32 nmol/g globin (about 30% of AA–Val), with a good linear relationship with AA–Val ($r = 0.96$) (Bergmark et al., 1993; Calleman et al., 1994).

AA–Val adducts were measured in laboratory workers in Sweden who used acrylamide in the preparation of polyacrylamide gels. The mean adduct level was 54 pmol/g, being significantly higher than the mean level (31 pmol/g) of non-smoking controls. The mean adduct level among smokers was higher (116 pmol/g), with a good correlation with the number of cigarettes smoked per day (Bergmark, 1997).

Acrylamide– and glycidamide–haemoglobin adducts were observed in blood collected from 11 Korean workers from an acrylamide production plant, and much lower levels were observed in all four outside control subjects (Licea-Perez et al., 1999). The Committee noted that in this study, much lower levels of glycidamide adducts relative to acrylamide adducts (3–12%) were reported than in the study by Bergmark et al. (1993).

Construction of a tunnel in Sweden led to the inadvertent exposure of workers to a grouting solution containing acrylamide and NMA over a 2-month period in 1997 (Hagmar et al., 2001). One to five weeks after cessation of exposure to the grouting agent, 210 workers underwent physical examinations, and blood samples were collected for analysis of acrylamide–haemoglobin adducts. In addition, blood samples were collected from 18 non-smoking control subjects with no known occupational exposure. The distribution of AA–Val adduct levels in workers was as follows: <0.08 nmol/g, $n = 47$; 0.08–0.29, $n = 89$; 0.30–1.0, $n = 36$; and >1.0, $n = 38$. There was a significant relationship between measured AA–Val adducts in groups of workers and the self-reported degree of exposure (high, some, none). There were also significant associations between AA–Val adduct levels and symptoms of peripheral neurotoxicity, skin irritation and general discomfort. The

control subjects had AA-Val adduct levels in the range of 0.02–0.07 nmol/g, which the Committee considered as being consistent with other studies of background exposure to acrylamide from the diet.

In a chemical plant in Germany, 62 workers (38 smokers, 24 non-smokers engaged in the production of surfactants for the textile industry) were investigated to assess exposure to several alkylating substances, including acrylamide. Ten persons not exposed occupationally to acrylamide (among which were two smokers) were used as a control group. Median values of AA-Val adducts (pmol/g globin) were 63 for exposed workers and 28 for controls; within workers, median values were 89 for smokers and 22 for non-smokers. Among smokers, the acrylamide–haemoglobin adduct level showed a good correlation with the number of cigarettes smoked per day — i.e. the daily consumption of one cigarette raises the level of acrylamide–haemoglobin adducts to about 3.4 pmol/g (Schettgen et al., 2002).

Acrylamide–haemoglobin adducts were measured in a group of 72 adults (63 males, 9 females), all of whom had no known contact to acrylamide, other than diet, including 47 smokers and 25 non-smokers. The median AA-Val level (pmol/g globin) was 85 (range 13–294) for smokers and 21 (range <12–50) for non-smokers. The reported levels of acrylamide–haemoglobin adducts are in line with results obtained from referents or unexposed groups in studies focusing on occupational exposure (Schettgen et al., 2003).

Acrylamide– and glycidamide–haemoglobin adducts were measured in control mice, rats and humans exposed through normal diets. The ratio of glycidamide/acrylamide adducts was 5.4, 1.8 and 1.0, respectively (Paulsson et al., 2003b).

An acrylamide-based grouting agent was used in railway tunnel works in Norway in 1997; shortly afterwards, several tunnel workers complained of symptoms of neurotoxicity. Eighty-four days (range 60–143) after cessation of grouting, mean acrylamide–haemoglobin adduct levels of 156 pmol/g globin in 23 exposed workers and of 63 pmol/g in 8 unexposed referents were found. Among the non-smoking workers, the mean AA-Val level was 82 pmol/g, compared with 33 pmol/g for the non-smoking referents, while the corresponding figures for smokers were 225 and 154 pmol/g for exposed workers and referents, respectively, the latter based on two measurements only. The extreme value for one exposed smoker was 890 pmol/g (Kjuus et al., 2004). The Committee noted that acrylamide–haemoglobin adduct levels among referents were compatible with the estimated background level of 20–70 pmol/g seen in other studies, but the increase seen among exposed persons, although significant, was lower than the increase observed among Swedish tunnel workers described by Hagmar et al. (2001). However, blood samples in the Swedish study were taken 1–5 weeks after cessation of exposure, while in the Norwegian study, the samples were collected, on average, 84 days (range 60–143) after cessation of grouting.

Acrylamide levels in urine of three men and three women were measured following consumption of potato chips containing 938 ± 1 µg acrylamide. Urinary elimination half-times were determined to be in the range of 1.8–2.8 h (mean

2.3 h) for total cumulative excretion of 2.2–6.2% (mean 4.5%) of the administered quantity of acrylamide (Pournara et al., 2004).

AA–Val adducts were measured in blood from 11 pregnant women (on a normal diet) a few hours prior to childbirth and in the corresponding umbilical cord blood of 11 neonates. There was a linear correlation between AA–Val adducts in mothers' blood and umbilical cord blood ($r = 0.86$). The highest value for both the mother and neonate was observed for the only smoking woman, as confirmed by simultaneous measurement of acrylonitrile–haemoglobin adducts. Among non-smokers, the median AA–Val adduct levels were 21 pmol/g and 10 pmol/g for mothers' blood and umbilical cord blood, respectively. However, considering the different life span of fetal and maternal erythrocytes and body weight differences, the authors concluded that the internal dose (per kg bw) in newborn infants is about the same as in their mothers (Schettgen et al., 2004).

Six volunteers consumed 85 g potato chips containing 115 µg acrylamide daily for 1 week (average exposure 1.9 µg/kg bw per day), and blood was drawn at baseline and at the end of the study for analysis of haemoglobin adducts. At baseline, the mean glycidamide/acrylamide–haemoglobin adduct ratio was 0.61. At the end of the study, levels of acrylamide–haemoglobin adducts had increased in one of six subjects, decreased in one of six and remained unchanged in four of six; by contrast, glycidamide–haemoglobin adducts increased in four of six subjects and decreased in two of six. The mean glycidamide/acrylamide–haemoglobin adduct ratio increased to 0.76. This study suggests that low dietary exposures to acrylamide result in efficient conversion to glycidamide by people (Vesper et al., 2004), similar to that observed in rodent studies conducted at low doses of acrylamide (Doerge et al., 2005a, 2005b).

Mercapturic acid conjugates of acrylamide and glycidamide were measured in the urine of 13 smokers and 29 non-smokers. The ratio of glycidamide/acrylamide mercapturates was in the range of 0.03–0.53 (mean 0.16, a value similar to that observed in rats). Haemoglobin adducts from acrylamide and glycidamide were also measured, and a high degree of correlation between mercapturic acid conjugates and the respective haemoglobin adduct was observed. The glycidamide/acrylamide–haemoglobin adduct ratio ranged between 0.4 and 1.7 (Boettcher et al., 2005).

Acrylamide in doses of 0.5–3.0 mg/kg bw was administered orally and dermally to 18 non-smoking sterile male subjects, and urine and blood were collected at baseline and at regular intervals for the determination of urinary metabolites and haemoglobin adducts. Of the orally administered dose (3.0 mg/kg bw), 30% was recovered in the urine, 26% being derived from acrylamide and 4% from glycidamide; however, no mercapturates from glycidamide were detected. A linear response between acrylamide– and glycidamide–haemoglobin adducts was observed after oral administration, and the ratios for adducts of glycidamide/acrylamide were in the range of 0.36–0.66 (Fennell et al., 2005). Data on urinary metabolites and haemoglobin adducts were also obtained from male F344 rats given an oral dose of 3 mg/kg bw. In contrast to men, 50% of the administered dose was detected in urine of rats, of which 29% was acrylamide mercapturic acid

conjugate and the remaining 21% was derived from glycidamide mercapturic acid conjugates. The haemoglobin adduct ratio for glycidamide/acrylamide in rats was 0.84. The authors concluded that men metabolize acrylamide to glycidamide to a lesser extent than do male rats and that dermal absorption in men was 6.6% of an equivalent oral dose (Fennell et al., 2005).

It seems that there is an important need to have a comparison of external dietary exposure with validated biomarkers of exposure and internal dose in order to assess the use of these biomarkers as exposure biomarkers at dietary exposure levels.

2.4.2 Biomarkers of effects

There are no biomarkers of effect for acrylamide, unless DNA adducts are considered as such (see section 2.4.1).

2.4.3 Clinical observations

No clinical observations were located in the literature.

2.4.4 Epidemiological studies

(a) Cancer

(i) Occupational exposure

A cohort of 371 male workers employed in the manufacture of acrylamide monomer and polyacrylamide was observed, with particular emphasis on cancers at sites identified from animal studies as being possibly relevant to acrylamide exposure, such as the central nervous system, thyroid gland, other endocrine glands and mesotheliomas. Exposure was categorized based on a review of worker job classification and personal 8-h time-weighted average concentration of acrylamide. Twenty-nine deaths were observed (up until 1982) out of 38 expected; after excluding workers not exposed to organic dyes, only four cancer deaths were observed versus 6.5 expected. The authors concluded that this study does not support a cause-effect relation between exposure to acrylamide at this work site and overall mortality, total malignant neoplasm or any specific cancers (Sobel et al., 1986).

An updated analysis of these data has been published (Marsh et al., 1999) for three plants in the United States, with an extended follow-up that was 11 years longer than in the previous report. The update also included a review of company records, allowing an estimation of exposure based on air concentrations that had been monitored from 1977. External comparisons were made with local (country) rates, while internal comparisons between exposed and non-exposed workers were carried out within the cohort. No association was found with mortality due to total malignant tumours (standardized mortality ratio [SMR] = 0.98). An excess mortality of at least 20% was found for cancers of the pancreas, thyroid, oesophagus, kidney and rectum, but none of them reached statistical significance. After further categorization of exposure using three levels, a significant excess risk was

found for pancreatic cancer (SMR = 2.26, 95% confidence interval [CI] 1.03–4.29) for subjects with cumulative exposure greater than 0.3 mg/m³-years; however, no consistent exposure–response relationship was observed. This estimate was based on nine pancreatic cancer cases (Marsh et al., 1999).

A reanalysis of the data proposed to combine the two intermediate categories of exposure (0.001–0.029 and 0.03–0.29 mg/m³-years) to avoid the small number of cases, obtaining a monotonically increasing risk with increasing exposure (Schulz et al., 2001).

The Committee noted that the main route of occupational exposure to acrylamide was inhalation and to a lesser extent dermal exposure.

(ii) *Dietary exposure*

A series of case–control studies has been carried out in several areas of Italy and Switzerland on cancers of the oral cavity and pharynx, larynx, oesophagus, colon and rectum, breast and ovary. Overall, they include a few more than 7000 cases and 13 000 controls. All the studies were hospital-based and used a food frequency questionnaire comprising 78 items to assess dietary intake. Two questions asking for the usual consumption of fried/baked potatoes were used as a surrogate for the dietary acrylamide intake. Subjects were categorized in three levels, showing whether their weekly consumption was zero, one or more than one portion of fried/baked potatoes. Odds ratios (ORs) estimated were adjusted by age, gender and several potential confounders, including alcohol consumption and smoking habits. All the ORs for the highest versus the lowest level of exposure ranged between 0.8 and 1.1; an inverse trend was observed for colon cancer and colon and rectal cancers combined (Pelucchi et al., 2003).

In a previous analysis of the same data, a positive association between laryngeal cancer risk and consumption of certain fried foods was found: beef/veal, fish/shellfish, eggs/omelet and potatoes. The OR for the highest to lowest levels of exposure for fried potatoes was 1.86 (95% CI 1.29–2.68), and the test for trend was significant ($P = 0.019$) (Bosetti et al., 2003). The Committee concluded that the different outcomes of the two analyses of laryngeal cancer may have been due in part to different categorization of potato consumption.

The results of Pelucchi et al. (2003) were criticized because of lack of inclusion of other foods important to total dietary intake of acrylamide, such as coffee (Beer et al., 2004). A reanalysis of previous data according to coffee consumption added a further 5500 cases and 7000 controls, approximately, from other Italian and Swiss studies on breast and colorectal cancer. Compared with the lowest level of consumption, significant ORs were found for the highest levels of coffee consumption for cancers of the oral cavity and pharynx (0.6), oesophagus (0.6), colon in Italy (0.7) and colon/rectum in Switzerland (0.4) (Pelucchi et al., 2004).

Already existing data from a population-based case–control study in Sweden, aimed at assessing the relation between heterocyclic amines in fried foods and large bowel and urinary tract cancers, were also used to assess the potential risk

of dietary acrylamide. The study included 591, 263 and 133 cases of histologically confirmed cases of cancers of large bowel, bladder and kidney, respectively, together with 538 age and gender frequency-matched population controls. Dietary habits for the 5 years prior to the study were assessed by means of a food frequency questionnaire of 188 items, including the majority of foods found to contain acrylamide. A score of acrylamide intake was assigned to every subject from his or her usual food consumption, and the median acrylamide content was determined by the Swedish National Food Administration. Potential confounders considered in the analysis were smoking, body mass index and the intake of alcohol, fruits and vegetables, red meat, saturated fat and total energy. Using quartiles of daily dietary intake of acrylamide, the relative risk for colorectal cancer decreased with increasing exposure with a significant trend, with a 40% reduction of risk for those in the highest compared with the lowest quartile of consumption. This inverse association was evident among non-smokers and suggestive (non-significant) among smokers (Mucci et al., 2003a).

The authors reanalysed the study including acrylamide content in coffee; the daily mean dietary acrylamide intake estimated was 34 µg for controls and 34.8, 36.8 and 34.5 µg for cases of colorectal, bladder and kidney cancer, respectively. The pattern of inverse association with colorectal cancer remained, and there was a trend to a decreased risk of kidney cancer with increasing exposure to acrylamide, although it did not reach statistical significance (Mucci et al., 2003b).

Data from a larger Swedish case-control study of renal cell cancer, which included 379 cases and 353 age and sex frequency-matched controls, were reanalysed. Dietary assessment was made by means of a food frequency questionnaire including 11 food items with potentially elevated acrylamide levels. Acrylamide food content was ascertained by search of food databases in Sweden and the United States. The estimated daily average intake of acrylamide was 27.6 µg per person, for both cases and controls. After adjusting for potential confounders (smoking, education, body mass index, total energy), no association was observed between renal cell cancer risk and dietary acrylamide; the relative risk (RR) comparing the highest versus the lowest quartile was 1.1 (95% CI 0.7–1.8) (Mucci et al., 2004).

(a) *Effects other than cancer*

(i) *Neurotoxicity*

Among 41 heavily exposed workers involved in the production of acrylamide in China, a neurotoxicity index was built based on neuropathic signs and symptoms and indicators of peripheral neural dysfunction, such as vibration threshold and electroneuromyography. This index adequately predicted clinical diagnosis of peripheral neuropathy and was significantly correlated with AA-Val haemoglobin adducts and mercapturic acid in urine (Calleman et al., 1994).

Tunnel workers exposed to acrylamide in Sweden were asked about symptoms such as numbness, tingling or pain in hands, feet or legs in an interview, and a physical examination was performed. A standardized neurophysiological

examination was carried out, comprising both motor (median and peroneal nerves) and sensory (median and sural nerves) neurography and measurement of sensory perception thresholds in the left foot. There was a significant dose–response association between prevalence of peripheral nervous symptoms and AA–Val haemoglobin adducts. The upper confidence limit for the threshold dose was 0.51 nmol/g for numbness or tingling and 1.86 nmol/g for leg cramps (Hagmar et al., 2001).

The Committee noted that there was a time lag in exposure measurement, as blood samples were drawn for haemoglobin adduct measurement 1–5 weeks after exposure ended, and that haemoglobin adduct levels may not have reached a steady state during the 2 months when most of the exposure occurred. This may have affected the internal dose estimation.

Neurophysiological measurements among tunnel workers exposed to acrylamide and NMA in Norway included motor nerve conduction velocity (NCV), amplitude and F-latency in the median, ulnar, peroneal and posterior tibial nerves as well as sensory NCV and amplitude in the median and ulnar nerves. Most neurophysiological measurements did not show any significant difference between exposed and non-exposed subjects; however, there was a slight but significant difference in the mean sensory NCV and motor distal delay in the ulnar nerve 4 months post-exposure and also a reduction in mean sensory NCV and amplitude in the sural nerve after 16 months. Early reversible changes were found in the upper but not in the lower extremities (Kjuus et al., 2004).

The Committee noted that exposure to acrylamide in these studies was inhalation and dermal, not dietary.

(ii) Genotoxicity

An unpublished report reported the frequency of chromosomal aberrations in tunnel workers in Norway with relevant exposure to acrylamide. Blood samples were collected from 25 tunnel workers with highest exposure to acrylamide-containing grout and 25 workers who had not been exposed, and four lymphocyte cultures per sample were carried out. Chromosome damage was scored per 200 cells and reported as number of cells with aberrations (excluding gaps), chromatid breaks, chromosome breaks, chromatid gaps and chromosome gaps. Furthermore, DNA from all subjects but two controls was genotyped to assess deletion polymorphisms in glutathione-S-transferases M1 and T1 (GSTM1 and GSTT1). All indicators of chromosome damage were higher among exposed, but only the difference in mean number of chromatid gaps (10.6 vs 6.4) was statistically significant. This pattern remained unchanged when subjects were stratified according to length of exposure and smoking habits. Among workers with GSTM1–/GSTT1–, higher frequencies for all effect parameters were evident in the exposed group, although none of them reached statistical significance, probably due to small numbers (four exposed vs three controls). Among subjects with GSTM1–/GSTT1+ and GSTM1+/GSTT1+, only the number of chromatid gaps was higher for exposed. The most consistent finding related to genotypes was the lower number of chromatid gaps in subjects with intact genes: among exposed, the mean

number of chromatid gaps was 16.3 for subjects with GSTM1-/GSTT1-, 10.4 for subjects with GSTM1-/GSTT1+ and 8.8 for those with GSTM1+/GSTT1+, respectively (Kjuus et al., 2005). The Committee noted that both GSTM1 and GSTT1 enzymes induce conjugation with glutathione, one of the potential pathways in the metabolism of acrylamide. The results may indicate that individuals with intact genes, and thus active enzymes, are less prone to such chromosomal aberrations when exposed to acrylamide. On the other hand, so far only increased chromosome aberrations including chromatid and/or chromosome breaks, but no chromatid gaps, have been found related to cancer.

3. ANALYTICAL METHODS

3.1 Chemistry

Acrylamide ($\text{CH}_2=\text{CH}-\text{CO}-\text{NH}_2$; 2-propenamide) is a white crystalline solid. It has a relative molecular mass of 71, a melting point of $84.5 \pm 0.3^\circ\text{C}$ and a high boiling point (136°C at 3.3 kPa) (Cyanamid, 1969; Habermann, 1991). Acrylamide is extremely soluble in water (2155 g/l at 30°C), in lower alcohols (1550 g/l in methanol and 860 g/l in ethanol) and in other polar organic solvents (396 g/l in acetonitrile, 126 g/l in ethyl acetate and 631 g/l in acetone). It is virtually insoluble in non-polar organic solvents such as heptane and carbon tetrachloride (<1 g/l) (Cyanamid, 1969; Habermann, 1991). The limited conjugation involving π -electrons means that acrylamide lacks a strong chromophore for ultraviolet (UV) detection and does not fluoresce. The solubility behaviour dictates the extraction strategy used for analysis, and the low molecular mass and low volatility have consequences with respect to the measurement techniques used.

Solid acrylamide is quite stable at ambient conditions and has a long storage life. Even at temperatures up to its melting point, no significant polymer formation is observed (e.g. for 1 day in the absence of light). Above its melting point, however, liquid acrylamide polymerizes rapidly and exothermically (Habermann, 1991).

Acrylamide is a bifunctional monomer containing a reactive electron-deficient double bond and an amide group, and it undergoes reactions typical of those two functionalities (Friedman, 2003). It exhibits both weak acidic and weak basic properties. The electron-withdrawing carboxamide group activates the double bond, although the activation is not as great as by a carbonyl or acid group in conjugation. Consequently, acrylamide reacts with nucleophilic reagents in a Michael addition reaction.

3.2 Description of analytical methods

3.2.1 Introduction

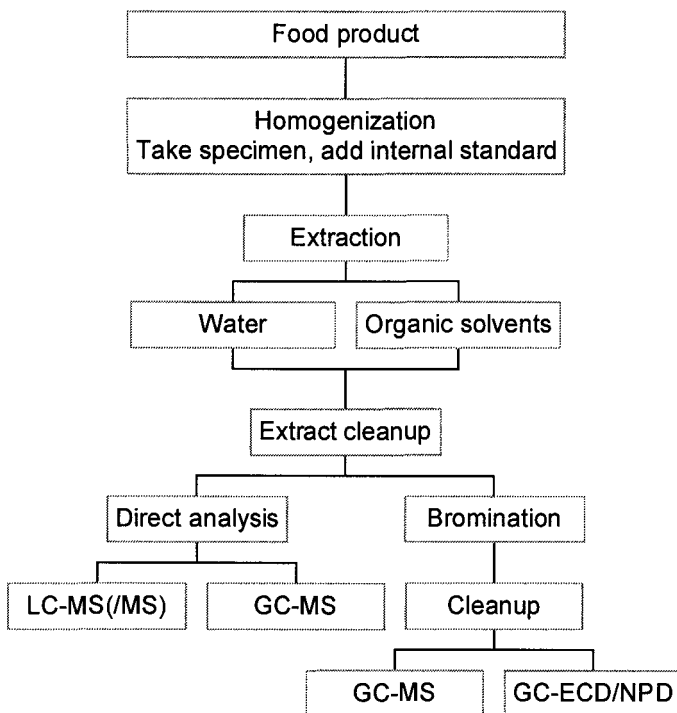
The formation of acrylamide is discussed in section 5.

3.2.2 Screening tests

Several research groups have undertaken developmental work to raise antibodies to acrylamide with the objective of deploying a test method in enzyme-linked immunosorbent assay (ELISA), dip-stick or lateral-flow formats. No publications have reported success. It seems likely that the reactivity of acrylamide allied to its small size has so far defeated attempts to raise useful antibodies.

A schematic of the main steps in analysis of foods for acrylamide is shown as Figure 3.

Figure 3. General schematic for acrylamide analysis



ECD, electron capture detection; GC, gas chromatography; LC, liquid chromatography; MS, mass spectrometry; NPD, nitrogen-phosphorus detection

3.2.3 Quantitative methods

(a) Addition of internal standard

Nearly all published methods use internal standards. Both deuterium-labelled ($^2\text{H}_3\text{-AA}$) and carbon-labelled ($^{13}\text{C}_3\text{-AA}$) substances are available commercially and are used widely. The triple label in each takes the internal standard well away from the mass ions used to measure acrylamide itself in methods that employ MS.

In most published methods, the internal standard is added to the specimen of food, and a period of incorporation is allowed before commencing extraction. It is then assumed that the internal standard behaves in the same way as the "native" acrylamide. This is usually checked by determining the recovery of acrylamide spiked into the matrix.

(b) Extraction using water

The high water solubility of acrylamide means that extraction of foods using plain water is effective. Water extraction is most usually conducted at room temperature (Rosén & Hellenäs, 2002; Tareke et al., 2002) with a mass ratio of about 1 part sample to 10 parts water (Rosén & Hellenäs, 2002; Tareke et al., 2002). Acrylamide is neither strongly acid nor basic, so adjustment of pH is not required. The sample needs to be finely divided to ensure efficient extraction. Extraction seems to be rather rapid, and just a few minutes' agitation of a dispersed sample in cold water is effective. Cereal and potato products are naturally hydrophilic and extract well. Extraction using hot water for 1–2 h does not seem to give any higher recovery (Owen et al., 2005). However, fatty matrices such as chocolate or peanut butter benefit from using hot water to promote dispersion and effective extraction (Ahn et al., 2002; Gutsche et al., 2002; Zyzak et al., 2003). Alternatively, room temperature extraction using a binary mixture of water and an organic solvent such as dichloroethane to break up and remove the fat phase (Zyzak et al., 2003) is very effective. Other organic solvents, including hexane, have been used by others for defatting the sample prior to water extraction (Gutsche et al., 2002; Hartig et al., 2003; Vatter & Shetty, 2003).

(c) Extraction using organic solvents

Accelerated solvent extraction with acetonitrile has been used as an alternative to water extraction for analysis of acrylamide in potato products and crispbreads (Cavalli et al., 2003). Other polar solvents, including methanol (Tateo & Bononi, 2003), propanol, acetonitrile and ethanol/dichloromethane mixture, have been employed to extract acrylamide from foods (Owen et al., 2005). Pre-swelling some foods with water is necessary to ensure efficient extraction (Biedermann et al., 2002c).

(d) Efficiency of extraction

When the same materials are analysed by laboratories using different extraction solvents and extraction conditions, such as in proficiency exercises (Clarke et al., 2002; Owen et al., 2005), there is a general consensus of results, with no evidence that one combination of solvents/conditions gives rise to better results than others. Some laboratories (Biedermann et al., 2002c; Jezussek & Schieberle, 2003) have used an enzymatic treatment of the food sample during water extraction, but treatment with amylase or protease did not liberate any additional acrylamide in the foods tested. The preponderance of evidence from different extraction studies indicates that extraction of acrylamide from the food sample is complete when using the normal analytical procedures (JIFSAN, 2004).

(e) *Determination by GC-MS after bromination*

Analysis for acrylamide using bromination and GC determination was relatively advanced even before acrylamide was discovered in heated foods, because of the need to test drinking-water, discharge waters and crops for acrylamide (Lande et al., 1979; Castle et al., 1991; Habermann, 1991; Bologna et al., 1999). Bromination affords an analyte that is much easier to analyse at trace levels than acrylamide itself. The product of bromination is 2,3-dibromopropionamide, which can be back-extracted from the aqueous bromination solution into a solvent such as ethyl acetate. Bromination is an addition reaction, and the isotope label (^2H or ^{13}C) on the internal standard is retained (Nemoto et al., 2002; Ono et al., 2003).

In some methods, the first-formed 2,3-dibromopropionamide is dehydrobrominated to form the more stable 2-bromopropenamide by treatment with triethylamine before GC-MS analysis. In this case, one of the three deuterium labels is lost if $^2\text{H}_3\text{-AA}$ is used as the internal standard, but quantification via the monobromo derivative is still successful (Andrawes et al., 1987; Nemoto et al., 2002; Pittet et al., 2004).

(i) *Confidence in the identification and quantification aspects*

It is possible to determine acrylamide by bromination followed by GC analysis using an alkali flame ionization detector (Schultzová & Tekel, 1996) or using an electron capture detector (Poole et al., 1981; Raymer et al., 1993). However, virtually all laboratories make use of the extra selectivity and confidence offered by MS coupled with the facility this provides to use an isotopically labelled internal standard.

The bromination-GC-MS (Br-GC-MS) method has a high intrinsic level of specificity originating from three attributes: 1) water extraction, bromination to make a less polar derivative and then back-extraction into ethyl acetate eliminate many water-soluble and unreactive components; 2) capillary GC is a high-resolution procedure with a large number of effective chromatographic plates; and 3) brominating the acrylamide gives a higher molecular mass analyte, which, especially with the characteristic $^{79}\text{Br}/^{81}\text{Br}$ isotope pair, gives several ions to monitor and confirms the identity of the analyte.

(ii) *Trueness of the Br-GC-MS analysis*

There was an initial concern that the harsh conditions used in the bromination procedure, with strong acid and potentially oxidizing conditions with elemental bromine, might give rise to acrylamide formation from precursors as an artefact of the method. These concerns were dispelled early on when parallel analysis of sample extracts using Br-GC-MS and underivatized LC-MS gave broad agreement for a range of food types (Ahn et al., 2002). More recently, when 74 different food samples were tested using both Br-GC-MS and LC-MS, the range of results spanned 2–3500 $\mu\text{g}/\text{kg}$. There was excellent correlation between the two approaches, with a correlation coefficient of $r^2 = 0.946$. The slope of the line was

near unity ($Y = 0.931X$), and the intercept was very close to the origin ($-2.2 \mu\text{g/kg}$) (Ono et al., 2003). Similarly, in check-sample exercises, there has been no evidence of any bias from Br-GC-MS methods compared with other test methods used (Owen et al., 2005).

(iii) Performance of the Br-GC-MS methods

In a typical procedure (Ahn et al., 2002), homogenized food is extracted by shaking with water in a 1:10 ratio. A portion of the filtered extract is brominated overnight at about 5°C , and the derivative is extracted into ethyl acetate. The organic extract is dried over sodium sulfate and then evaporated to a small volume. Analysis on a normal bench-top GC-MS instrument fitted with a capillary column with a 50:50 phenyl/methyl silicone phase gave a limit of quantification (LOQ) of less than $10 \mu\text{g/kg}$. At this level, the brominated acrylamide gave a good response in all 4 m/z channels monitored. In this procedure, 1 ml of final analysis solution corresponded to 1 g of food.

It is possible to increase the effective concentration for GC-MS analysis, for example, using a procedure described by Ono et al. (2003), in which a final analysis solution of $25 \mu\text{l}$ corresponds to 0.28 g food, or an 11-fold concentration. The limit of detection (LOD) and LOQ were said to be $1 \mu\text{g/kg}$ and $3 \mu\text{g/kg}$, respectively (Ono et al., 2003). Other cleanup steps have been described, using Carrez solutions I & II, cleanup of the brominated derivative using Florisil, dehydrobromination using triethylamine and then GC-MS (Pittet et al., 2004). The concentration factor was 34 (final extract of 0.44 ml corresponding to the 15-g food specimen), and the LOD and LOQ were quoted to be $2 \mu\text{g/kg}$ and $5 \mu\text{g/kg}$, respectively. Cleanup of the brominated food extract on silica (Castle et al., 1991; Castle, 1993), on Florisil (Nemoto et al., 2002; Pittet et al., 2004) and by size exclusion chromatography (Tareke et al., 2000) have also been employed to good effect in order to achieve lowered detection limits. An LOQ of $5\text{--}10 \mu\text{g/kg}$ is more than adequate for testing most individual food items, although improved sensitivity is desirable when testing composite diet samples.

(iv) Br-GC-MS: Conclusions

It can be concluded that methods using bromination of acrylamide followed by GC-MS are well developed and have been demonstrated to be accurate in check-sample exercises. LOQs down to $5 \mu\text{g/kg}$ are well within the reach of the average laboratory equipped with a standard benchtop GC-MS instrument.

(f) Determination by GC-MS with no derivatization

Acrylamide is not a natural candidate for direct GC-MS analysis, for several reasons. Firstly, the polar solvents (and preferably water) that are required to effect a good extraction of acrylamide out of foods are not well suited for pre-concentration and injection onto a capillary column. Secondly, acrylamide is very polar and has a low volatility compared with its molecular mass, and so selection

of the column phase is critical. Lastly, such a small molecule with a relative molecular mass of 71 does not give a very convincing or unique mass spectrum.

Nevertheless, a number of laboratories have persisted with the difficult task of developing a direct GC method for acrylamide (Biedermann et al., 2002c), largely because it offers higher sample throughput (avoiding the time-consuming bromination step) and it reduces the use of corrosive and hazardous chemicals. Given the limited solubility of acrylamide in most organic solvents, a polar solvent such as methanol (Tateo & Bononi, 2003), propanol or butanone (Biedermann et al., 2002c) is needed for efficient extraction. Water is still required, however, since swelling of many food samples with water is necessary; otherwise, the recovery is very poor. Fatty samples require defatting, normally by extraction into hexane. Due to the high polarity of acrylamide, a polar column such as Carbowax is used, and on-column injection is preferable. Chemical ionization is said to offer better sensitivity than electron impact ionization, with an LOD down to 5 µg/kg (Robarge et al., 2003).

Extract cleanup for underivatized GC-MS is likely to be more demanding than for the Br-GC-MS procedure, although little comparative work has been reported. The extra requirement is because of the possibility that the precursors of acrylamide may be extracted and thus that extra acrylamide may be formed as an artefact during the GC analysis. It has been noted by others (DeVries & Post, 2004; Grob et al., 2004; Tanaka et al., 2004) that extraction of acrylamide precursors from foods can lead to extra acrylamide formation as an artefact during subsequent heating, and this may occur also in the hot injection port of a gas chromatogram. In proficiency check-sample exercises, it has been noted (Anklam & Wenzl, 2005; Owen et al., 2005) that some laboratories using the direct GC-MS approach report high results.

(g) Determination by LC

LC has been used as the determination step after bromination (similar to GC) using a UV detector measuring at 196 nm (Brown & Rhead, 1979) in aqueous samples and in sugar using a column switching technique and with a thermospray interface to MS (Cutié & Kallos, 1986). LC-UV has also been used to test directly for residual acrylamide in polyacrylamides, soil and other environmental samples, by measuring in the rather universal range 208 up to 254 nm (Skelly & Husser, 1978; Shanker et al., 1990; Smith & Oehme, 1993; Ver Vers, 1999; Saroja et al., 2000). LC-UV exhibits rather poor sensitivity and selectivity, because acrylamide possesses a poor UV chromophore. Nevertheless, it has found application, especially in the testing of food samples prepared in the laboratory to simulate home cooking or industrial cooking and so for which blank (uncooked) samples are readily available to help guard against interferences in the analysis.

LC-UV has been used to test mainly potato products and instant noodles, for which acrylamide levels can be rather high, and so sensitivity is not a major issue. A drawback with this technique is that isotope-labelled acrylamide cannot be used as internal standard. To compensate for the lack of selective detection, column switching techniques have been used to get better separation (Terada & Tamura,

2003). For french fries and other foods, LC-UV at low wavelengths gave broadly the same results as LC-MS (Cavalli et al., 2003; Peng et al., 2003). Using LC with a diode array detector at 200, 214 and 240 nm for the analysis of potato chips showed higher results than expected for uncooked potato, but the results were not confirmed by another technique (Vattem & Shetty, 2003).

(h) Determination by LC-MS

(i) Extract cleanup requirements for LC-MS

As stated above, the best extraction solvent for acrylamide in foods is water, and this extract is directly compatible with reversed-phase LC using an aqueous mobile phase with a small amount of organic modifier. However, some prior cleanup of the aqueous extract is required. Cleanup for LC-MS methods has three approaches that are often used in combination: 1) using solid-phase extraction (SPE) with complementary stationary phases; 2) using chemical deproteination with Carrez I and II solutions (Gutsche et al., 2002; Hartig et al., 2003); and 3) removing unwanted co-extractives by physical methods of freeze-thaw precipitation or membrane filtration. No difference in results depending on the way in which the samples have been purified has been reported.

For purification of the extracts, different kinds of SPE columns have been used, including graphitized carbon, ion-exchange resins and mixed-mode materials. In most cases, the choice of any particular cleanup SPE cartridge has been made largely by trial and error until the problem has been solved. Consequently, a large number of SPE materials have been reported (Ahn et al., 2002; Gutsche et al., 2002; Rosén & Hellenäs, 2002; Tareke et al., 2002; Becalski et al., 2003; Leung et al., 2003; Ono et al., 2003; Peng et al., 2003; Riediker & Stadler, 2003; Roach et al., 2003; Andrzejewski et al., 2004; Shih et al., 2004). In many cases, the SPE cleanup step has been combined with a molecular size cut-off filter (3–5 kDa) to remove larger molecules that would otherwise interfere.

(ii) Chromatographic performance in LC-MS

The chromatographic resolving power of LC columns is much lower than for GC columns, so column choice is critical for a successful analysis. This is especially true because acrylamide is so water soluble and it is a challenge to get useful retention on most reversed-phase LC columns.

As with the SPE cleanup, a number of different stationary-phase chemistries have been used to effect the LC separation of acrylamide from other co-extractives. These include graphitic carbon (Gutsche et al., 2002; Rosén & Hellenäs, 2002; Tareke et al., 2002; Becalski et al., 2003; Hartig et al., 2003; Inoue et al., 2003; Leung et al., 2003; McHale et al., 2003; Shih et al., 2004), octyl decyl-modified silica (Ahn et al., 2002; Hartig et al., 2003; Ono et al., 2003; Peng et al., 2003; Roach et al., 2003; Zyzak et al., 2003; Andrzejewski et al., 2004), other modified silicas (Hartig et al., 2003; Jezussek & Schieberle, 2003) and ion-exchange resins (Cavalli et al., 2003; Riediker & Stadler, 2003) — some with a supplementary size-exclusion mode too (Terada & Tamura, 2003). No difference

in results has been shown depending on the type of column used. The column type used most frequently is graphitic carbon.

(iii) Detection by LC-MS

Triple-quadrupole mass spectrometers for LC-MS are quite expensive, but single-stage instruments are not usually sensitive enough to conduct acrylamide analysis on water extracts of foods unless a degree of pre-concentration is used. Even so, most modern LC-MS/MS instruments perform relatively poorly below about 100 Da unless tuned specifically for low mass/charge ratio ions; acrylamide has a relative molecular mass of 71. Most LC-MS/MS methods use ESI in the positive ion mode (Ahn et al., 2002; Gutsche et al., 2002; Rosén & Hellenäs, 2002; Tareke et al., 2002; Ahn & Castle, 2003; Becalski et al., 2003; Hartig et al., 2003; Leung et al., 2003; Ono et al., 2003; Riediker & Stadler, 2003; Roach et al., 2003; Andrzejewski et al., 2004; Shih et al., 2004).

(iv) Confidence in LC-MS identification of acrylamide

LC-MS/MS identification of acrylamide rests on the chromatographic retention time and on the presence and relative abundance of characteristic ions (JIFSAN, 2004). The main ions observed for acrylamide are m/z 72 (protonated molecular ion), 55 (loss of amino) and 27 (subsequent loss of carbon monoxide). A typical identification criterion is that the relative abundance values should agree to within $\pm 10\%$ for acrylamide to be considered to be detected (Roach et al., 2003; Andrzejewski et al., 2004). In a similar vein, acrylamide in food was confirmed if at least two positive selective-reaction monitoring (SRM) responses were obtained with matching ion ratios within an acceptable tolerance (mean ± 10 –20%) compared with the ratios obtained from acrylamide standards. Three SRM traces were routinely recorded, although the acquisition of just two SRM traces fulfils the criteria required in the Commission Decision 2002/657/EC (Riediker & Stadler, 2003). Another criterion that has been used is to examine the full mass spectrum obtained. So, for example, the ion m/z 55 was used for quantification; for identification, the spectra should be identical for the sample and for the standard at 10 eV and 20 eV collision energy (Tareke et al., 2002).

(v) Quantitative aspects of LC-MS analysis

In many reports, it is not clear exactly how the reported LOD and LOQ values were derived. Some laboratories have derived the values from standards, and others from sample extracts. It is also frequently unclear if the LOD and LOQ values cited are for the main qualifying ion only or if they take proper account of the need to record and measure the qualification ions so that the relative abundance ratios can be checked. For acrylamide, the qualification ions are often much weaker (less abundant) than the main quantification ion, depending on the instrument and the conditions used. Most laboratories are still within the same magnitude as Rosén & Hellenäs (2002) and Tareke et al. (2002), independent of which LC-MS method they have used. LOD values are 3–20 $\mu\text{g/kg}$, LOQ values

are 10–50 µg/kg and the analysis is linear over the range 10–10 000 µg/kg (with some variations).

(vi) *LC-MS methods: conclusions*

Most survey data for acrylamide have been obtained using LC-MS/MS analysis. The technique has proved to be well correlated with GC-MS measurements and is accurate.

(i) *Summary of the performance of analytical methods used*

During the past 3 years since acrylamide formation in heated foods was discovered and reported, there has been a tremendous development of analytical methods for the determination of levels in foods. There are both GC-MS and LC-MS/MS techniques, which fulfil the requirements for today's acrylamide analysis (Wenzl et al., 2003). There is currently an absence of certified reference materials and methods that have been tested collaboratively according to the harmonized guidelines of the International Union for Pure and Applied Chemistry (IUPAC). Consequently, the performance of methods is judged using in-house (single laboratory) validation data and performance in check-sample exercises and performance assessment schemes. The methods show good agreement generally and are likely to be accurate. There is a need for improvements in precision (within-laboratory) and repeatability (between-laboratory). However, given the very wide range of acrylamide concentrations that can be achieved using different cooking and heat-processing methods, and given the very high number of survey data available, it is not expected that the analytical uncertainty will be a large factor in the overall uncertainty in estimates of exposure.

4. **SAMPLING**

Acrylamide formation is largely a surface phenomenon where the conditions of high temperature and low moisture pertain. The affected foods are mostly solids. Acrylamide is not distributed homogeneously throughout these foods, but it is concentrated at the surface. There can be very large differences between individual food pieces, such as potato crisps or chips. There can also be large differences in acrylamide content within individual crisps or chips, since the edges and tips brown more than the rest of the item. So the whole portion or serving of food as eaten should be homogenized thoroughly before a specimen is taken for extraction and analysis. Acrylamide is freely water-soluble and virtually insoluble in oil and fat phases, so if there is any tendency for foods to separate, this should be avoided. This said, most of the affected foods can be homogenized quickly and effectively.

Acrylamide levels have been reported to decline slowly in some food products during long-term storage. One example is ground coffee, for which a 40–65% decline was found after 6 months' storage of three brands at room temperature (Andrzejewski et al., 2004). In contrast, there was no significant decline of acrylamide levels in a coffee sample stored at –40 °C (Andrzejewski et al., 2004).

Also in the work of Andrzejewski et al. (2004), the level of acrylamide in coffee was measured when freshly brewed and after standing heated for up to 5 h. No significant change in the concentration was found. In general, however, for home-cooked, take-away and restaurant meals that are freshly cooked and then consumed hot, there seem to have been no systematic studies reported on the short-term stability of acrylamide. The current view is that these products can be allowed to cool and then analysed later, but this should be verified.

Given that acrylamide seems to be rather stable in the large majority of the affected foods, any discrepancy between the date of analysis of retail samples compared with the “normal” date of consumption by the consumer is not expected to be a major source of error in estimates of intake via the diet.

5. EFFECTS OF PROCESSING

5.1 Heat-induced formation of acrylamide in foods

Acrylamide formation has been discussed and reviewed recently (European Commission, 2003; Friedman, 2003; European Food Safety Authority, 2004; JIFSAN, 2004; Taeymans et al., 2004).

The free amino acid asparagine, present as such in many raw food commodities, has been identified as a major precursor of acrylamide in foods. Heat-induced formation from asparagine, involving reducing sugars or other carbonyl compounds as necessary co-reactants, has been demonstrated in a number of studies on mixtures of pure chemicals or simple food models. The importance of asparagine is further supported by observations from real foods — e.g. through conformity between acrylamide and asparagine levels in foods due to variations in the natural asparagine content or as a result of addition or enzymatic removal.

From a very simplistic view, the conversion from asparagine to acrylamide merely represents elimination of the carboxyl and the amine groups from the asparagine molecule. However, the general view is that the most important formation mechanism in food is through complex Maillard reactions. Several reaction pathways have been suggested and supported by experimental evidence from model experiments. Some controversy still exists on the quantitative importance of these different pathways.

Acrylamide formation from other precursors and/or by other reaction mechanisms has been demonstrated in chemical model systems. No firm data have been presented indicating their importance to acrylamide levels in foods. Alternative mechanisms could be minor contributors to the acrylamide formation in common and important food sources, working in parallel with Maillard reactions of asparagine, and possibly of more significant importance in certain specific food commodities and with specific cooking/production conditions.

Much of the present knowledge on formation mechanisms and factors has been obtained from test tube studies on chemical mixtures or simple food models. Differences in results from different studies might be due to model setup — e.g. dry or aqueous environment, open or closed system, choice of

temperature/heating time, etc. Often, a small amount of dry test sample in a sealed system is submitted to temperature treatment at temperatures at 180 °C or higher. This represents a well defined model allowing for repeatable and conclusive experimentation. On the other hand, the results might not be applicable to real food. The process of cooking is a dynamic and highly complex process. For example, the temperature of the actual food item in a hot oven does not significantly exceed 100 °C until most of the water has evaporated, a process that starts at the surface and is delayed by the diffusion rate of water from the inner parts.

5.2 Chemical mechanisms

5.2.1 Formation from amino acid and sugar by Maillard reactions

The classical Maillard reaction system represents a complex reaction cluster leading to browning and formation of the flavour and aroma compounds associated with fried or baked foods. Low moisture, high temperature and alkaline pH typically favour the reaction. However, Maillard compounds can be formed at slow rates under less favourable conditions.

Acrylamide formed from asparagine and sugar in Maillard model experiments using labelled reagents was shown to have incorporated all three carbon atoms, as well as the nitrogen atom, from the amide side-chain of asparagine (Becalski et al., 2003; Zyzak et al., 2003). Labelled carbon atoms in the glucose co-reactant were not incorporated (Stadler et al., 2002; Yaylayan et al., 2003).

In the first reaction steps, condensation between the amine group of asparagine and the carbonyl group of a co-reactant (e.g. a reducing sugar) can form a Schiff's base via an *N*-substituted glycosylamine (Stadler et al., 2002). Several different reaction routes have been proposed for the further reaction to acrylamide — e.g. decarboxylation of the Schiff's base followed by deamination through loss of an imine or formation of a 3-aminopropionamide (3-APA) intermediate and subsequent loss of ammonia (Yaylayan et al., 2003; Zyzak et al., 2003; Stadler et al., 2004).

Strecker degradation of asparagine, involving dicarbonyl compounds formed in classical Maillard reactions, has been suggested as an alternative formation mechanism (Mottram et al., 2002).

Maillard system studies with asparagine and sugars added to flour have pointed to the possible importance of free ammonia, especially at lower temperatures and in high-moisture systems. Addition of ammonium bicarbonate was shown to dramatically increase acrylamide formation. The relative molar yield increased from about 0.1% to 5% (Biedermann & Grob, 2003). It was suggested that ammonia reacts with the reducing sugar, forming an amino-sugar that is more prone to react with asparagine in the Maillard reaction than the intact sugar (Weisshaar, 2004).

Sugar-assisted formation of acrylamide has also been demonstrated in pyrolysates from aspartic acid and glucose (Yaylayan et al., 2004). To a lesser extent,

this amino acid was also able to form acrylamide via the acrylic acid pathway discussed in the next section.

5.2.2 Formation via acrylic acid

It is known that heating of acrylic acid in the presence of free ammonia yields acrylamide (e.g. Yasuhara et al., 2003). In food systems, the acrylic acid can be formed in well known heat-induced reactions from various common food components, including fats (via acrolein), amino acids and carbohydrates. The ammonia can likewise be formed from deamination of proteins and amino acids. Recent experiments have demonstrated the formation of acrylamide from β -alanine and the dipeptide carnosine (*N*- β -alanyl-L-histidine) in yields comparable to the asparagine–glucose system in pyrolysis experiments (Yaylayan et al., 2004). The mechanism(s) might be of specific relevance to meat, where free asparagine levels are low. The pyrolysis experiments were done at high temperature and short time (350 °C, 20 s).

5.2.3 Formation from 3-aminopropionamide

As mentioned above (section 5.2.1), 3-APA has been identified as a late intermediate in acrylamide formation from asparagine in the Maillard reaction (Zyzak et al., 2003). More recently, 3-APA has been detected in raw potatoes at concentrations in the range 0.2–2 mg/kg fresh weight (Granvogl et al., 2004). The presence was proposed to be due to the enzymatic decarboxylation of asparagine. The relative turnover of 3-APA to acrylamide at 180 °C in aqueous model systems was up to 60%, compared with 0.1% for the asparagine–glucose system. Even at temperatures down to 100 °C, the turnover was above 0.1%.

5.2.4 Elimination of acrylamide

Acrylamide concentrations in fried foods can be seen as the net result of two competing processes: formation and elimination. By adding labelled acrylamide before heating in model experiments, both processes could be followed simultaneously (Biedermann et al., 2002a, 2002b). Elimination was demonstrated in all models, including potato, flour and, in particular, meat. Similar studies with coffee (Taeymans et al., 2004) and gingerbread (Amrein et al., 2004) have also been performed. Although elimination was seen in gingerbread during baking, it was concluded that the rate was low in comparison with the formation rate within normal baking times. In roasting of coffee, on the other hand, acrylamide is accumulated during an early phase followed by a dramatic reduction before the final product is obtained (Taeymans et al., 2004).

No experimental data are available concerning the chemical mechanism(s) for, or end-products of, acrylamide elimination in foods. It has been hypothesized that the elimination could be due to reaction with nucleophilic sites on proteins — i.e. through mechanisms similar to the formation of protein and DNA adducts in humans and other animals (Friedman, 2003).

5.2.5 Analogues: formation of other compounds

Model studies on acrylamide formation have resulted in the identification of other heat-induced compounds, including, for example, *N*-methylacrylamide, *N,N*-dimethylacrylamide, 2-pyrrolidone and styrene (Stadler et al., 2004). The two former were identified in pyrolysates of carnosine/creatine mixtures, and *N*-methylacrylamide was also detected in meat pyrolysates in amounts estimated to be comparable with acrylamide levels in potato products (Yaylayan et al., 2004). 2-Pyrrolidone was detected as a conspicuous peak during acrylamide analysis of foods and later in model systems containing glutamine and sugar (Biedermann et al., 2003; Stadler et al., 2003). The identity was first mistaken for 3-butenamide, the four-carbon analogue to acrylamide (Weisshaar & Gutsche, 2002).

5.3 Formation factors

5.3.1 Heating temperature and time

Formation of acrylamide has been demonstrated in asparagine–sugar model systems at temperatures from 120 °C to 350 °C (Mottram et al., 2002; Stadler et al., 2002, 2004; Becalski et al., 2003). Maximum yields (0.1–0.3% relative asparagine turnover) were obtained with time/temperature combinations ranging from 20 min/155 °C to 5 min/180 °C. In several of the studies, more vigorous heating resulted in lower yields. Thus, temperature and the time of heating appear to be interrelated parameters, affecting both formation and elimination rates. Covariance of temperature and time was recently demonstrated with asparagine–glucose dissolved in dimethylsulfoxide — i.e. a liquid non-aqueous system. Fixed heating times of 5 and 60 min showed sharp and well separated temperature optima for acrylamide formation at 160 °C and 120 °C, respectively, with fairly similar maximum yields (approximately 1%) (Robert et al., 2004).

Although increased acrylamide levels at long heating times were also seen in experiments with simple food models (Biedermann & Grob, 2003; Rydberg et al., 2003), it is likely that the upper end-points for heat treatment in these studies were considerably higher than those in real food production. In a study on deep-fried potato pieces of various well defined sizes, the upper limit for heat treatment was related to an anticipated consumption threshold for surface browning (Taubert et al., 2004). Decreased accumulation in the high temperature range (total range 120–230 °C) was seen only in small-sized potato pieces. The effect was suggested to be due to a smaller surface-to-volume ratio, and thereby a smaller precursor reservoir, in small compared with large pieces.

Consistently increased acrylamide levels with increased time and temperature were observed within realistic intervals for heat treatment in production of bread (Surdyk et al., 2004), potato crisps (Haase et al., 2003; Kita et al., 2004; Pedreschi et al., 2004) and french fries (Gertz et al., 2003; Grob et al., 2003; Taeymans et al., 2004).

For coffee, the levels in the finished product were about 5–10 times lower than at an early stage of the roasting process. The pattern was similar at temperatures of 210–250 °C (Taeymans et al., 2004).

5.3.2 Precursors

The ability of several sugars (e.g. glucose, fructose, galactose, lactose, ribose and sucrose), as well as other carbonyl compounds (e.g. glyceraldehydes, glyoxal and ascorbic acid), to assist the formation of acrylamide from asparagine has been demonstrated in model experiments (Stadler et al., 2002, 2004; Weisshaar & Gutsche, 2002; Becalski et al., 2003; Pollien et al., 2003; Zyzak et al., 2003). Fructose was more efficient than glucose in most studies, typically giving 30–100% higher yields (Becalski et al., 2003; Biedermann & Grob, 2003; Rydberg et al., 2003; Yaylayan et al., 2003; Stadler et al., 2004; Taeymans et al., 2004). Experiments in crystalline model systems demonstrated higher yields from fructose, while glucose was the more efficient in an anhydrous liquid system (dimethyl sulfoxide). This was explained by a higher molecular mobility, linked to the sugars' melting behaviour and phase transition temperatures being more important than the chemical reactivity in the crystalline system (Robert et al., 2004). Inconsistent data on the effect of sucrose might be due to temperature differences. The non-reducing disaccharide sucrose is not supposed to take part in the formation reaction until thermally decomposed. In comparison with glucose, the molar acrylamide yields with sucrose were 50% lower or 30% and 370% higher in experiments at 175 °C, 200 °C and 350 °C, respectively (Becalski et al., 2003; Yaylayan et al., 2003; Taeymans et al., 2004). By contrast, no increase in acrylamide levels was detected from sucrose addition when dried potato was heated at 120 °C (Biedermann & Grob, 2003).

Addition of asparagine and fructose to the dough prior to baking wheat bread showed that the acrylamide levels were strongly correlated to the asparagine concentration, while the fructose addition had no effect (Surdyk et al., 2004). Addition of asparagine in gingerbread production showed similar results (Amrein et al., 2004). The findings are supported by model experiments with wheat and rye flour (Weisshaar, 2004).

The potential for acrylamide formation in a large number of potatoes has been investigated in relation to the natural variation in levels of asparagine and sugars in the raw tubers. Linear regression of acrylamide levels versus contents of reducing sugars (fructose + 0.5 × glucose) gave a correlation coefficient (r^2) of 0.88. By including the asparagine levels in the regression, the correlation was only slightly improved to $r^2 = 0.91$ (Amrein et al., 2003). Similar results were obtained for french fries when the added amount of glucose, fructose and sucrose was used in the regression equation (Becalski et al., 2004).

The fact that sugar, and not asparagine, appears to be a major limiting factor for acrylamide formation in potato products is likely to be due to the high natural content of free asparagine in potato and the fact that the variation of the asparagine levels is low in comparison with the variation of sugar levels in potatoes (Biedermann & Grob, 2003; Becalski et al., 2004). The average concentration of glucose in the study cited above (Becalski et al., 2004) was 1.7 mg/g (fresh weight basis), with a relative standard variation of 90%; the corresponding values for asparagine were 5.6 mg/g and 38%, respectively. By comparison, the mean value for the asparagine content in samples from a range of European wheat varieties

averaged 0.23 mg/g (dry weight basis), with a relative standard deviation of 42% (Taeymans et al., 2004).

Literature data on the levels of free asparagine in food raw materials or agricultural commodities have been reviewed in response to the discovery of its role in acrylamide formation (Elmore & Mottram, 2002; Doyle, 2003). The availability of data on factors behind the variability is limited for some commodities. Asparagine in European wheat varieties from a single crop year showed a 5-fold variation range between varieties and at least a 2-fold range within varieties (Taeymans et al., 2004). Rye contains more asparagine than wheat, and whole grain flour has a higher asparagine content than sifted white flour (Fredriksson et al., 2004). The levels of glucose and fructose in potatoes are well studied and known to be influenced by several factors, including temperature and time of storage, growing conditions and variety characteristics (Haase & Weber, 2003; Olsson et al., 2004).

5.3.3 *Acidity*

The acrylamide accumulation in asparagine–glucose solutions at 150 °C increased exponentially with increasing pH in the range 4–7 (Jung et al., 2003). The acrylamide content of heated potato tissue adjusted to pH values from 6 to 10 exhibited a maximum around pH 8 (Rydberg et al., 2003).

5.3.4 *The food matrix*

High acrylamide levels have mainly been detected in carbohydrate-rich foods. However, there is no evidence that starch is directly involved in the formation mechanism; instead, indirect effects have been proposed. Starchy foods generally provide an inert matrix, high in dry matter, which might entrap and stabilize precursors, intermediates and/or acrylamide itself. It is also possible that the link is merely circumstantial — e.g. that starchy foods often contain high levels of the free asparagine and reducing sugars (European Food Safety Authority, 2004).

By contrast, animal-derived food matrices seem to promote elimination reactions rather than formation (Biedermann et al., 2002b). Addition of fish tissue to potato prior to frying was shown to give a concentration-dependent decrease of acrylamide levels (Rydberg et al., 2003).

5.3.5 *Water activity*

Acrylamide accumulation is known to accelerate at the final stages of the frying process of, for example, potato chips and to occur mainly in the outer “dry” tissues (Grob et al., 2003; Sell et al., 2004). It is not known to what extent this is a direct effect of decreased moisture content or of the increased heat transfer occurring concomitantly. No investigations on the specific role of water activity on the reaction rate for acrylamide formation have so far been reported.

6. LEVELS AND PATTERNS OF CONTAMINATION OF FOOD COMMODITIES

6.1 Surveillance data

Acrylamide concentration data for different food items were evaluated for the current meeting from 24 countries. Most of the samples provided by Europe have been collected from the European Union (EU) monitoring database of acrylamide levels in food (European Union, 2004). Updated data from this June 2004 status have been collected for the meeting from Austria, Belgium, Denmark, France, Sweden, Switzerland and the United Kingdom. All data have been submitted on an individual basis. The total number of analytical results (single or composite samples) was 6752, with 67.6% from Europe (179 from Austria, 158 from Belgium, 30 from Denmark, 4 from Finland, 292 from France, 2898 from Germany, 9 from Greece, 22 from Ireland, 6 from Italy, 337 from The Netherlands, 1 from Spain, 124 from Sweden, 424 from Switzerland and 79 from the United Kingdom), 21.9% from North America (141 from Canada and 1337 from the United States), 8.9% from Asia (103 from China, 170 from China, Hong Kong Special Administrative Region, 3 from Israel, 156 from Japan, 50 from Syria, 2 from Turkey and 116 from the United Arab Emirates) and 1.6% from the Pacific region (111 from Australia/New Zealand). No data from Latin America and Africa were submitted.

The Committee noted that the occurrence data evaluated at this meeting were more comprehensive than those available at the FAO/WHO (2002) consultation (240 samples).

The choice of food items analysed for acrylamide concentration was based on what was known since 2002–2003 on the occurrence of acrylamide in foodstuffs and also based on recommendations made at the last consultation (FAO/WHO, 2002), especially concerning other foodstuffs that undergo similar processing and that might also contain acrylamide, such as meat, milk, rice, cassava, soya products, vegetables and processed fruits. Table 7 shows the summary for individual occurrence data, including the percentage of censored data (below reporting limits) collected from 24 countries during 2002–2004.

Acrylamide concentrations are found to be highest in starchy foods cooked by methods such as grilling, roasting, baking, frying and deep-frying. Improved knowledge of acrylamide formation during the food process revealed that the acrylamide content in the food product depended on the oil temperature, the moisture, the total fat and the time of frying (Leung et al., 2003). As contamination with acrylamide is known to occur during heat treatment in several commodities, the cooking methods have been noted. The list includes cereals and pasta, raw and boiled; cereals and pasta, processed (rice, corn tortilla, popcorn; toasted, fried, grilled); breakfast cereals; bread and rolls; pastry and biscuits ("cookies" in the USA); pizza; potato puree/mashed/boiled; potato and potato products baked; potato crisps ("chips" in the USA); potato chips ("french fries" in the USA); potato chips and croquettes frozen not ready-to-eat; coffee brewed ready-to-drink; coffee ground, instant or roasted not brewed; coffee extracts; coffee decaffeinated; coffee substitutes; cocoa products; green tea ("roasted"); vegetables raw, boiled and canned; vegetables processed (toasted, baked, fried, grilled); fruits fresh; fruits

Table 7. Summary of acrylamide occurrence data from various countries from 2002 to 2004

Country	Number of samples ^a	% of values below LOR ^b
Australia/New Zealand	111	51
Austria	179	17
Belgium	158	6
Canada	141	4
China	103	29
China, Hong Kong Special Administrative Region	170	38
Denmark	30	0
Finland	4	0
France	292	20
Germany	2898	7
Greece	9	0
Ireland	22	0
Israel	3	0
Italy	6	0
Japan	156	10
Netherlands	337	40
Spain	1	100
Sweden	124	20
Switzerland	424	26
Syria	50	0
Turkey	2	0
United Arab Emirates	116	24
United Kingdom	79	23
United States	1337	34

^a Number of analytical results for individual samples and for composite samples.

^b LOR means limit of reporting (detection and quantification limit).

processed (dried and fried); nuts and oilseed (peanuts, olives, almonds, etc.); pulses (beans, pea, taro and yam canned, roasted or fried); meat and products (including beef, veal, pork, sheep, chicken, sausage, e.g. coated, cooked, fried); milk and milk products (including, e.g., pasteurized milk, butter, ice cream, processed cheeses and yoghurts); fish and seafood (including, e.g., breaded, fried, baked); sugars and confectionary (fruit paste, chocolate bar, etc.); infant

formula; baby food (canned, jarred); baby food (dry powder); baby food (biscuits, rusks, etc.); condiments and sauces; dried foods; and alcoholic beverages (beer, gin, wine).

6.2 National occurrence

Six countries (Finland, Greece, Israel, Italy, Spain and Turkey) submitted very few data (less than 10 per country) according to the European Union (2004) report. For this reason, no description is included for these countries.

6.2.1 Australia

Australia submitted the results of acrylamide occurrence analysed in carbohydrate-based foods from a recent publication (Croft et al., 2004). Acrylamide concentrations were obtained from 111 food composite samples based on an individual food sampling approach where each sample represents between 1 and 11 samples of the same food item purchased from a range of commercial shops in Sydney during November and December 2002 and being blended to form one single composite sample for analysis. Types of food were selecting according the Australian diet and derived from foods consumed in the 1995 National Nutrition Survey. Analyses were performed using the LC–tandem mass spectrometry (LC-MS-MS) technique. The limits of the method are, respectively, 25 and 50 µg/kg for detection and quantification. Fifty-one per cent of values were reported as being below the limit of reporting (LOR). The mean concentrations in the primary food items analysed were as follows: potato crisps (394–882 µg/kg), savoury biscuits (96–1270 µg/kg) and sweet potatoes (25–545 µg/kg).

6.2.2 Austria

Austria submitted the results of acrylamide occurrence analysed in 179 individual Austrian foods purchased in local stores during the years 2002 and 2003 (Murkovic, 2004). Analyses were performed using the high-performance liquid chromatography (HPLC)-MS technique. The limits of the method are, respectively, 15 and 30 µg/kg for detection and quantification. Seventeen per cent of values were reported below the LOR. The mean concentrations in the primary food groups analysed were as follows: potato crisps (627 µg/kg), cookies (275 µg/kg), coffee not brewed (204 µg/kg) and crispbread and potato chips (152–153 µg/kg).

6.2.3 Belgium

Belgium submitted the results of occurrence of acrylamide in foods from a recent publication (Matthys et al., 2005). Acrylamide concentrations were obtained from 158 individual food samples purchased in 2003 from different supermarkets and restaurants. Analyses were performed using the LC-MS-MS technique. The limits of the method are, respectively, 15 and 30 µg/kg for detection and quantification. Six per cent of values were reported below the LOR. The median concentrations in the primary food groups analysed were gingerbread (1403

µg/kg), potato crisps (676 µg/kg), baby's biscuits (324 µg/kg), french fries (254 µg/kg) and sweet spiced biscuits (204 µg/kg).

6.2.4 Canada

Canada submitted the results of a preliminary survey on occurrence of acrylamide in foods for the sixty-fourth JECFA meeting (Becalski et al., 2003). Acrylamide concentrations were obtained from 141 individual food samples purchased from a range of commercial shops during 2002 and 2003. Analyses were done using the LC-MS-MS technique. The limit of detection ranged from 2 to 15 µg/kg. Four per cent of values were reported below the LOR. The mean concentrations in the primary food groups analysed were as follows: coffee substitute (2733 µg/kg), potato chips (1320 µg/kg), french fries (795 µg/kg), breakfast cereals (112 µg/kg), bread (49 µg/kg), cocoa products (48 µg/kg) and coffee ready-to-drink (15 µg/l).

6.2.5 China

China submitted data on the occurrence of acrylamide in foods for the sixty-fourth JECFA meeting (Chinese Center for Disease Control and Prevention, 2005) using the Global Environment Monitoring System Food Contamination Monitoring and Assessment Programme (GEMS/Food) format. Acrylamide concentrations were obtained from 103 individual food samples based on an individual food sampling approach with samples purchased in 2004 from a range of food outlets in China. Analyses were performed using the LC-MS-MS technique. The limits of the method are, respectively, 3 and 10 µg/kg for detection and quantification. Twenty-nine per cent of values were reported below the LOR. The mean concentrations in analysed food groups were potato crisps (641 µg/kg), cereal-based rice (123 µg/kg) and pastry and biscuits (87 µg/kg).

6.2.6 China, Hong Kong Special Administrative Region

China, Hong Kong Special Administrative Region (SAR) submitted the results of acrylamide occurrence in foods from recent publications (FEHD, 2003; Leung et al., 2003) using the GEMS/Food format. Acrylamide concentrations were obtained from 170 food composite samples based on an individual food sampling approach using samples purchased in 2003 from a range of retail outlets and restaurants over Hong Kong SAR. Sampling includes analytical results of 99 individual samples where each sample was analysed individually as well as 71 composite samples where each sample represents five samples of the same food item from different sources blended to form one single composite sample for analysis. Analyses were performed using the LC-MS-MS technique. The limits of the method are, respectively, 3 and 10 µg/kg for detection and quantification. Thirty-eight per cent of values were reported below the LOR. Higher acrylamide levels up to above 1000 µg/kg were found in the food group biscuits-related products and crisps (Leung et al., 2003). The highest levels were found in potato crisps, with a range from 1500 to 1700 µg/kg.

6.2.7 Denmark

Denmark submitted the results of occurrence of acrylamide in coffee from a recent evaluation (Grandby & Fagt, 2004). Acrylamide concentrations were obtained from 30 individual food samples of ground and instant coffee purchased from four major supermarkets during 2003, representing the brands consumed by the Danish population. Analyses were performed using the HPLC-MS-MS technique. The limit of detection was estimated to be 2 µg/kg. All the samples have been quantified. Mean concentrations in brewed coffee ranged from 3.8 to 5.2 µg/l (dark roasted coffee), from 7.2 to 8.0 µg/l (instant coffee) and from 9.1 to 10.0 µg/l (medium roasted coffee).

6.2.8 France

France submitted the results of occurrence of acrylamide in foods from a recent evaluation (AFSSA, 2004). Acrylamide concentrations were obtained from 292 individual food samples purchased from a range of commercial shops during 2002 and 2003. Analyses were performed using the LC-MS and LC-MS-MS technique. The limits of the method ranged from 3 to 20 µg/kg and from 10 to 40 µg/kg, respectively, for detection and quantification. Twenty per cent of values were reported below the LOR. Mean concentrations in analysed food groups were as follows: potato chips (786 µg/kg), salted biscuits (390 µg/kg), potato crisps (298 µg/kg), sweet biscuits (227 µg/kg) and breakfast cereals and rolls (133–154 µg/kg).

6.2.9 Germany

Germany submitted the results of occurrence of acrylamide in foods from two recent publications (Mosbach-Schulz et al., 2003; European Union, 2004). Acrylamide concentrations were provided from 2898 individual composite food samples based on an individual food sampling approach where each sample represents from three to nine samples of the same food item purchased from a range of commercial shops from 2002 to 2004, blended to form one single composite sample for analysis. Analyses were mainly performed using the GC-MS and LC-MS-MS techniques. The limits of the method respectively range from 10 to 40 µg/kg and from 20 to 80 µg/kg for detection and quantification. Seven per cent of values were reported below the LOR. Mean concentrations reported in the main analysed food groups were as follows: potato fritters/crisps (634–697 µg/kg), potato snack foods (906 µg/kg), coffee not brewed and substitute (586 µg/kg), bread and rolls (503 µg/kg), pastry and biscuits (397 µg/kg) and chips fried/cooked (238–253 µg/kg).

6.2.10 Japan

Japan submitted the results of occurrence of acrylamide in foods for the sixty-fourth JECFA meeting (Japan Ministry of Agriculture, Forestry and Fisheries, 2004) using the GEMS/Food format. Acrylamide concentrations were obtained from 156 individual food samples based on a composite food sampling approach.

Samples were purchased in 2004 in seven randomly selected supermarkets in six major cities in Japan. Analyses were performed using the GC-MS-MS technique. The limits of the method are, respectively, 5 and 20 µg/kg for detection and quantification. Ten per cent of values were reported below the LOR. Mean concentrations in analysed food groups were potato crisps (1183 µg/kg), green tea ("roasted") (323 µg/kg), cereals and pasta processed (121 µg/kg) and cereal and pasta raw and boiled (23 µg/kg).

6.2.11 The Netherlands

The Netherlands submitted the results of occurrence of acrylamide from recent publications (Konings et al., 2003; European Union, 2004). Acrylamide concentrations were obtained from 337 individual food samples purchased from a range of commercial shops during 2002 on the basis of market shares for the most current brands. Analyses were performed using the GC-MS and LC-MS-MS techniques. The limits of the methods ranged, respectively, from 10 to 30 µg/kg and from 30 to 60 µg/kg for detection and quantification. Forty per cent of values were reported below the LOR. Mean concentrations in analysed food groups were potato crisps (1249 µg/kg), biscuits and pastry (391 µg/kg), french fries (292 µg/kg), toast (183 µg/kg), breakfast cereals (89 µg/kg) and rusks and crackers (92 µg/kg).

6.2.12 Norway

Norway published the results of occurrence of acrylamide in foods from a recent publication (Dybing & Sanner, 2003). Acrylamide concentrations were obtained from 38 individual food samples purchased from a range of commercial shops during 2002. Analyses were performed using the LC-MS-MS technique. No information on reporting limits or mean concentrations in analysed food groups was reported.

6.2.13 Sweden

Sweden submitted the results of occurrence of acrylamide in foods from a recent publication (Svensson et al., 2003). Acrylamide concentrations were obtained from 124 individual food samples purchased from supermarkets in Uppsala during 2002; each sample was analysed individually. Analyses were done using the LC-MS-MS technique. The limits of the method were 15 µg/kg and 30 µg/kg for detection and quantification, respectively. Twenty per cent of values were reported below the LOR. Reported mean concentrations in analysed food groups were as follows: potato crisps (1360 µg/kg), french fries (540 µg/kg), popcorn (500 µg/kg), fried potato/baked products (310 µg/kg), cookies/biscuits (300 µg/kg) and crispbread (300 µg/kg).

Data were also available from Sweden for four breast milk samples taken from pooled archives, one for each of the years 1998–2001. Each of the four samples was a pooled sample from 10 mothers. A further 15 samples collected from individual mothers in 2000–2004 were also analysed. No information on

sampling times or on the food consumption by the mothers was available. One of the 19 samples of milk contained acrylamide at 0.5 µg/kg, which was just above the LOQ. The other 18 samples were below the LOQ (i.e. <0.5 µg/kg).

6.2.14 Switzerland

Switzerland submitted the results of occurrence of acrylamide in foods from a recent publication (Swiss Federal Office of Public Health, 2002). Acrylamide concentrations were obtained from 424 individual food samples purchased from a range of commercial shops during 2002 and 2004. Analyses were done using the GC–high-resolution mass spectrometry (HRMS) technique. The limits of the method ranged from 0.5 to 10 µg/kg and from 1 to 20 µg/kg, respectively, for detection and quantification. Twenty-six per cent of values were reported below the LOR. Mean concentrations in analysed food groups were as follows: potato crisps (503 µg/kg), rösti (450 µg/kg), potato chips (330 µg/kg), crispbread (381 µg/kg) and coffee ready-to-drink (20 µg/l).

6.2.15 Syria

Syria submitted the results of occurrence of acrylamide in foods for the sixty-fourth JECFA meeting (Zayzafoon & Odeh, 2004). Acrylamide concentrations were obtained from 50 food samples based on an individual food sampling approach; samples were purchased in 2004 from a range of all brands of potato chips and biscuits in local supermarkets. Fresh potatoes were also examined before and after frying. Analyses were performed using the HPLC-UV technique. The limits of the analytical method were not given. All the samples have been quantified. Mean concentrations reported in analysed food groups were as follows: fried potatoes (2060 µg/kg) and potato chips (1644 µg/kg).

6.2.16 United Arab Emirates

United Arab Emirates submitted the results of occurrence of acrylamide in foods from a recent report (Madduri & Ragaei, 2004). Acrylamide concentrations were obtained from 116 individual food samples purchased from retail outlets and households in several towns during 2003; each sample was analysed individually. Analyses were performed using the GC-MS technique. The limits of the method were 10 µg/kg and 24 µg/kg for detection and quantification, respectively. Twenty-four per cent of values were reported below the LOR. Reported mean concentrations in analysed food groups were as follows: snack fried foods (e.g. potato chips, potato crisps) (1063 µg/kg), deep fried foods (191 µg/kg) and bakery products (132 µg/kg).

6.2.17 United Kingdom

The United Kingdom submitted occurrence data from European Union (2004) and from the 2003 Total Diet Study (TDS) survey of acrylamide occurrence in food groups (FSA, 2004). Acrylamide concentrations were obtained from a composite group sampling approach where samples are purchased from 3–24 locations

throughout the United Kingdom. Seventy-nine samples were analysed using the GC-MS technique. The limits of the method are, respectively, 10 µg/kg and 20 µg/kg for detection and quantification. Twenty-three per cent of values were reported below the LOR. Reported mean concentrations in analysed TDS food groups were as follows: potatoes (53–112 µg/kg) and miscellaneous cereals (57 µg/kg).

6.2.18 United States

The United States submitted the results of occurrence of acrylamide in foods from the 2002–2004 TDS survey of acrylamide (DiNovi & Howard, 2004; US FDA, 2005). Acrylamide concentrations were obtained from 1337 food composite samples based on an individual food sampling approach; samples were purchased from a range of commercial shops in different locations throughout the United States during 2002 and 2004. Sampling includes analytical results of 938 individual samples where each sample was analysed individually as well as 399 composite samples where each sample represents three samples of the same food item from three different sources being blended to form one single composite sample for analysis. Analyses were performed using the LC-MS-MS technique. The limits of the method were 3 µg/kg and 20 µg/kg for detection and quantification, respectively. Thirty-four per cent of values were reported below the LOR. Reported mean concentrations in analysed foods were as follows: potato chips (548 µg/kg), french fries (322–664 µg/kg), cookies (222 µg/kg), toast (208 µg/kg), breakfast cereals (133 µg/kg), soft bread (44 µg/kg) and brewed coffee (8.5 µg/kg).

6.2.19 Summary of national occurrence data

The range of highest average levels of contamination was found for the following foods: potato crisps ("chips" in USA) (298–1644 µg/kg), potato chips ("french fries" in USA) (152–2060 µg/kg), breads and rolls (183–503 µg/kg) and pastry and biscuits ("cookies" in USA) (132–397 µg/kg).

6.3 International occurrence

The empirical distribution of contamination from individual food items, taking into account the weighting of samples from individuals' composite samples, has been described according to the GEMS/Food categorization (WHO, 2003). The total number of analytical results (single or composite samples) was 6752, corresponding to a total of 25 812 individual food samples purchased. Each individual food sample has been analysed either individually (40% of the total samples) or as a single composite sample (60% of the total samples) representative of 2 of 24 of the same food items collected from different locations being blended for analysis. To obtain the best possible mean and variance from food commodities analysed, the weighting (numbers of food samples being blended into composite) of the composite sample in the calculation has been taken into account. When information was not available (15% of the total sampled), the mean numbers of samples purchased to form the individual composite sample for analysis have

been introduced into the model according to mean values reported by countries at the level of the food commodity.

Most of the samples reported were analysed by LC-MS, GC-MS or LC-MS-MS; the LOD or LOQ was $<30 \mu\text{g/kg}$. In order to take into account the censored data in the calculation of dietary exposure, international recommendations described in the GEMS/Food report have been applied for data below the detection limit (LOD) and below the quantification limit (LOQ) (WHO, 1995). As the percentage of non-quantified values was less than 60% for major contributing foods, the following treatment was used: data below LOD = $\frac{1}{2}$ LOD and data below LOQ = $\frac{1}{2}$ LOQ. This approach concerns 13% of the data from Europe, 31% from North America, 51% from the Pacific region and 32% from Asia.

The summary of the distribution-weighted concentrations of acrylamide found in several food commodities from 2002 to 2004 is presented in Table 8. Each food group has been subdivided into subgroups according to the cooking process. A differentiation has been made between raw, boiled and canned products and processed food (fried, baked, grilled). Highest average levels of contamination were found for the following food commodities: coffee extracts, $1100 \mu\text{g/kg}$; coffee substitutes, $845 \mu\text{g/kg}$; potato crisps ("chips" in USA), $752 \mu\text{g/kg}$; coffee (decaffeinated coffee, not brewed), $668 \mu\text{g/kg}$; breads and rolls, $446 \mu\text{g/kg}$; pastry and biscuits, $350 \mu\text{g/kg}$; potato chips ("french fries" in USA), $334 \mu\text{g/kg}$; green tea ("roasted"), $306 \mu\text{g/kg}$; coffee (ground, instant or roasted, not brewed), $288 \mu\text{g/kg}$; cocoa products, $220 \mu\text{g/kg}$; baby foods (biscuits, rusks, etc.), $181 \mu\text{g/kg}$; potato baked, $169 \mu\text{g/kg}$; fruits processed (dried, fried), $131 \mu\text{g/kg}$; cereals and pasta processed (toasted, fried, grilled), $123 \mu\text{g/kg}$; and dried foods, $121 \mu\text{g/kg}$. Other food commodities have mean levels of approximately $100 \mu\text{g/kg}$ or less.

7. DIETARY INTAKE ASSESSMENT

7.1 Exposure to acrylamide from non-food sources

The CERHR (2004) expert panel on the reproductive and developmental toxicity of acrylamide have reported human exposure data from several non-food routes (i.e. personal care products, cigarette smoking, drinking-water and occupational exposures):

- **Personal care products:** Exposure to acrylamide from dermal contact with cosmetics, consumer products, some gardening products, paper and pulp products, coatings and textiles is possible because the polyacrylamide used in these products may contain some free acrylamide, which is most often estimated to be $<0.01\%$ w/w. The expert panel selected $1.1 \mu\text{g/kg bw per day}$ and $0.5 \mu\text{g/kg bw per day}$ as conservative estimates of upper-bound and mean dermal acrylamide exposures, respectively, from contact with personal care products.
- **Cigarette smoking exposure:** Studies conducted on acrylamide-haemoglobin adduct concentrations in smokers and non-smokers have shown values (median to 95th percentile) ranging from 20 to 70 pmol/g globin in non-smokers

Table 8. Summary of the distribution-weighted concentration of acrylamide in several food commodities, 2002–2004

Commodities	Number of individual samples (single or composite)	Number of weighted individual samples	% < LOR ^a	Mean concentration (µg/kg) ^b	CV (%) ^c	90th percentile (µg/kg)	97.5th percentile (µg/kg)	Reported maximum concentration (µg/kg)
<i>Cereals and cereal-based products^d</i>	3304	12 346	17	343	156	886	1811	7834
- Cereals and pasta, raw and boiled	113	372	72	15	71	30	40	47
- Cereals and pasta, processed (toasted, fried, grilled)	200	634	15.5	123	110	288	450	820
- Cereal-based processed products, all	2991	11 327	15.1	366	151	978	1843	7834
Breads and rolls	1294	5145	14	446	130	1247	2134	3436
Pastry and biscuits (USA = "cookies")	1270	4980	9.8	350	162	798	1691	7834
Breakfast cereals	369	1130	31.4	96	131	213	391	1346
Pizza	58	85	50	33	270	51	269	763
<i>Fish and seafood (including breaded, fried, baked)^d</i>	52	107	56	25	180	93	208	233
<i>Meat and offals (including coated, cooked, fried)^d</i>	138	325	55	19	174	51	128	313
<i>Milk and milk products^d</i>	62	147	89	5.8	119	15	25	36
<i>Nuts and oilseeds^d</i>	81	203	37	84	233	250	477	1925
<i>Pulses^d</i>	44	93	43	51	137	190	210	320

Table 8. (contd)

Commodities	Number of individual samples (single or composite)	Number of weighted individual samples	% < LOR ^a	Mean concentration (µg/kg) ^b	CV (%) ^c	90th percentile (µg/kg)	97.5th percentile (µg/kg)	Reported maximum concentration (µg/kg)
<i>Roots and tubers^d</i>	2068	10 077	6.0	477	108	1069	1915	5312
- Potato puree/mashed/boiled	33	66	82	16	92	35	69	69
- Potato baked	22	99	4.5	169	150	368	1270	1270
- Potato crisps (USA = chips)	874	3555	1.4	752	73	1434	2291	4080
- Potato chips (USA = french fries)	1097	6309	6.4	334	128	729	1500	5312
- Potato chip, croquettes (frozen, not ready-to-serve)	42	48	33	110	145	253	743	750
<i>Stimulants and analogues^{d,e}</i>	469	1455	2.1	509	120	1060	2164	7300
- Coffee (brewed), ready-to-drink	93	101	2.2	13	100	24	44	116
- Coffee (ground, instant or roasted, not brewed)	205	709	0	288	51	472	618	1291
- Coffee extracts	19	119	0	1100	93	2376	4948	4948
- Coffee decaffeinated	26	34	0	668	169	2282	5399	5399
- Coffee substitutes	73	368	0	845	90	2013	2355	7300
- Cocoa products	23	23	0	220	111	672	909	909
- Green tea ("roasted")	29	101	28	306	67	640	660	660
<i>Sugars and honey (mainly chocolate)^d</i>	58	133	47	24	88	90	106	112

Table 8. (contd)

Commodities	Number of individual samples (single or composite)	Number of weighted individual samples	% < LOR ^a	Mean concentration (µg/kg) ^b	CV (%) ^c	90th percentile (µg/kg)	97.5th percentile (µg/kg)	Reported maximum concentration (µg/kg)
<i>Vegetables^d</i>	84	193	63	17	206	57	158	202
- Raw, boiled and canned	45	146	87	4.2	103	6.6	24	25
- Processed (toasted, baked, fried, grilled)	39	47	36	59	109	148	199	202
<i>Fruits, fresh</i>	11	57	9	0.8	188	0.7	7.8	10
<i>Fruit, dried, fried, processed</i>	37	49	43	131	125	355	708	770
<i>Alcoholic beverages (beer, gin, wine)</i>	66	99	61	6.6	143	16	39	46
<i>Condiments and sauces</i>	20	22	75	71	345	123	1168	1168
<i>Infant formula</i>	82	117	100	<5	82	15	15	15
<i>Baby food (canned, in sealed jars)</i>	96	226	21	22	82	46	73	121
<i>Baby food (dry powder)</i>	24	34	33	16	125	57	73	73
<i>Baby food (biscuits, rusks, etc.)</i>	32	58	0	181	106	348	866	1217
<i>Dried food</i>	13	13	31	121	266	127	1184	1184

^a LOR means limit of reporting (detection and quantification limit).

^b Results that were below the reporting limits (LOD or LOQ) have been assumed to be half of those limits.

^c Coefficient of variation (standard deviation divided by the mean, %).

^d According to correspondence with the GEMS/Food commodities group, only the mean concentrations of acrylamide given in bold type were used to estimate international intake.

^e Concentrations for brewed coffee ([concentration of acrylamide in coffee-as-consumed] × 28, to convert concentration in beverage into concentration in coffee powder).

and from 85 to 159 pmol/g in smokers. Based on adduct concentration, estimated median acrylamide intake was 0.85 µg/bw per day in non-smokers, and it was estimated that the value in smokers was about 4 times higher, 3.4 µg/kg bw per day. Cigarette smoke contains acrylamide at 1–2 µg/cigarette. In combination with the estimates of adducts, there are approximately 3–8 pmol/g globin formed per microgram of acrylamide in cigarette smoke. Using concentrations of acrylamide measured in cigarette smoke, the panel estimated mean and upper-bound acrylamide exposures at 0.67 and 1.63 µg/kg bw per day, respectively, in smokers.

- **Drinking-water intake:** Exposure from contamination of drinking-water by industrial releases into domestic water supplies is not a general exposure source because acrylamide is rapidly biodegraded. There is no bioaccumulation in the aquatic food-chain because acrylamide is highly water-soluble and not lipophilic. Drinking-water is commonly treated with polyacrylamide resins to remove suspended particulates. This practice was estimated to produce acrylamide concentrations much lower than 0.5 µg/l. The estimated upper-bound exposure is 0.01 µg/kg bw per day from drinking 2 litres of water per day.
- **Occupational exposures:** Exposure to acrylamide is possible for workers in a wide range of industries that use polyacrylamide: paper and pulp, construction, foundry, oil drilling, textiles, cosmetics, food processing, plastics, mining and agricultural occupations. Workers could be exposed by inhaling dusts or vapours and through dermal contact with monomers and polymers. Researchers or technicians who prepare polyacrylamide gels may also experience variable and intermittent exposures to acrylamide. Based on geometric means of 0.01–0.13 mg/m³ and an upper-bound exposure of 0.3 mg/m³ (permissible exposure level), the expert panel estimated mean and upper-bound workplace (United States and Europe) acrylamide inhalation exposures at 1.4–18.6 µg/kg bw per day and 43 µg/kg bw per day, respectively. Skin exposure and uptake are unknown and difficult to measure.

7.2 *National assessments of intake from diet*

Dietary intakes have been reported for 17 countries (Australia, Belgium, Canada, China, China, Hong Kong SAR, Czech Republic, Denmark, France, Germany, The Netherlands, New Zealand, Norway, Sweden, Switzerland, United Arab Emirates, the United Kingdom and the United States). No dietary intakes were available for Latin America and Africa. In general, countries have calculated national intake using deterministic modelling by combining national individual consumption data with national mean occurrence data obtained from surveys they have conducted in their own country. Three countries (Belgium, The Netherlands and the United States) have conducted intake estimates using Monte Carlo sampling techniques on the full available distribution of occurrence and consumption data.

7.2.1 Australia

Australia submitted the results of the survey of acrylamide in carbohydrate-based foods from a recent publication (Croft et al., 2004). Intake estimates were obtained by a deterministic method using the dietary modelling computer program DIAMOND, combining mean acrylamide concentrations in food and food consumed by each individual reported in the 1995 National Nutrition Survey. Concentration values reported below the reporting limits ($<LOR = 51\%$) have been assigned a value equal to 0 and the LOR. The intake estimates for the whole population older than 2 years of age ranged from 0.4–0.5 $\mu\text{g/kg bw per day}$ (average) to 1.4–1.5 $\mu\text{g/kg bw per day}$ (95th percentile). Children aged 2–6 years have exposures ranging from 1–1.3 $\mu\text{g/kg bw per day}$ (mean) to 3.2–3.5 $\mu\text{g/kg bw per day}$ for the 95th-percentile consumer. Major contributing foods to the total exposure are hot potato chips (25–27%), toast (9–13%), breakfast cereals (7–14%) and potato crisps (6–12%).

7.2.2 Belgium

Belgium submitted the results of the survey from a recent publication (Matthys et al., 2005). An individual food consumption survey carried out in 1997 on 347 adolescents was used. Dietary exposure distributions for acrylamide were generated in a probabilistic way using the Monte Carlo program according to the distribution of food consumption and available distribution of acrylamide levels. The intake estimates for adolescents aged from 13 to 18 years ranged from 0.5–0.6 $\mu\text{g/kg bw per day}$ (median) to 0.9–1.3 $\mu\text{g/kg bw per day}$ (95th percentile). Main food contributors to total exposure for the adolescent population are french fries (30%), bread (11%) and biscuits (11%).

7.2.3 Canada

Canada submitted the results of intakes from a preliminary survey (Vavasour, 2005). Food consumption data were collected from a 24-h dietary recall survey. Dietary exposures to acrylamide were generated in a deterministic fashion using mean concentration levels and food consumption data. Concentration values reported below the reporting limits ($<LOR = 4\%$) have been assigned a concentration equal to 0 and the LOR. The mean intake estimates were approximately 0.4 $\mu\text{g/kg bw per day}$ for adults aged 20–39 years and 1 $\mu\text{g/kg bw per day}$ for the pre-teen and teen population (10–19 years). No information was available on the primary foods contributing to total exposure.

7.2.4 China

China submitted the results of intakes during the JECFA meeting (Chen, 2005). Food consumption data of the Chinese people have been collected in 2002 from the National Nutrition and Health Survey covering 55 768 persons aged 15–70+ years. It covered all the 31 provinces, municipalities and autonomous regions in mainland China. Dietary exposures for acrylamide were generated in a deterministic conservative fashion using mean concentration levels of food commodities

and mean food consumption data at the food group commodities level. Concentrations reported below the reporting limits ($<LOR = 29\%$) have been assigned to a concentration equal to $\frac{1}{2}$ LOR. The mean intake for adult Chinese has been estimated to be $1.1 \mu\text{g/kg bw per day}$. The main food contributors to total exposure are cereal-based foods: rice (40%), roots and tubers (42%) and wheat and wheat products (18%).

7.2.5 China, Hong Kong Special Administrative Region

Hong Kong SAR submitted the results of intake from recent publications (FEHD, 2003; Leung et al., 2003). Food consumption data were collected in 1995 from the Hong Kong adult survey and in 2000 from a secondary school student survey. Dietary exposures of acrylamide were generated in a deterministic fashion using mean concentration levels and food consumption data. Concentrations reported below the reporting limits ($<LOR = 38\%$) have been assigned a concentration equal to $\frac{1}{2}$ LOR. The intake estimates for adults and students are, respectively, $0.3 \mu\text{g/kg bw per day}$ and $0.4 \mu\text{g/kg bw per day}$ for the average consumer. No information was available on the primary food contributors to total exposure.

7.2.6 Czech Republic

Czech Republic published the results of a 2003 survey of acrylamide (National Institute of Public Health, 2003). The intake estimates for the whole population older than 1 year was $0.3 \mu\text{g/kg bw per day}$ for the average consumer. The basis of the intake study is unknown because the publication on the web site was not available in English.

7.2.7 Denmark

Denmark published the results of a survey of acrylamide in coffee from a recent evaluation (Grandby & Fagt, 2004). Intake estimates were performed in a probabilistic fashion using @risk software by combining the distribution of the acrylamide concentration in coffee ready-to-drink by each individual reported in the 1995 Danish nationwide dietary survey of 3098 participants aged from 1 to 80 years. The mean intake estimates from coffee ranged from $6.5 \mu\text{g/day}$ (equivalent to $0.09 \mu\text{g/kg bw per day}$, assuming a mean body weight of 70 kg) for the adult consumer to $18 \mu\text{g/day}$ for the 95th-percentile consumer. Using a coffee consumption comparable with that of the other Scandinavian countries ($0.4\text{--}0.5 \mu\text{g/kg bw per day}$), coffee contributes 20% of the total exposure.

7.2.8 France

France submitted the results of intake of acrylamide from a recent evaluation (AFSSA, 2004). Intake estimates were performed in a deterministic fashion combining mean acrylamide concentrations of food with food consumed by each individual as reported in the national individual food consumption survey completed in 1999 covering 3003 participants aged from 3 to 98 years. Concentrations

reported below the reporting limits ($<LOR = 20\%$) have been assigned a concentration equal to $\frac{1}{2}$ LOR. The intake estimates for the whole population older than 15 years is $0.5 \mu\text{g/kg bw per day}$ for the average consumer and $1.3 \mu\text{g/kg bw per day}$ for the 95th-percentile consumer. Children aged from 3 to 14 years have exposures ranging from 1 to $2.5 \mu\text{g/kg bw per day}$. The main food contributors to total exposure are potato chips (27–28%), bread (12–29%), pastry and sweet biscuits (20%) and rolls (9–11%).

7.2.9 Germany

Germany submitted the results of intakes from two recent publications (Mosbach-Schulz et al., 2003; European Union, 2004). Using a deterministic approach, intakes reported in the Mosbach-Schulz et al. (2003) report are based on the mean results from the acrylamide measurement programme performed by the German food surveillance programme and from the national individual consumption study covering 25 000 participants from 1985 to 1989. Concentrations reported below the reporting limits ($<LOR = 7\%$) have been assigned to a concentration equal to $\frac{1}{2}$ LOR. The intake estimates for the population aged from 4 to 79 years ranged from 0.6 to $1.2 \mu\text{g/kg bw per day}$ for the average consumer to $3.2 \mu\text{g/kg bw per day}$ and $5.1 \mu\text{g/kg bw per day}$, respectively, for the 95th- and 99th-percentile child consumer. No information was available on the main food contributors to total exposure.

7.2.10 The Netherlands

The Netherlands submitted the results of the survey of acrylamide from a recent publication (Konings et al., 2003). The individual food consumption survey, called the National Food Consumption Survey, as carried out in 1998 on 6257 non-institutionalized persons aged 1–97 years, was used. Dietary exposure distributions for acrylamide were generated in a probabilistic fashion using the Monte Carlo Risk Analysis Program (Monte Carlo Risk Analysis Program version 1.2, RIKILT, Wageningen) combining the distribution of food consumption and available distribution of acrylamide levels. Concentrations reported below the reporting limits ($<LOR = 39\%$) have been assigned to a concentration equal to $\frac{1}{2}$ LOR, while samples likely to contain no acrylamide at all were assumed to have acrylamide concentrations equal to 0. The intake estimates for the whole population aged from 1 to 97 years ranged from 0.5 to $1 \mu\text{g/kg bw per day}$ for the average consumer and from 0.6 to $1.1 \mu\text{g/kg bw per day}$ for the 95th-percentile consumer. Main food contributors to total exposure in the adult and children populations are potato crisps (31–46%), potato chips (18–23%), Dutch spiced cake (11–20%), coffee (13%), biscuits (10%) and bread (10%).

7.2.11 New Zealand

New Zealand submitted the results obtained from the individual dietary records approach (Food Standards Australia New Zealand, 2004). The concentration data used for the dietary exposure assessment were derived from the Australian Government Analytical Laboratory's analytical data on the concentration of

acrylamide in a range of Australian carbohydrate-based foods (Croft et al., 2004), together with New Zealand food consumption data derived from the 1997 National Nutrition Survey. Intake estimates were obtained in a deterministic fashion using the dietary modelling computer program DIAMOND, combining mean acrylamide concentrations of food with food consumed by individuals as reported in the National Nutrition Survey. The same concentration data and modelling as done for Australia have been done for New Zealand. The intake estimates for the adult population older than 15 years ranged from 0.4–0.5 µg/kg bw per day for the average consumer to 1.2–1.4 µg/kg bw per day for the 95th-percentile consumer. The main food group contributors to total exposure are hot chips (25%), sweet biscuits (15%), baked potatoes (14%) and toasted bread (10%).

7.2.12 Norway

Norway submitted the results of a survey of acrylamide from a recent publication (Dybing & Sanner, 2003). No information on treatment of censored data was reported. Intake estimates were obtained in a deterministic fashion combining mean acrylamide concentrations of food with food consumed by each individual reported from the national individual food consumption survey NORKOST, performed in 1997 with 2672 participants aged from 16 to 79 years and, for children, the UNGKOST, performed in 2000 with 2957 participants aged 9 years and 3779 aged 13 years. The intake estimate for the adult population is 0.5 µg/kg bw per day for the average and 1.5–1.6 µg/kg bw per day for the 97.5th-percentile consumer. Children aged 9 and 13 years have an exposure range, respectively, from 0.3–0.4 µg/kg bw per day and 0.5 µg/kg bw per day for the average to 1.1–1.5 µg/kg bw per day and 2.1–2.9 µg/kg bw per day for the 97.5th-percentile consumer. The main food group contributors to exposure are coffee (28–29%), potato crisps (17–18%), soft bread (12–13%) and other bread (8–12%).

7.2.13 Sweden

Sweden submitted data on intake of acrylamide from a recent publication (Svensson et al., 2003). Intake estimates were obtained in a deterministic fashion combining mean acrylamide concentration in foods with foods consumed by each individual reported from the Swedish national individual food consumption survey performed in 1997–1998 with 1200 subjects aged from 18 to 74 years. Concentrations reported below the reporting limits (<LOR = 20%) were assigned as being equal to the LOR. The intake estimates for the adult population range from 0.4 µg/kg bw per day for the average consumer to 0.9 µg/kg bw per day for the 95th-percentile consumer. The main food contributors to total exposure are coffee (39%, calculated with two data points of contamination), french fries (16%), bread (11%), fried potato products (not including french fries or potato crisps) (11%) and potato crisps (9%). For infants, dietary intakes were estimated at 0.04 µg/kg bw per day for birth up to 6 months with breastfeeding or infant formula to 0.5 µg/kg bw per day (7–12 months) (Fohgelberg et al., 2005).

7.2.14 Switzerland

Switzerland submitted the results of intake of acrylamide from a recent publication (Swiss Federal Office of Public Health, 2002). Assessment of acrylamide intake was performed by a 2-day duplicate diet study performed in 2002 with 27 subjects aged from 16 to 57 years. Analyses were performed on 72 samples (9 groups \times 4 meals \times 2 days) by a GC-HRMS and LC-MS-MS technique. The mean daily intake for the population surveyed was estimated at 0.5 $\mu\text{g}/\text{kg}$ bw per day. The main food group contributors to exposure are potatoes fried/baked or roasted (35%) and brewed coffee (22%).

7.2.15 United Arab Emirates

United Arab Emirates submitted the results of intake of acrylamide from a recent report (Madduri & Ragae, 2004). Intake estimates were obtained in a deterministic fashion combining mean acrylamide concentrations in food with food consumed as measured by a household survey of 76 families (637 persons) aged from 12 to 65 years and more. Concentrations in samples below the reporting limits ($<\text{LOR} = 24\%$) have been assigned as being equal to 0. The average intake estimate for the adult population (>20 years) ranged from 0.9 to 1.0 $\mu\text{g}/\text{kg}$ bw per day. For adolescents (12–20 years) and children (<12 years), intakes are, respectively, 1.2 $\mu\text{g}/\text{kg}$ bw per day and 2.0 $\mu\text{g}/\text{kg}$ bw per day. The main food contributors to total exposure are potato chips and crisps (44%), pizza (14%), fried grilled potatoes (13%) and pastry and cookies (11%).

7.2.16 United Kingdom

The United Kingdom submitted the results of the 2003 TDS survey intakes of acrylamide (FSA, 2004). Intake estimates were obtained in a deterministic fashion by combining mean acrylamide concentrations in food groups with food groups consumed by each individual reported in the 1995–2002 National Diet and Nutrition Survey. Concentrations reported below the reporting limits ($<\text{LOR} = 23\%$) have been assigned as being equal to 0 and the LOQ, depending on the food group and the knowledge of acrylamide formation in foods. The intake estimate for the adult population (19 to over 65 years) ranged from 0.3–0.4 $\mu\text{g}/\text{kg}$ bw per day in the average consumer to 0.6–0.7 $\mu\text{g}/\text{kg}$ bw per day for the 97.5th-percentile consumer. For young people (4–18 years) and toddlers (1.5–4.5 years), intakes ranged from 0.5–1 $\mu\text{g}/\text{kg}$ bw per day for the average consumer to 0.9–1.8 $\mu\text{g}/\text{kg}$ bw per day for the 97.5th-percentile consumer. No information was available on the main contributing foods to exposure.

7.2.17 United States

The United States submitted the intakes from the 2002–2004 TDS survey of acrylamide (DiNovi & Howard, 2004; US FDA, 2005). The food consumption survey called the Continuing Survey of Food Intakes by Individuals (CSFII) was carried out in 1998 with 20 000 participants aged 2+ years; the Market Research Corporation of America carried out a survey in 1982–1987 with 26 000 participants

aged 2+ years. Both surveys were used. Dietary exposure distributions for acrylamide were generated in a probabilistic way using a Monte Carlo sampling combining the distribution of acrylamide levels and the distribution of food consumption obtained in each survey. Concentrations reported as being below the reporting limits ($< \text{LOR} = 34\%$) have been assigned as being equal to $\frac{1}{2}$ LOR. The intake estimates for the whole population aged 2+ years ranged from 0.4–0.5 $\mu\text{g/kg bw}$ per day for the average consumer to 0.8–0.9 $\mu\text{g/kg bw}$ per day for the 90th-percentile consumer. For children (2–5 years), intakes ranged from 1.0–1.3 $\mu\text{g/kg bw}$ per day for the average consumer to 2.2–2.3 $\mu\text{g/kg bw}$ per day for the 90th-percentile consumer. The main food group contributors to total exposure are french fries (23–30%), breakfast cereals (11–23%), toast (12–13%), cookies (10–13%), potato chips (11–15%), cereals (29%) and starchy vegetables (52%).

7.2.18 Summary of national intake estimates

National dietary intake data for 17 countries were evaluated at this meeting. All regions were represented except Latin America and Africa, where no dietary intakes were available. National intakes were calculated mainly using deterministic modelling by linking national individual consumption data with national mean occurrence data obtained from national surveys, using the actual consumer body weights reported in consumption surveys.

A summary of the results is presented in Table 9, assuming the consumer body weights reported in consumption surveys. Intake estimates at national levels ranged from 0.3 to 2.0 $\mu\text{g/kg bw}$ per day for the average in the general population. Intake estimates ranged from 0.6 to 3.5 $\mu\text{g/kg bw}$ per day for high-percentile (90th–97.5th) consumers, including children, and up to 5.1 $\mu\text{g/kg bw}$ per day for the 99th-percentile consumer. Based on the available data, children had intakes of acrylamide that were about 2–3 times those of adult consumers when expressed on a body weight basis. The Committee noted that these estimates are consistent with the long-term dietary intake assessment performed by the FAO/WHO (2002) consultation, which was based on a limited data set of analytical results representing only a fraction of the diet.

In the absence of a health-based guidance value for acrylamide, the relative contribution of food commodities to the total intake is reported. The relative contribution of each food group may be different between studies depending on the numbers of food categories considered in the intake evaluation.

The major contributing foods to total exposure for most countries were potato chips ("french fries" in North America) (16–30%), potato crisps ("chips" in North America) (6–46%), coffee (13–39%), pastry and sweet biscuits ("cookies" in North America) (10–20%) and bread and rolls/toast (10–30%). Other food items contributed less than 10% of the total exposure.

The Committee concluded that based on national estimates, an intake of 1 $\mu\text{g/kg bw}$ per day of acrylamide could be taken to represent the average for the general population and that an intake of 4 $\mu\text{g/kg bw}$ per day could be taken to

Table 9. Summary of dietary intake assessments for acrylamide in various countries

Country	Population group	Average/50th percentile ($\mu\text{g/kg}$ bw per day)	90th, 95th, 97.5th, 99th percentile ($\mu\text{g/kg}$ bw per day)	Reference	Comments
Australia	Whole population (>2 years)	0.4–0.5	1.4–1.5 (P95)	Croft et al. (2004)	National individual food consumption
	Children (2–6 years)	1.0–1.3	3.2–3.5 (P95)		Deterministic modelling (mean occurrence data, <LOR = 0 and LOR) Major contributing foods: hot potato chips (25–27%), toast (9–13%), breakfast cereals (7–14%), potato crisps (6–12%)
Belgium	Adolescents M (13–18 years)	0.6 (P50)	1.3 (P95)	Matthys et al. (2005)	National individual food consumption
	Adolescents F (13–18 years)	0.5 (P50)	0.9 (P95)		Probabilistic modelling (MCRA, <LOR = $\frac{1}{2}$ LOR) Major contributing foods: french fries (30%), breads (11%) and biscuits (11%)
Canada	Adults (20–39 years)	0.4	–	Vavasour (2005)	National individual food consumption
	Pre-teens and teens (10–19 years)	1.0	–		Deterministic modelling (mean occurrence data, <LOR = 0 and LOR) No information on major contributing foods
China	Adults (15–>70 years)	1.1	–	Chen (2005)	National individual food consumption Deterministic modelling (mean occurrence data, <LOR = $\frac{1}{2}$ LOR) Major contributing foods are roots and tubers (42%), cereal-based rice (40%) and wheat and wheat products (18%)

Table 9. (contd)

Country	Population group	Average/50th percentile ($\mu\text{g/kg}$ bw per day)	90th, 95th, 97.5th, 99th percentile ($\mu\text{g/kg}$ bw per day)	Reference	Comments
China, Hong Kong Special Administrative Region	Adults (>18 years)	0.3	–	FEHD (2003)	National individual food consumption
	School students	0.4	–		Deterministic modelling (mean occurrence data, <LOR = $\frac{1}{2}$ LOR) No information on major contributing foods
Czech Republic	Whole population (>1 year)	0.3	–	National Institute of Public Health (2003)	The basis of the intake study is unknown because the publication on the web site was not available in English No information on major contributing foods
Denmark	Adults (15–80 years)	6.5 $\mu\text{g/day}$ (0.09 ^a)	18 $\mu\text{g/day}$ (P95)	Grandby & Fagt (2004)	National individual food consumption Probabilistic modelling (@risk software) Study implemented to estimate the dietary intake from coffee; contribution was estimated to be 20% of the total exposure found in Scandinavian countries
France	Adults (>15 years)	0.5	1.3 (P95)	AFSSA (2004)	National individual food consumption
	Children (3–14 years)	1.0	2.5 (P95)		Deterministic modelling (mean occurrence data, <LOR = $\frac{1}{2}$ LOR) Major contributing foods: potato chips (27–28%), bread (12–29%), pastry and sweet biscuits (20%), rolls (9–11%)

Table 9. (contd)

Country	Population group	Average/50th percentile ($\mu\text{g/kg}$ bw per day)	90th, 95th, 97.5th, 99th percentile ($\mu\text{g/kg}$ bw per day)	Reference	Comments
Germany	Adults (18–79 years)	0.6		Mosbach-Schulz et al. (2003)	National individual food consumption
	Children (15–18 years)	0.9	3.2–5.1 (P99)		Deterministic modelling (mean occurrence data)
	Children (4–6 years)	1.2			No information on major contributing foods
	Adolescents (19–24 years)	0.7			
The Netherlands	Whole population (1–97 years)	0.5	0.6 (P95)	Konings et al. (2003)	National individual food consumption
	Children (1–6 years)	1.0	1.1 (P95)		Probabilistic method (MCRA-RIKILT, <LOR = $\frac{1}{2}$ LOR)
	Children (7–18 years)	0.7	0.9 (P95)		Major contributing foods: potato crisps (31–46%), potato chips (18–23%), Dutch spiced cake (11–20%), coffee (13%), biscuits (10%) and bread (10%)
New Zealand	Whole population (>15 years)	0.3–0.5		Food Standards Australia New Zealand (2004)	National individual food consumption
	Whole population, consumers only (>15 years)	0.4–0.5	1.2–1.4 (P95)		Deterministic modelling (Australian mean occurrence data, <LOR = 0 and LOR) Major contributing foods: hot chips (25%), sweet biscuits (15%), baked potatoes (14%) and toasted bread (10%)

Table 9. (contd)

Country	Population group	Average/50th percentile ($\mu\text{g/kg}$ bw per day)	90th, 95th, 97.5th, 99th percentile ($\mu\text{g/kg}$ bw per day)	Reference	Comments
Norway	Adults M (16–79 years)	0.5	1.5 (P97.5)	Dybing & Sanner (2003)	National individual food consumption
	Adults F (16–79 years)	0.5	1.6 (P97.5)		Deterministic modelling (mean occurrence data)
	Children (9 years and 13 years)	0.3–0.5 ^a	1.1–2.9 (P95)		Major contributing foods: coffee (28–29%), potato crisps (17–18%), soft bread (12–13%) and other bread (8–12%)
Sweden	Whole population (18–74 years)	0.4	0.9 (P95)	Svensson et al. (2003)	National individual food consumption
	Infants (birth up to 6 months)	0.04		Fohgelberg et al. (2005)	Deterministic modelling (mean occurrence data, <LOR = LOR)
	Infants (7–12 months)	0.5			Major contributing foods: coffee (39%, calculated with two occurrence data), french fries (16%), bread (11%), fried potato products (11%) and potato crisps (9%)
Switzerland	Adults (16–57 years)	0.5	–	Swiss Federal Office of Public Health (2002)	Duplicate-diet study Major identified contributing foods: potato fried/baked or roasted (35%) and coffee (22%)
United Arab Emirates	Adults (>20 years)	0.9–1.0	–	Madduri & Ragaee (2004)	National individual food consumption
	Adolescents (12–20 years)	1.2	–		Deterministic modelling (mean occurrence data)

Table 9. (contd)

Country	Population group	Average/50th percentile (µg/kg bw per day)	90th, 95th, 97.5th, 99th percentile (µg/kg bw per day)	Reference	Comments
United Arab Emirates (contd)	Children (<12 years)	2.0	–		Main contributing foods are potato chips and crisps (44%), pizza (14%), fried grilled potatoes (13%) and pastry and cookies (11%)
United Kingdom	Adults (19–>65 years)	0.3–0.4	0.6–0.7 (P97.5)	FSA (2004)	National food consumption survey
	Young people (4–18 years)	0.5–1	0.9–1.6 (P97.5)		Deterministic modelling (mean occurrence data, <LOQ = 0 and LOQ, depending on food group)
	Toddlers (1.5–4.5 years)	1	1.8 (P97.5)		No information on major contributing foods
United States	Whole population (2+ years)	0.4–0.5	0.8–0.9 (P90)	DiNovi & Howard (2004)	National individual food consumption
	Children (2–5 years)	1.0–1.3	2.2–2.3 (P90)		Probabilistic modelling (Monte Carlo sampling) Major contributing foods: french fries (23–30%), breakfast cereals (11–23%), toast (12–13%), cookies (10–13%), potato chips (11–15%)

F, female; LOR, limit of reporting; M, male; MCRA, Monte Carlo Risk Analysis; P50, 50th percentile; P90, 90th percentile; P95, 95th percentile; P97.5, 97.5th percentile; P99, 99th percentile

^a Assuming a mean body weight of 70 kg.

represent high consumers. In these intake estimates for average to high intake, children are also included.

7.3 Regional estimates of intake from GEMS/Food diet

As acrylamide occurs in every part of the world, data on food consumption obtained from the GEMS/Food regional diets and data on food contamination collected from countries around the world are considered the most relevant for risk assessment. In general, the food items analysed were well characterized, and it was possible to combine them with the GEMS/Food classification. In total, 6372 values were included (94.4%). For certain other food items, analytical data were not used because food items for which residue data were submitted did not directly match the GEMS/Food categorization. This category included 380 specific individual food items (5.6%) having a lower mean level of acrylamide and regrouped in the following food groups: fruits (48 values), dried foods (13 values), alcoholic beverages (66 values), baby and infant food (234 values) and condiments and sauces (19 values).

Finally, to take into account the food cooking process, which is important for acrylamide occurrence, and to avoid as much as possible a source of uncertainty in the resulting exposure estimates, matching of acrylamide residue data to GEMS/Food consumption data was conducted according to the following criteria:

- To be in concurrence with the GEMS/Food regional diet unit for the consumption of coffee (in grams per person per day) and according to Stegen et al. (1997), a multiplicative factor for brewed coffee (coffee as consumed multiplied by 28 to convert contamination in beverage into concentration on a coffee roasted or ground basis) has been applied in order to express the mean acrylamide concentration for the stimulant and analogue group in the units of the GEMS/Food regional diet.

As it was difficult to increase precision in the food group consumption for certain GEMS/Food sub-items, in order to get the best correspondence between contamination and consumption, a more conservative approach consisting of reporting the total food consumption of the five GEMS/Food regional diets was chosen, even if occurrence data reported for certain sub-items were not available. International estimates of intake were prepared by combining the international weighted means of contamination levels (Table 8) with the food consumption values reported in the GEMS/Food database (WHO, 2003) for each of the five GEMS/Food regional diets (Middle Eastern, Far Eastern, African, Latin American and European). Each weighted mean concentration was multiplied by the total mean consumption of the corresponding food category reported to derive mean total intakes of acrylamide per regional diet. For this reason, major contributing foods to total exposure are expressed here as food group commodities (e.g. roots and tubers).

The Committee noted that these estimates are conservative, as the foods considered are raw commodities, while the acrylamide levels are for specifically processed foods (e.g. the intake of all raw potatoes is being combined with

contaminant levels taken from fried or baked potato products). Additionally, in regions with few or no acrylamide concentration data, the use of this broad assumption may result in a mismatch between the foods considered and the acrylamide concentration data employed (e.g. cassava consumption combined with acrylamide levels from processed potato products). Based on only two submitted results, cassava chips seem to have a lower mean concentration than the processed potato product (45 µg/kg vs 170 µg/kg).

The summary of the results is presented in Table 10. The range for the international mean intakes was estimated to be 3.0 µg/kg bw per day up to 4.3 µg/kg bw per day for the five GEMS/Food regional diets, assuming a body weight of 60 kg. Cereals and roots and tubers are the main contributors to the total exposure calculations for each regional diet. Intakes from cereals are about 1.3–2.6 µg/kg bw per day. Intakes from roots and tubers are about 0.5–2.6 µg/kg bw per day. Other GEMS/Food groups contribute less than 5% to the total exposure calculations.

8. PREVENTION AND CONTROL

Based on the growing knowledge on formation mechanisms and factors, various strategies for lowering the levels of acrylamide in food products as well as in catered and home-cooked foods can be anticipated. These might aim at lowering the amount of precursors, chemical and physical interference with formation and elimination reactions, and removing highly contaminated items or other actions on the final products. Mitigation actions may be introduced at various stages, from plant breeding and cultivation to the final product and its consumption, as exemplified by the following points:

- Raw materials: Selection and development of varieties, optimized cultivation and storage conditions, etc.
- Recipe and additives: Proteins or amino acids, pH-lowering compounds, etc.
- Pretreatment and process conditions: Washing, soaking or blanching, fermentation or enzyme treatment, pre/post-drying, thermal input and profile, etc.

Several experiments on food models, in particular potato-based, are reviewed in section 8.2 below. Significant reductions in acrylamide levels, up to 99%, have been reported in these studies. However, it should be emphasized that the feasibility of adapting these methods to large-scale food processing has not been fully studied. Furthermore, any major changes would need to be checked for consumer acceptability, nutritional quality and the possible increased formation of other undesirable substances. Negative effects on nutritional quality could be, for example, a higher fat content that might follow from frying at lower temperatures or a lower content of dietary fibre in bread and cereal products if a decreased asparagine content in bread and cereal products were to be achieved by using less whole grain flour. A lower content of reducing sugars could be accomplished by storage of potato tubers at higher temperature, but this might, on the other hand, require an increased use of chemicals that inhibit sprouting.

Table 10. Summary of dietary intake assessments for acrylamide according to commodities evaluated from GEMS/Food regional diets^a

GEMS/Food commodity	Middle Eastern regional diet			Far Eastern regional diet			African regional diet			Latin American regional diet			European regional diet		
	mean consumption (g/person per day)	mean intake (µg/kg bw per day)	% mean contribution	mean consumption (g/person per day)	mean intake (µg/kg bw per day)	% mean contribution	mean consumption (g/person per day)	mean intake (µg/kg bw per day)	% mean contribution	mean consumption (g/person per day)	mean intake (µg/kg bw per day)	% mean contribution	mean consumption (g/person per day)	mean intake (µg/kg bw per day)	% mean contribution
Cereals	429.9	2.46	77.0	450.6	2.58	70.8	291.7	1.67	38.3	254.4	1.45	48.3	221.9	1.27	34.9
Fish and seafood	13.2	0.01	0.2	31.5	0.01	0.4	36.5	0.02	0.4	46.7	0.02	0.6	46.8	0.02	0.5
Meat and offals	43.3	0.01	0.4	47.0	0.01	0.4	30.4	0.01	0.2	78.0	0.02	0.8	217.3	0.07	1.9
Milk and milk products	132.4	0.01	0.4	32.7	0.00	0.1	42.2	0.00	0.1	167.9	0.02	0.5	336.1	0.03	0.9
Nuts and oilseeds	12.8	0.02	0.6	50.0	0.07	1.9	34.2	0.05	1.1	57.5	0.08	2.7	29.9	0.04	1.2
Pulses	21.2	0.02	0.6	14.5	0.01	0.3	17.6	0.01	0.3	20.6	0.02	0.6	9.4	0.01	0.2
Root and tubers	61.8	0.49	15.4	108.5	0.86	23.7	321.3	2.55	58.6	159.3	1.27	42.1	242.0	1.92	53.0
Stimulants and analogue ^b	8.2	0.07	2.2	1.7	0.01	0.4	0.6	0.01	0.1	5.5	0.05	1.6	14.4	0.12	3.4
Sugars and honey	95.8	0.04	1.2	50.5	0.02	0.6	42.7	0.02	0.4	104.3	0.04	1.4	107.3	0.04	1.2
Vegetables	233	0.07	2.1	178.9	0.05	1.4	77.0	0.02	0.5	150.4	0.04	1.4	371.6	0.11	2.9
Total		3.2			3.6			4.3			3.0			3.6	

From WHO (2003)

^a Mean body weight = 60 kg.^b According to Stegen et al. (1997), a multiplicative conversion factor of 28 (1 g for 30 ml) has been applied for brewed coffee to convert contamination data into ground and roasted coffee.

8.1 Mitigation achievements

The Confederation of Food and Drink Industries of the European Union (CIAA) presented a review on the mitigation achievements made in real food production up to December 2004 (Ashby et al., 2004). An average reduction in acrylamide levels by 30–40% in potato crisps was stated to have been achieved by introducing several adjustments to the existing production procedures — e.g. optimized patterns for thermal input and a 0.5% increase in product moisture. The origin of data used for these calculations was not clearly specified, and it is not known to what extent food producers have implemented such measures. Significant reduction was also reported from process optimization for non-fermented crispbread, while little progress has been obtained so far in reducing levels of acrylamide in various other important intake sources (e.g. roasted coffee and cooked cereals).

It should be noted that long-term overall effects of various prevention and control measures are very difficult to evaluate. This is due to the very high variation of acrylamide levels between different items of some foods. For example, significant fluctuations in the acrylamide levels in potato products can appear over a season or between different seasons, due to varying sugar levels in potatoes caused by agricultural and storage factors.

8.2 Mitigation experiments in food models

8.2.1 Lowering the amount of precursors

Variety selection and plant breeding as well as optimized growth and storage conditions might be used to lower the amounts of critical acrylamide precursors — e.g. reducing sugars in potatoes and asparagine in cereals. Cold storage of potato tubers is well known to significantly increase the sugar levels. Lower acrylamide levels have been demonstrated in fried potatoes previously stored at 8 °C or higher compared with 4 °C (Noti et al., 2003; De Wilde et al., 2004; Matthäus et al., 2004). French fries made from frozen fresh prefabricates were for the same reason lower in acrylamide than prefabricates stored at 4 °C (Fiselier et al., 2004).

Measures to control sugar levels in raw potatoes have been in place in the food industry for quite a long time in order to avoid excessive browning of the products. The largest potential for improvements is therefore likely to be with household and restaurant potatoes.

Soaking or blanching of potato slices prior to frying removed sugars and asparagine to varying degrees, depending on the duration and temperature of the treatment, and lowered the acrylamide levels in the product (Haase et al., 2003; Kita et al., 2004; Pedreschi et al., 2004). Cold water soaking for 1–90 min gave an acrylamide reduction of 10–40% in potato crisp models. Higher temperatures were required for stronger effect. The extraction of asparagine and sugars was significantly enhanced by using solutions of sodium hydroxide, acetic acid and citric acid (Kita et al., 2004). Soaking also reduced the acrylamide levels in french fries, while blanching at 80 °C or higher increased the levels in some cases, possibly due to increased mobility of precursors from inner tissues (Grob et al., 2003).

The enzyme asparaginase converts asparagine to aspartic acid and ammonia. Application of asparaginase to cracker products reduced the acrylamide levels by at least 70% (Vass et al., 2004). Pretreatment of potato tissue with asparaginase reduced the levels of asparagine and acrylamide in a model snack product by 88% and 99%, respectively (Zyzak et al., 2003). Similar effects have been demonstrated in model experiments with flour from wheat, rye and potato (Weisshaar, 2004).

Yeast fermentation resulted in reduced levels of asparagine and acrylamide in a realistic bread-making model. Prolonged fermentation times (180 min + 180 min) resulted in reduced acrylamide levels by 87% and 77% in breads with whole grain wheat and rye bran, respectively (Fredriksson et al., 2004).

8.2.2 Chemical interference with formation or elimination

By lowering the pH in corn chips or french fries through the addition of citric acid prior to baking, acrylamide levels were reduced by up to 80% (Jung et al., 2003). Later studies on various potato products demonstrated more modest effects (Gama-Baumgartner et al., 2004; Kita et al., 2004; Pedreschi et al., 2004). Significantly decreased levels in potato crisps and french fries, pretreated by soaking in solutions of citric or acetic acid, could be attributed mainly to the extraction of precursors from the potato tissue (Kita et al., 2004). Addition of citric acid to the dough significantly lowered the acrylamide content of gingerbread (Amrein et al., 2004).

Replacing ammonium bicarbonate by sodium bicarbonate as a baking agent and replacing reducing sugars by sucrose both reduced the acrylamide content in gingerbread (Amrein et al., 2004) and crackers (Vass et al., 2004).

Addition of various amino acids (e.g. glycine) was shown to reduce acrylamide formation in a potato model (Rydberg et al., 2003) and in gingerbread (Amrein et al., 2004).

8.2.3 Optimized time/temperature regimen

Several authors have suggested that frying potato crisps (Haase et al., 2003; Kita et al., 2004; Pedreschi et al., 2004) and french fries (Gertz et al., 2003; Grob et al., 2003; Matthäus et al., 2004; Taeymans et al., 2004) at low temperature (e.g. 170 °C) for long times gives up to 50% less acrylamide compared with high temperature (e.g. 190 °C), when the frying times were adapted to produce comparable products. By contrast, lower temperature/longer time increased acrylamide levels in gingerbread (Amrein et al., 2004).

Reduced end temperature for baking was reported to lower the acrylamide content in crackers (Vass et al., 2004).

8.2.4 Drying

The moisture content of potato crisps should be approximately 2% to obtain a desirable texture and sufficiently long shelf life. Application of a drying step at

100 °C after frying enabled the temperature/time to be reduced, thereby lowering the acrylamide content by 70–80% in an experimental preparation of potato crisps (Kita et al., 2004). A similar approach was effective with french fries (Sell et al., 2004).

Low-temperature vacuum frying reduced acrylamide levels in potato crisps (Granada et al., 2004).

9. DOSE-RESPONSE ANALYSIS AND ESTIMATION OF CARCINOGENIC/TOXIC RISK

9.1 Contribution of above data to assessment of risk

9.1.1 Pivotal data from biochemical and toxicological studies

(a) Metabolism and activation

The chemistry and metabolism of acrylamide have been the subject of a recent review (Friedman, 2003). Chemically reactive towards nucleophiles, including amino and thiol groups in amino acids and proteins, acrylamide acts by Michael addition to the carbon–carbon double bond. Acrylamide is, however, less reactive than many other vinyl monomers, including acrylonitrile. Acrylamide reacts with the *N*-terminal valine residue in haemoglobin; this adduct has proved a useful biomarker of exposure to acrylamide in experimental animals and in humans (Bergmark et al., 1993; Tareke et al., 2000).

Acrylamide is metabolized *in vivo* to its chemically reactive epoxide, glycidamide. This reaction is mediated exclusively by CYP2E1 in mice. The reaction competes with direct conjugation of acrylamide with glutathione (Calleman et al., 1990; Sumner et al., 1992, 1999; Rice, 2005). Glycidamide has been reported to be 100–1000 times more reactive with DNA than acrylamide (Segerbäck et al., 1995). Glycidamide adducts with purine bases of DNA have been described, and these have been found in DNA of liver, lung and kidney of mice treated with acrylamide (Segerbäck et al., 1995; Gamboa da Costa et al., 2003). In adult mice dosed with glycidamide, glycidamide–DNA purine adduct levels were somewhat higher than in mice that received acrylamide. Treatment of neonatal mice with glycidamide yielded 5- to 7-fold higher whole-body DNA adduct levels than treatment with acrylamide, which was consistent with lower P450 levels in immature tissues (Gamboa da Costa et al., 2003). In adult mice treated with acrylamide, DNA adduct formation showed a supralinear dose–response relationship; this result is consistent with saturation of oxidative biotransformation of acrylamide at higher doses (Gamboa da Costa et al., 2003; Rice, 2005). The ratio of glycidamide adducts to acrylamide adducts in haemoglobin from animals dosed with acrylamide was higher in mice than in rats (Paulsson et al., 2002). This reflects less efficient conversion of acrylamide to glycidamide in rats (Bergmark et al., 1991). However, at lower oral doses of acrylamide, similar levels of glycidamide–DNA adducts were measured in rat and mouse liver (Doerge et al., 2005b).

(b) *Neurotoxicity*

The nervous system is a principal site of the toxic actions of acrylamide. Existing knowledge of the nature of acrylamide neurotoxicity comes from numerous experiments conducted in a range of animal models, as well as epidemiological accounts of human industrial and accidental exposures. A number of reviews have been published on the subject of acrylamide neurotoxicity (Spencer & Schaumburg, 1974a; Tilson, 1981; Hattis & Shapiro, 1990; Gold & Schaumburg, 2000; LoPachin et al., 2003; Tyl & Friedman, 2003; LoPachin, 2004). The cumulative evidence described in these reviews indicates that sufficient, repeated exposure to acrylamide by any route (dermal, oral, intraperitoneal, etc.) eventually results in peripheral neuropathy. Crofton et al. (1996) suggested that the observed neurotoxicity after acrylamide exposure results from an accumulation of toxic damage from repeated exposures, since acrylamide has not been shown to accumulate at the sites of toxicity.

Often the neurobehavioural deficits associated with acrylamide peripheral neuropathy (hindlimb weakness, foot splay and gait abnormalities) occur relatively early during exposure in rats and in the absence of detectable axonal degeneration (LoPachin et al., 2000). Acrylamide-induced nerve terminal degeneration in the cerebellum may contribute to these characteristic gait abnormalities (Lehning et al., 2002). Nerve terminal damage in the brain stem and spinal cord may develop prior to axonopathy and the appearance of significant gait disturbances. These and observations in the forebrain — where there is a clear absence of axonal degeneration in the presence of significant terminal degeneration — led to the suggestion that nerve terminals, rather than axons, may be the primary site of acrylamide intoxication (Lehning et al., 2002; LoPachin et al., 2002). These studies also demonstrated that with continued dosing, terminal degeneration emerged in brain areas critical for learning, memory and other cognitive functions (i.e. cerebral cortex, thalamus and hippocampus). Subsequent studies led to the hypothesis that acrylamide reacts with target-specific thiol-containing presynaptic proteins (e.g. synaptosomal-associated protein of 25 kDa; *N*-ethylmaleimide-sensitive factor) to disrupt normal neurotransmission and pre-synaptic membrane turnover (LoPachin et al., 2002, 2003). Others have suggested that inhibition of fast, bidirectional axonal transport by acrylamide might serve to cause or contribute to the noted terminal degeneration (Sickles et al., 2002). Regardless of mechanism, degeneration of nerve terminals appears to precede the observation of axonopathy originally reported by Spencer and colleagues (Spencer & Schaumburg, 1975, 1977), and it is now thought that the axonopathy is secondary to nerve terminal degeneration.

The lowest effect levels that are associated with degenerative peripheral nerve changes (light microscopy) in rats exposed to acrylamide in drinking-water for 90 days are 5 mg/kg bw per day (Burek et al., 1980) and, for 2 years, 2 mg/kg bw per day (Johnson et al., 1986) and 2–3 mg/kg bw per day (Friedman et al., 1995). Acrylamide-induced neurological impairment has been observed in other species and in mice exposed via parenteral administration or oral exposure at higher dose levels.

(c) *Reproductive and developmental toxicity*

Oral studies in mice, in which acrylamide was given via the drinking-water, have shown clear effects on male reproductive capacity at doses of around 7 or 15 mg/kg bw per day. Sperm count, sperm morphology, male fertility and intrauterine survival of embryos were adversely affected. The effects were considered to be dominant lethal effects mediated by a mutagenic mechanism.

Acrylamide does not appear to have any effect on female reproductive capacity in the mouse at doses up to around 7 or 15 mg/kg bw per day.

In male rats, similar effects, including dominant lethality, lower fertility and lower sperm count, have been seen at doses of about 7 and 12 mg/kg bw per day when acrylamide is given in the drinking-water or at oral gavage doses at or above 15 mg/kg bw per day. In addition, adverse effects on copulatory behaviour have been found, which may be secondary to acrylamide neurotoxicity and may account for some of the impact on male reproductive success. A NOEL for neurotoxicity, copulatory and reproductive effects of about 5 mg/kg bw per day has been identified for acrylamide given via the drinking-water or via oral gavage.

When female rats were given acrylamide via the drinking-water, there were no effects on reproductive capacity. Slight developmental toxicity was evident from reductions in birth weight and subsequent pup body weight; while this could have been secondary to maternal toxicity (including neurotoxicity), a direct effect of acrylamide on the pups via the milk could not be ruled out. The lowest-observed-adverse-effect level (LOAEL) for this effect was about 2.5–10 mg/kg bw per day.

In one study, the effects of treatment on both male and female rats were investigated by giving acrylamide via the drinking-water over two generations. Reductions in offspring numbers and body weight were seen at 5 mg/kg bw per day, with a NOEL of 2 mg/kg bw per day for dominant lethal and developmental effects. Signs of neurotoxicity but no effects on reproduction were seen at 0.5 mg/kg bw per day, the lowest dose tested.

In developmental toxicity studies in which acrylamide was given by oral gavage to rats and mice, no teratogenicity was seen. Fetotoxic effects (reduced fetal weight and increase in rib variants) were observed at a maternally toxic dose of 45 mg/kg bw per day in mice. A NOEL of 15 mg/kg bw per day for developmental toxicity was identified for both mice and rats.

In developmental neurotoxicity studies in rats, transient effects on dopamine receptors were observed in 2-week-old pups in a study using an oral gavage dose of 20 mg/kg bw per day during organogenesis and possible effects on biogenic amines in developing and adult brain following oral gavage doses of 25 mg/kg bw per day during the suckling period. Others showed that this dosing regimen caused progressive and severe neurotoxicity in adult females, suggesting that effects on pups, including mortality and reduced body weight gain, could have been secondary to the maternal toxicity. In a comprehensive developmental neurotoxicity study in rats, in which acrylamide was given by oral gavage from GD 6 to LD 10, the NOEL for maternal toxicity was 5 mg/kg bw per day and the NOEL

for maternal neurotoxicity was 10 mg/kg bw per day. The overall NOEL for developmental toxicity was ≤ 5 mg/kg bw per day, based on effects on transient female pup body weight at 5 mg/kg bw per day, and the NOAEL for developmental neurotoxicity was 10 mg/kg bw per day. This indicates that acrylamide is not a selective developmental neurotoxicant.

The overall oral NOEL for reproductive and developmental effects taken from these studies is 2 mg/kg bw per day.

(d) *Mutagenicity and clastogenicity*

Acrylamide is not mutagenic in the *Salmonella* mutagenicity assay, either without or with an exogenous metabolic activation system (Hashimoto & Tanii, 1985). However, abundant evidence has demonstrated that both acrylamide and glycidamide are mutagenic and clastogenic in mammalian cells. Acrylamide induces gene mutations and chromosomal aberrations in germ cells of mice and chromosomal aberrations in germ cells of mice in vivo, induces chromosomal aberrations in somatic cells of rodents in vivo and induces gene mutations and chromosomal aberrations in cultured cells in vitro (IARC, 1994b; Dearfield et al., 1995).

A recent study (Besaratina & Pfeifer, 2004), discussed in more detail above, compared the genotoxic effects of acrylamide and glycidamide in mouse and human cells in vitro by using DNA sequencing to identify mutated sites within specific genes. Glycidamide was more mutagenic than acrylamide at any given dose. The mutagenicity of acrylamide in human and mouse cells was shown to be based on the capacity of its metabolite glycidamide to form promutagenic DNA adducts. These results are consistent with the profile of guanine and adenine base adducts previously identified (Gamboa da Costa et al., 2003) and with observations of acrylamide and glycidamide mutagenicity in transgenic mice (Von Tungeln et al., 2005; Manjanatha et al., in press).

The formation of micronuclei in mouse and rat bone marrow cells by acrylamide demonstrated the clastogenicity of acrylamide in vivo (Paulsson et al., 2002). Rats appear to be less sensitive than mice to the clastogenic effects of acrylamide, which are mediated by metabolism to glycidamide. This conclusion was supported by the following: acrylamide induced dose-dependent increases in acrylamide- and glycidamide-haemoglobin adducts in both mice and rats; although acrylamide-haemoglobin adducts were comparable in the two species, glycidamide-haemoglobin adduct levels were approximately 4-fold lower in rats; and micronucleus formation in peripheral erythrocytes was observed in mice, but not in rats.

In contrast, glycidamide given intraperitoneally to mice and rats induced a linear dose-dependent increase in haemoglobin adduct levels in both species; rats showed 30% higher levels of haemoglobin adducts per administered amount of glycidamide than mice (Paulsson et al., 2003a). At the lower doses administered, a small but significant increase in micronuclei frequency was observed in rats. Glycidamide seemed equally potent whether injected as preformed material or

generated *in vivo* by metabolism. Thus, glycidamide appears to be the predominant clastogenic agent in rodents exposed to acrylamide (Paulsson et al., 2002, 2003a; Rice, 2005).

(e) Carcinogenicity

Acrylamide has been tested for carcinogenicity in two 2-year drinking-water studies in Fischer 344 rats (Johnson et al., 1986; Friedman et al., 1995). Positive results in these bioassays establish that acrylamide is a multiorgan carcinogen in rats. These studies, including tumour incidences, have been discussed in detail above in section 2.2.3. In the first bioassay (Johnson et al., 1986; Rice, 2005), acrylamide increased the incidence of follicular adenomas of the thyroid, peritesticular mesotheliomas and adrenal gland pheochromocytomas in males and of thyroid follicular tumours, mammary tumours, glial tumours of the central nervous system, oral cavity papillomas, uterine adenocarcinomas, pituitary adenomas and clitoral gland adenomas in females. In the second bioassay (Friedman et al., 1995; Rice, 2005), acrylamide increased the incidences of peritesticular mesotheliomas in males, thyroid follicular cell tumours in both sexes and mammary gland tumours in females and would have been interpreted as increasing the incidence of primary tumours of the central nervous system if all such tumours identified in treated rats had been included in the data analysis. Primary brain tumours were considered under-reported by a recent review (Rice, 2005).

Acrylamide has been shown in short-term screening bioassays when given either orally or intraperitoneally to increase the incidence, number and multiplicity of lung tumours in strain A mice. Further evidence for the carcinogenicity of acrylamide in mice comes from mouse skin tumour initiation–promotion assays. These findings are consistent with a genotoxic mode of action and are consistent with the previously discussed positive findings for acrylamide and glycidamide genotoxicity (Bull et al., 1984a, 1984b; Rice, 2005).

Acrylamide has been hypothesized to induce tumours in hormonally sensitive tissue by dopamine agonist activities that promote age-related hormonal changes, which may then promote sustained cell proliferation in the tunica vaginalis and mammary gland, progressing eventually to mesothelioma and fibroadenomas, respectively. Similarly, acrylamide has been hypothesized to alter a signal transduction pathway to persistently stimulate cell proliferation in thyroid follicular cells, eventually progressing to follicular cell adenomas. A genotoxic mechanism in combination with a hormonal mechanism for the carcinogenicity of acrylamide has been discussed by the report of the Institutet for Miljomedicin (1998) and the European Commission (2002a).

The patterns of tumour sites in rats and mice that result from exposure to acrylamide resemble the distributions of target sites in each species for carcinogenesis by a number of known potent genotoxic carcinogens (Rice, 2005). As has been pointed out by Rice & Wilbourn (2000), the occurrence of tumours of thyroid gland, mammary gland, etc., in a bioassay does not necessarily imply that hormonal dysregulation is the mode of carcinogenesis of the test agent.

9.1.2 Pivotal data from human clinical/epidemiological studies

Studies of manufacturing workers exposed to acrylamide are the only studies that have been analytically designed. These studies did not anticipate exposure to dietary acrylamide. Exposure to acrylamide was not associated with any statistically significant dose-related increase for cancer risk at any organ site, except that a statistically significant doubling of risk for pancreatic cancer was found for workers with the highest cumulative exposure (based on only nine cases).

In the case of dietary exposure to acrylamide and increased risk for cancer, the only studies available are case-control studies originally designed to evaluate the possible contribution of dietary factors other than acrylamide to human cancer risk (Rice, 2005). These studies have found no increased cancer risk attributable to acrylamide intake, but they would only have been able to detect approximately a doubling of risk across the exposure categories from low to high.

9.2 General modelling considerations

The Committee analysed cancer dose-response data by dose-response modelling, in accordance with the International Programme on Chemical Safety document *Principles for modelling dose-response for the risk assessment of chemicals* (IPCS, 2005). The statistical methods of dose-response modelling as applied at this meeting are briefly described below. For each tumour end-point considered relevant, the quantal dose-response models shown in Table 11 were fitted to the dose-incidence data.

Table 11. Dose-response models used

Model	Model equation ^a	Constraints
One-stage	$R = a + (1-a) (1-\exp(-x/b))$	$0 \leq a \leq 1$,
Two-stage	$R = a + (1-a) (1-\exp(-(x/b)-c(x/b)^2))$	$0 \leq a \leq 1$
Log-logistic	$R = a + (1-a) / (1 + \exp(c \log_{10}(b/x)))$	$0 \leq a \leq 1$, $c \geq \ln(10)$
Log-probit	$R = a + (1-a) \Phi(c \log_{10}(x/b))$	$0 \leq a \leq 1$
Weibull	$R = a + (1-a) (1-\exp(-(x/b)^c))$	$0 \leq a \leq 1$, $c > 1$
Proast M2	$y = \exp(bx)$, th1	
Proast M3	$y = \exp(b x^d)$, th1	$d \geq 1$
Proast M4	$y = c - (c-1)\exp(-bx)$, th1	

^a Φ denotes the (cumulative) standard normal distribution function.

The first five of these models directly relate the incidence (R , expressed as a fraction) to the dose (x). In these models, the parameter a (also expressed as a fraction) reflects the incidence in the controls, the parameter b denotes the slope and parameter c can be considered as a shape parameter. The last three models

(Proast M2–M4) are a specific family of models that assume an underlying continuous response (indicated by y), which is translated into a binary response (incidence) by incorporating a cut-off point ($th1$) in the normal distribution around y , below which an animal does not respond, and above which it does respond.

Some of the models are nested members of a larger family of models. Two models are nested, when the one model can be seen as an extension of the other (simpler) model, by incorporating one or more parameters. For instance, the two-stage model is an extension of the one-stage model by including parameter c . Also, the Proast models are a nested family of models. Nested models can be formally compared with each other as follows. Inclusion of an extra model parameter should result in a higher log-likelihood value; if this increase is larger than 1.92, inclusion of the parameter has resulted in a significantly better fit (log-likelihood ratio test). If the increase is less than 1.92, the fit is not significantly better, and the parameter is omitted.

When dose–response data are available from more than one study or for both sexes, these models are fitted simultaneously to both such subgroups. This was done either by assuming all parameters in the model being the same for all subgroups or by assuming only the background response parameter (a) or only the slope (b) being different. When all parameters are assumed to be the same, a single curve results; otherwise, different curves for the subgroups will result. A model in which a parameter is assumed to be different represents a model that is nested to the same model with the parameter assumed to be the same for the subgroups. Hence, the log-likelihood ratio test can be used for testing if an additional background or slope parameter results in a significantly better fit.

9.2.1 Selection of models

In general, those models that do not result in a significantly worse fit than the saturated model (one parameter per data point) are considered to be acceptable. For instance, when the saturated model has eight parameters (i.e. eight observed incidences available), a fitted dose–response model with three parameters should result in a log-likelihood that is no more than 5.54 lower than the log-likelihood associated with the saturated model. Table 12 summarizes the critical differences in log-likelihood values for various numbers of degrees of freedom (i.e. difference in number of parameters between the models to be compared).

For those models that were considered acceptable according to the criteria just mentioned, the benchmark dose (BMD) values as well as the benchmark dose lower confidence limit (BMDL) values were calculated. All BMD and BMDL values were calculated for a 10% extra risk, defined as:

$$\text{extra risk} = \frac{R(\text{BMD}) - R(0)}{1 - R(0)}$$

This represents the additional response fraction divided by the tumour-free fraction in the controls.

Table 12. Critical differences in log-likelihood values for various numbers of degrees of freedom

Number of degrees of freedom	Critical difference in log-likelihood ($\alpha = 0.05$)
1	1.92
2	3.00
3	3.91
4	4.74
5	5.54
6	6.30
7	7.03
8	7.75

The BMD and BMDL values were estimated by the bootstrap method, usually performing 500 bootstrap runs. These values therefore contain some random error, but usually no more than about 10% for the BMDL.

The calculations were performed using the dose-response software package PROAST, version V07 (developed at the National Institute of Public Health and the Environment [RIVM], Bilthoven, The Netherlands), which is freely available.

In general, dose-response modelling of toxicological data is used to determine a point of departure for further risk assessment that is within the range of observation. For cancer bioassays, the observable (additional or extra) tumour incidence usually is around 10%. Therefore, the BMD₁₀ could be used as an appropriate point of departure.

9.2.2 Selection of data

Several regulatory and international scientific groups have determined that acrylamide is most likely a carcinogen in humans. Acrylamide was evaluated by the International Agency for Research on Cancer (IARC) in 1994 (IARC, 1994b) and classified as "probably carcinogenic to humans" (Group 2A) on the basis of the positive cancer bioassay results in rats (Johnson et al., 1986), supported by evidence that acrylamide is efficiently transformed to a chemically reactive genotoxic metabolite, glycidamide, in both rodents (Calleman et al., 1990) and humans (Bergmark et al., 1993).

A number of carcinogenic risk assessments of acrylamide done by various expert groups between 1976 and 2002 have been summarized by Ruden (2004). These include the American Conference of Governmental Industrial Hygienists (ACGIH, 1991), the Swedish National Chemicals Inspectorate (1989), the Institutet for Miljomedicin (1998) and the European Commission (2002a). The US EPA (1993) determination was done for acrylamide in drinking-water. The Norwegian Food Control Authority (2002), the Australian assessment (NICNAS, 2002), the

FAO/WHO consultation on acrylamide in foods (FAO/WHO, 2002) and the Dutch assessment by Konings et al. (2003) were performed more recently. The most recent US EPA Integrated Risk Information System (IRIS) assessment is still in draft form as of December 2004 (US EPA, 2004).

9.2.3 Measure of intake

This risk assessment will use acrylamide levels from human dietary intake surveys as well as administered doses in animal studies as the metrics for acrylamide exposure. From the dietary surveys, an acrylamide intake of 0.001 mg/kg bw per day represents the average intake of the general population based on national estimates, and an intake of 0.004 mg/kg bw per day represents the intake by high consumers.

This risk assessment will not use biomarker levels — either haemoglobin adduct or DNA adduct levels — as a metric of exposure, although information on biomarker levels and their possible relationship to exposure is accumulating.

This risk assessment will also not use levels of acrylamide or acrylamide metabolites — e.g. acrylamide in urine, blood or tissues, or glycidamide in urine — as a metric for exposure. The one existing PBPK model for acrylamide (Kirman et al., 2003) that was based primarily on older rodent data derived from studies of total radioactivity from relatively high doses of acrylamide.

9.2.4 Measure of response

A number of measures of response have been used in various risk assessments. In its cancer risk assessment, the US EPA used combined incidence data for tumours in the central nervous system, mammary and thyroid glands, uterus and oral cavity in female F344 rats exposed to acrylamide in drinking-water for 2 years (Johnson et al., 1986; US EPA, 1993). In a revised cancer risk assessment (McClure et al., 2004), the US EPA used thyroid tumours in male and female rats, mammary gland tumours (predominantly benign) in female rats and tunica vaginalis (scrotal sac) mesotheliomas in male rats from the Friedman et al. (1995) study.

The US Food and Drug Administration used the Johnson et al. (1986) study and based its cancer risk assessment on the significantly increased incidence of thyroid, testicular, mammary and central nervous system tumours (US FDA, 1998).

Dybing & Sanner (2003) used testicular mesotheliomas and mammary gland adenomas as reported from the Johnson et al. (1986) study as the basis for their risk assessment.

The Committee used mammary gland fibroadenomas from the studies by Johnson et al. (1986) and Friedman et al. (1995), but noted that although both studies showed a dose-response-related increase, the dose-response information in the data is limited, as background response was high and maximum response was low.

This risk assessment will not use responses in epidemiological studies as a measure of response, because a dose–response has not been demonstrated in these studies.

The Committee considered the rat subchronic drinking-water study (Burek et al., 1980) as the pivotal study with respect to neurotoxic effects.

9.2.5 Selection of mathematical model

After fitting the models described in section 9.2, the fits of the models are compared, and the best-fitting models are considered as plausible models (for criteria, see general section on modelling). The BDM(L)s associated with the various plausible models are summarized in the form of a range of values.

9.3 Estimates of BMDs and BMDLs

Tables 3 and 4 in section 2.2.3 present the tumour data from Johnson et al. (1986) and Friedman et al. (1995), as described in section 9.2.4. These studies have been described in detail in section 2.2.3.

Dose–response analysis was performed for mammary tumours, thyroid tumours, testicular tumours and central nervous system tumours. For each of these end-points, the total number of animals was considered most relevant for modelling and deriving the BMDs.

In general, the models described in section 9.2 were fitted to the dose–response data from both studies simultaneously, while allowing for particular parameters in the models to be different between the studies.

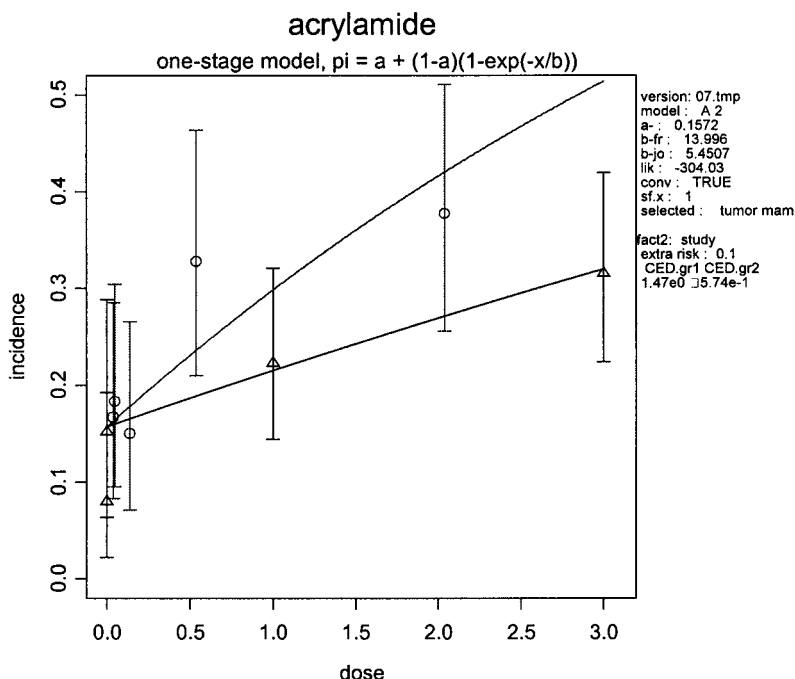
9.3.1 Mammary tumours

Figure 8 shows the observed incidences for mammary tumours and the one-stage model fitted to them. The background response is quite high, and the maximum observed response is below 40%, making the range between minimum and maximum response small.

The two studies showed statistically significantly different dose–response relationships, although it could not be decided from the dose–response analysis whether the studies differed in background responses or in slopes (see Table 13 below). The results for the analyses assuming different slopes were selected for further evaluation, as this situation resulted in lower BMDs for the Johnson et al. (1986) study.

Most models resulted in a best fit where the slope at dose zero was infinite, if the shape parameter was not constrained in the fitting process. The associated BMDLs were very low and varied considerably between models. When, however, a constraint was imposed on the shape parameter such that the slopes of the dose–response curves were forced to be finite, the resulting confidence intervals of the BMDs were quite small, and the BMDLs were close to the BMDs.

Figure 8. Incidences of total mammary tumours, with fitted one-stage model. Circles: Johnson et al. (1986); triangles: Friedman et al. (1995). Dose is expressed in mg/kg bw per day.



Therefore, it may be concluded that the uncertainty in the dose–response data was large (resulting in very low BMDLs for the unconstrained model fits) and that the BMDLs as reported in Table 13 strongly hinge on the assumption of finite slope at dose zero. This analysis seems to indicate that the dose–response relationship may in fact be (close to) linear for this end-point.

Table 13 summarizes the results of the various models fitted. The BMD(L)s for the models that were considered most appropriate are printed in bold in this table.

The log-probit model and Proast M4 were not included in Table 13, since the constraints needed for avoiding infinite slopes at dose zero were unknown at the point of this evaluation for these two models. The values for BMD_{10s} (and particularly for BMDLs) are evaluated for the most relevant models only; therefore, some cells are empty. In order to integrate the results from all the models used for both mammary tumour data sets, a composite analysis was conducted in which the model outputs were combined. This resulted in a BMD of 1.0 mg/kg bw per day and a BMDL of 0.4 mg/kg bw per day, which supports the other analysis.

Table 13. Modelling results for total number of mammary tumours

Model	Study-dependent parameter	log-lik	No. of pars	mg/kg bw ^a			
				BMD ₁₀	BMD ₁₀	BMDL ^b	BMDL ^b
				Friedman et al.	Johnson et al.	Friedman et al.	Johnson et al.
One-stage	None	-306.37	2				
One-stage	Background	-303.98	3	1.01			
One-stage	Slope	-304.03	3	1.47	0.57	0.98	0.38
Two-stage	Slope	-304.03	4				
Log-logistic ^c	None	-306.13	3	—	1.03	—	
Log-logistic ^c	Background	-303.70	4	—	0.90	—	
Log-logistic ^c	Slope	-303.72	4	1.37	0.48	0.89	0.30
Weibull ^c	None	-306.37	3	—	1.14	—	
Weibull ^c	Background	-303.98	4	—	1.01	—	
Weibull ^c	Slope	-304.03	4	1.48	0.57	1.09	0.46
Saturated model		-300.82	9				

log-lik, log-likelihood value; pars, parameters

^a The BMD(L)s for the models that were considered most appropriate are printed in bold.

^b Lower 5% confidence bound, based on 500 bootstrap runs.

^c With constraint on shape parameter, to avoid infinite slope at dose zero.

9.3.2 Testis tumours

Figure 9 shows the observed incidences of total testis tumours and the one-stage model fitted to them. Incidences do not exceed 20%; in the Johnson et al. (1986) study, they even seem to level off at this level, again resulting in a small range between minimum and maximum response.

The two studies appeared to show statistically significantly different dose-response relationships, the Johnson et al. (1986) study showing increased incidences at lower doses than the Friedman et al. (1995) study. As Figure 9 shows, the fit of the model is not very good. This is confirmed by comparing the log-likelihood of this model with that of the saturated model (see Table 14).

Fitting the one-stage model to the Johnson et al. (1986) data separately resulted in a better fit, but the BMD estimate was similar. The problem with the Johnson et al. (1986) data for total testis tumours is that only models allowing infinite slopes at dose zero result in a substantially better fit.

Figure 9. Incidences of total testis tumours with fitted one-stage model. Circles: Johnson et al. (1986); triangles: Friedman et al. (1995). The Johnson et al. (1986) data are plotted with a small shift, to avoid overlap between confidence intervals. Dose is expressed in mg/kg bw per day.

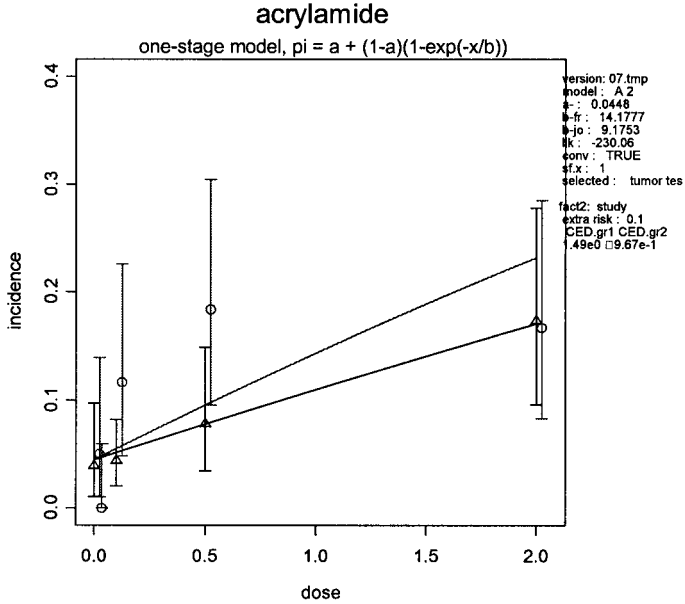


Table 14. Modelling results for total testis tumours^a

	Study-dependent parameter	log-lik	No. of pars	mg/kg bw ^b	
				BMD ₁₀	BMDL ₁₀ ^c
One-stage	Slope	-230.06	3	0.97	0.63
Two-stage					
Log-logistic ^d	Slope	-229.75	4		
Log-probit					
Weibull	Slope	-228.12	4	0.52	0.19
Weibull ^d	Slope	-230.06	4	0.97	0.97
Proast M4					
Saturated model		-222.41	10		

log-lik, log-likelihood value; pars, parameters

^a In all models, only the slope was assumed to differ between studies. BMD(L) values relate to Johnson et al. (1986).
^b The BMD(L)s for the models that were considered most appropriate are printed in bold.
^c Lower 5% confidence bound, based on 500 bootstrap runs.
^d With constraint on shape parameter, to prevent infinite slope at dose zero.

These data leave considerable model uncertainty, and the results again hinge on the finite slope assumption, which is not biologically plausible.

9.3.3 Central nervous system tumours

The reported central nervous system tumours in Friedman et al. (1995) did not show any dose–response, and the analysis was restricted to the data from Johnson et al. (1986) (Table 15). Here, only the top dose resulted in an increased incidence, for both sexes (see Figure 10).

Table 15. Modelling results for total central nervous system tumours

	log-lik	No. of parameters	mg/kg bw ^a	
			BMD ₁₀	BMDL ₁₀ ^b
One-stage	-116.97	2	2.01	1.25
Two-stage	-116.97	3		
Log-logistic	-115.82	3	1.91	1.44
Log-probit	-115.82	3	1.88	
Weibull	-115.82	3		
Proast M2	-116.82	2	1.87	1.49
Proast M3	-115.82	3	1.94	1.59
Saturated model	-110.97	10		

Data from Johnson et al. (1986)

log-lik, log-likelihood value

^a The BMD(L)s for the models that were considered most appropriate are printed in bold.

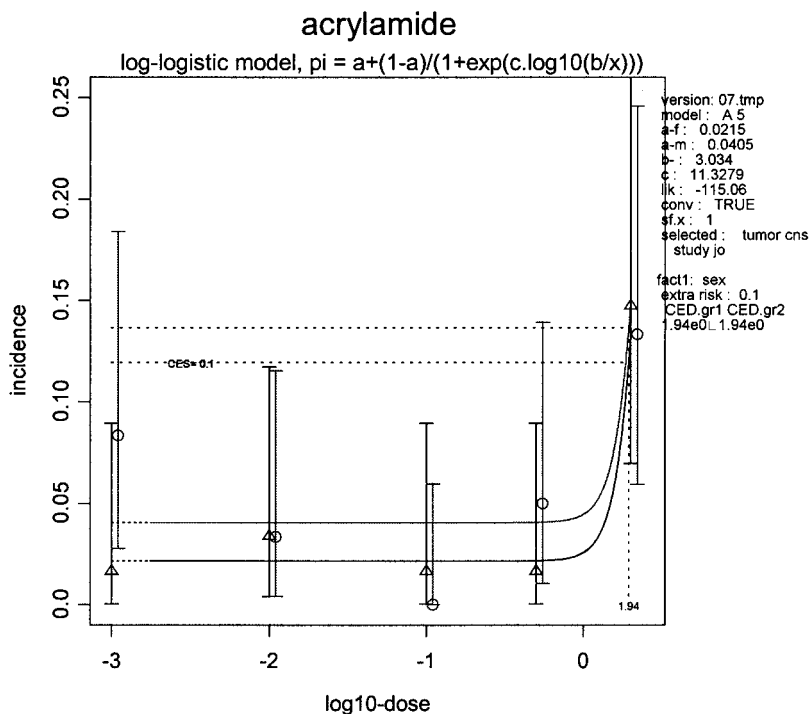
^b Lower 5% confidence bound, based on 500 bootstrap runs.

9.3.4 Thyroid tumours

Figure 11 shows the total thyroid tumour incidences observed, as a function of dose, with Proast M4 fitted to them. The dose–response data from Friedman et al. (1995) show a steeper dose–response than the data from Johnson et al. (1986), and the BMDs are derived for the Friedman et al. (1995) study for this end-point (Table 16).

The narrow range of BMDL values indicates that the result was not influenced greatly by the different mathematical models used, but inspection of the incidence data shows that there was little increase in response between 0.5 and 2.0 mg/kg bw per day in the modelled data. A similar small increase in response was found between 1.0 and 3.0 mg/kg bw per day in the study of Friedman et al. (1995).

Figure 10. Incidences of total central nervous system tumours (against log-dose), with fitted log-logistic model. Data from Johnson et al. (1986). Circles: males, triangles: females. Data for males are plotted with a small shift, to avoid overlap between confidence intervals.



9.4 Potency estimates in humans

Since epidemiological data were inadequate to establish a dose-response curve, no cancer potency estimates based on epidemiological data could be generated. In addition, as previously discussed, biomarker data are at present inadequate or inappropriate to generate a dose-response curve; hence, no potency estimates based on biomarker data were generated.

10. COMMENTS

10.1 Absorption, distribution, metabolism and excretion

In animals, acrylamide administered orally is rapidly and extensively absorbed from the gastrointestinal tract and is widely distributed to the tissues, as well as the fetus. It has also been found in human milk. Acrylamide is metabolized to a chemically reactive epoxide, glycidamide, in a reaction catalysed by CYP2E1. An

Figure 11. Incidences of total thyroid tumours, with fitted Proast M4. Circles: Johnson et al. (1986); triangles: Friedman et al. (1995). Data for Johnson et al. (1986) are plotted with a small shift, to avoid overlap between confidence intervals.

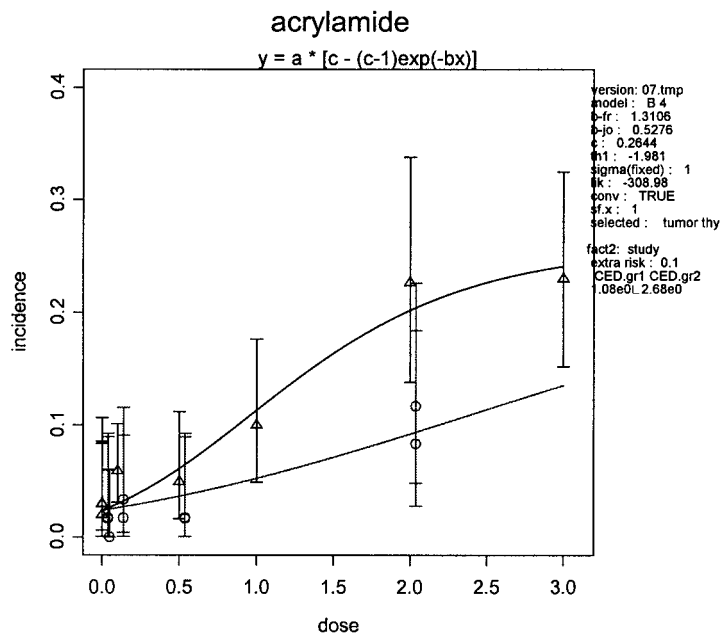


Table 16. Modelling results for thyroid tumours^a

	log-lik	No. of parameters	mg/kg bw	
			BMD ₁₀	BMDL ₁₀ ^b
One-stage	-309.24	2	1.16	0.93
Two-stage		3		
Log-logistic	-309.02	3	0.98	0.63
Log-probit	-309.24	3	0.88	0.62
Weibull	-309.00	3		
Weibull ^c	-309.24	3	1.16	0.97
Proast M2	-311.80	2		
Proast M4	-308.98	3	1.08	0.74
Saturated model	-301.81	19		

log-lik, log-likelihood value

^a BMD(L)s relate to Friedman et al. (1995).

^b Lower 5% confidence bound, based on 500 bootstrap runs.

^c With constraint on shape parameter, to prevent infinite slope at dose zero.

alternative pathway for the metabolism of acrylamide is conjugation with glutathione. Acrylamide and its metabolites are rapidly eliminated in the urine, primarily as mercapturic acid conjugates of acrylamide and glycidamide. The absolute bioavailability of acrylamide (i.e. the fraction entering the circulation as parent compound) is in the range of 23–48% in rodents for a dose of 0.1 mg/kg bw administered in the diet over a period of 30 min. The relative internal exposure to glycidamide is much higher after dietary administration than after intravenous administration, owing to extensive first-pass metabolism of acrylamide to glycidamide.

Glycidamide is much more reactive than acrylamide with DNA, and several purine base adducts have been identified *in vitro*. Studies in knockout and wild-type mice have shown that CYP2E1-mediated oxidation is the predominant pathway leading to the formation of glycidamide–DNA adducts. In rodents given acrylamide, glycidamide–DNA adducts are formed at comparable levels in all tissues examined and accumulate to apparent steady-state levels after regimens involving repeated dosing. DNA adducts have been found in the liver, lung, testis, leukocytes and kidney of mice and in the liver, thyroid, testis, mammary gland, bone marrow, leukocytes and brain of rats treated with either acrylamide or glycidamide. The formation of DNA adducts in mice shows a monotonic dependence on the dose of acrylamide administered, from measurable levels of adduct at background exposure to evidence for saturation of levels of adduct at higher doses. Kinetic studies of adduct loss from DNA *in vitro* and *in vivo* showed that spontaneous depurination, as opposed to active repair, is operative.

Both acrylamide and glycidamide also bind covalently to amino acids in haemoglobin, and adducts with the *N*-terminal valine residue have been widely used to estimate internal exposures in biomonitoring studies in humans. Preliminary studies measuring concentrations of acrylamide–haemoglobin and glycidamide–haemoglobin adducts in rodents and humans with background exposure to acrylamide in the diet suggested that there may be species differences in the relative formation of glycidamide (relative formation, mice > rats > humans). However, the long half-life of haemoglobin means that the measured levels of adduct reflect a time-weighted average over the lifetime of the erythrocyte. Thus, the same total exposure over an extended period of time or over a short period of time could produce similar levels of adducts. This has limited the usefulness of these biomarkers for dose–response modelling under circumstances where there is variability in the magnitude and frequency of exposure.

10.2 Toxicological data

Single oral doses produced acute toxic effects only at doses of >100 mg/kg bw, and reported LD₅₀s are generally >150 mg/kg bw.

Numerous studies in a number of animal species have shown that the nervous system is a principal target site for the toxic effects of acrylamide. Sufficient repeated exposure to acrylamide causes degenerative peripheral nerve changes that result from an accumulation of damage at the sites of toxicity (see Table 6 in section 2.2.6). For example, the same degree of neurotoxicity was observed in

rats given acrylamide at a dose of 50 mg/kg bw per day by intraperitoneal administration for 11 days and in rats given drinking-water containing acrylamide at a dose of 21 mg/kg bw per day for 40 days. Continued dosing with acrylamide has been shown to induce degeneration of nerve terminals in brain areas (i.e. cerebral cortex, thalamus and hippocampus) critical for learning, memory and other cognitive functions, and these lesions may precede the morphological changes in nerves. In rats given drinking-water containing acrylamide for 90 days, the NOEL was 0.2 mg/kg bw per day for morphological changes in nerves, detected by electron microscopy, and no exposure-related non-neoplastic lesions were found in other tissues at doses of <5 mg/kg bw per day.

In studies of reproductive toxicity, male rodents given acrylamide showed reduced fertility, dominant lethal effects and adverse effects on sperm count and morphology at oral doses of ≥ 7 mg/kg bw per day. In female rodents, no adverse effects on fertility or reproduction were observed, apart from slight reductions in the body weight of rat offspring at oral doses of 2.5 mg/kg bw per day (the LOEL) and above. In studies of developmental toxicity, acrylamide was fetotoxic in mice only at a maternally toxic oral dose of 45 mg/kg bw per day and was not teratogenic in mice or rats. In a study of developmental neurotoxicity, in which rats were given acrylamide orally from day 6 of gestation until day 10 of lactation, the NOEL for developmental neurotoxicity was 10 mg/kg bw per day. The overall NOEL for reproductive and developmental effects was 2 mg/kg bw per day.

10.3 Genotoxicity

Although acrylamide was not mutagenic in the Ames assay in *Salmonella*, glycidamide clearly was. Acrylamide was both clastogenic and mutagenic in mammalian cells in vitro and in vivo. In addition, studies of dominant lethality have shown that acrylamide is a germ cell mutagen in male rodents. The mutational spectra produced by acrylamide and glycidamide in transgenic mouse cells are consistent with the formation of promutagenic purine DNA adducts in vivo.

The metabolism of acrylamide to glycidamide appears to be a prerequisite for the genotoxicity caused by acrylamide in vitro and in experimental animals. Studies using knockout and wild-type mice showed that CYP2E1-mediated oxidation is the predominant pathway leading to the formation of DNA adducts. Estimates of internal exposures to glycidamide, based on measurements of haemoglobin adducts after administration of either acrylamide or glycidamide, indicated that glycidamide was the active clastogen responsible for induction of micronuclei in mice. Studies in wild-type and CYP2E1 knockout mice have also shown that glycidamide is the active metabolite of acrylamide responsible for germ cell mutations and dominant lethality in spermatids of male mice. Glycidamide is the presumed active mutagen because dosing with glycidamide produced increases in the frequency of mutation at the *Hprt* and *cII* loci in Big Blue transgenic mice that were comparable to or greater than those resulting from dosing with acrylamide.

10.4 *Carcinogenicity*

Acrylamide, administered in drinking-water, has been tested for carcinogenicity in two experiments in Fischer 344 rats. There were increases in the incidence of tumours at a variety of sites (see Tables 3 and 4 in section 2.2.3). Information about the total number of tumour-bearing animals was not available for either study.

Acrylamide was evaluated by IARC in 1994 and classified as "probably carcinogenic to humans (IARC Group 2A)" (IARC, 1994b) on the basis of a positive result in a bioassay for cancer (see Table 3), supported by evidence that acrylamide is efficiently biotransformed to a chemically reactive genotoxic metabolite, glycidamide, in both rodents and humans. The endocrine-responsive nature of several tumour sites from the two long-term bioassays with acrylamide in F344 rats has elicited speculation about neuroendocrine-mediated mechanisms. However, no published studies have linked hormonal changes with the carcinogenicity of acrylamide in any tissue, nor is there any indication of hormonal effects in studies of reproductive toxicity. Moreover, the wide body of evidence supporting a genotoxic mechanism is not incompatible with hormonal dysregulation by acrylamide, because it is clear that other factors beyond DNA damage are probably required for the observed target tissue specificity of tumorigenesis caused by acrylamide.

10.5 *Observations in humans*

Epidemiological studies in humans exposed in industry or accidentally suggest that the nervous system is a principal target site for toxicity caused by acrylamide in humans.

In workers exposed occupationally to acrylamide, exposure was not associated with an increase in overall mortality caused by cancer, nor with any statistically significant dose-related increase in risk of cancer at any organ site, except for a statistically significant doubling of risk for pancreatic cancer in workers with the highest cumulative exposure. These studies, however, were based on small numbers of cases, measurements of dietary intake of acrylamide were not made and potential confounders, such as tobacco smoking, were not considered.

The only information available that included dietary intake of acrylamide came from case-control studies originally designed to assess the potential risk of cancer associated with dietary factors other than acrylamide. The available results from epidemiological studies that estimated oral exposure to acrylamide were not suitable for use in risk assessment for acrylamide.

The formation of acrylamide-haemoglobin adducts has been used as a biomarker of exposure in humans. Although levels of acrylamide adducts were often found to be higher among exposed workers and smokers, and there was a positive correlation with the amount of tobacco product smoked, some uncertainties remained that precluded use of this measure as a marker of dietary intake of acrylamide at the present time. Because analytical methods may vary between laboratories, there is a need for improved and validated analytical methodology. At

the time of the present meeting, it was not possible to link biomarkers of exposure to acrylamide in humans with measurements of toxicity in experimental animals.

10.6 Analytical methods

The analytical methodology used to measure concentrations of acrylamide in food appeared to be adequate, although no methods for rapid screening had yet been developed. Acrylamide is freely soluble in water, and studies indicated that the extraction procedures employed gave complete extraction. Most survey data for acrylamide have been obtained using either LC-MS-MS or GC-MS. Stable isotope-labelled derivatives of acrylamide are used widely as internal standards. Both LC-MS-MS and GC-MS methods have been found to be accurate in the many schemes for proficiency tests and exercises for checking samples that have been conducted. There are currently no certified reference materials or analytical methods that have been tested collaboratively to internationally recognized standards. There is a need for improvement in analytical precision, but given the large number of data available on acrylamide concentrations in food, this does not affect the current estimates of intake. LC-MS-MS methods can routinely quantify acrylamide at concentrations as low as 10 µg/kg. Similarly, an LOQ of 5 µg/kg is well within the reach of the average laboratory equipped with a standard bench-top GC-MS instrument.

10.7 Formation of acrylamide during cooking and heat processing

Acrylamide may be formed when dietary items, typically plant commodities high in carbohydrates and low in protein, are subjected to high temperatures during cooking or other thermal processing. The most important precursor is the free amino acid asparagine, which reacts with reducing sugars in the Maillard reactions that also form colour and flavour. Alternative mechanisms might be important in some speciality foods.

Although trace amounts of acrylamide can be formed by boiling, formation of more significant quantities of acrylamide generally requires a processing temperature of 120 °C or higher. Concentrations are likely to represent a balance of competing complex processes of formation and destruction of acrylamide. Most acrylamide is accumulated during the final stages of baking, grilling or frying processes as the moisture content of the food falls and the surface temperature rises, with the exception of coffee, where levels of acrylamide fall considerably at later stages of the roasting process. Acrylamide seems to be stable in the large majority of the affected foods, again with the exception of ground coffee, in which concentrations of acrylamide can decline during storage over months.

Since the formation of acrylamide is dependent on the exact conditions of time and temperature used to cook or to heat process a food, there can be large variations between different brands of the same product and between different batches of the same brand. Large variations are also to be expected during home-cooking, although this aspect has been less well documented. The composition of the food also has an influence — crucially, the content of free asparagine and reducing sugars. Varietal, storage and seasonal variations can occur. Within the

ranges of natural variation, the limiting precursor in cereals is asparagine, while fructose and glucose are more important in potatoes. Other important factors are pH and water content. Addition of ammonium bicarbonate, a leavening agent used in some bakery products, significantly increases acrylamide formation. High concentrations of other amino acids or proteins that compete with asparagine in the Maillard reaction or that react with already-formed acrylamide reduce the concentration of acrylamide.

10.8 Prevention and control

Research into the formation and mitigation of acrylamide is ongoing and has been the subject of several international scientific meetings and reviews. The European food industry (CIAA) submitted a review on the mitigation achievements of food producers up to December 2004 (Taeymans et al., 2004). An average reduction of acrylamide of 30–40% in potato crisps was stated to have been achieved by introducing several adjustments into the existing production procedures. The detailed data behind this calculation were not reported, and it was not known to what extent these adjustments had been applied by producers of crisps. Significant reduction was also reported from process optimization for non-fermented crispbread, while little progress had been obtained so far in reducing concentrations in various other foods making an important contribution to intake, e.g. roasted coffee and breakfast cereals.

Experiments in food models have indicated a number of possible options for mitigation. The most efficient reduction has been achieved by using the enzyme asparaginase to selectively remove asparagine prior to heating. Although tested in models in cereals and potatoes, use is probably limited to specific food products manufactured from liquidized or slurried materials. Several other means of lowering the levels of precursor can be applied at various stages of the food chain, e.g. by variety selection and plant breeding, controlling growth and storage factors affecting concentrations of sugar in potatoes, pretreatment of potato pieces by soaking or blanching and prolonged time for yeast fermentation in bread-making. Other possibilities for mitigation include alteration of the composition of the product, e.g. addition of competing amino acids or acidic compounds, and alteration of process conditions, e.g. lowering the frying temperature. The feasibility of adapting these methods to large-scale food processing had not been studied sufficiently in most cases. Furthermore, any major changes would need to be checked for consumer acceptability, nutritional quality and the possible increased formation of other undesirable substances.

10.9 Levels and pattern of food contamination

At its present meeting, the Committee reviewed data provided by 24 countries on the occurrence of acrylamide in different food items analysed between 2002 and 2004 (see Table 8 in section 6.3). The total number of analytical results (single or composite samples) was 6752, with 67.6% coming from Europe, 21.9% from North America, 8.9% from Asia and 1.6% from the Pacific. No data from Latin America or Africa were submitted. The Committee noted that the occurrence data

evaluated at its present meeting were more comprehensive than those available at the FAO/WHO consultation in 2002 (FAO/WHO, 2002) (240 samples).

The choices of food items analysed for concentration of acrylamide were mainly made on the basis of knowledge acquired since 2002–2003 on the occurrence of acrylamide in foods and beverages and also on the basis of recommendations made at the last FAO/WHO consultation, especially concerning other foods and beverages that undergo similar processing and that might also contain acrylamide, such as meat, milk, rice, cassava, soya products, vegetables and processed fruits.

Data were available from Sweden for four archived samples of human milk, one for each of the years 1998–2001. Each of the four samples comprised a pool of samples from 10 mothers. A further 15 samples collected from individual mothers in 2000–2004 were also analysed. No information on sampling times or on food consumption by the mothers was available. One of the 19 samples of milk contained acrylamide at a concentration of 0.5 µg/kg, which was just above the LOQ; the other 18 samples were below the LOQ, i.e. <0.5 µg/kg.

10.10 Dietary intake assessment

Data on national dietary intake for 17 countries were evaluated at this meeting. All regions were represented except for Africa and Latin America, for which no dietary intakes were available. National intakes were calculated mainly using deterministic modelling, by linking data on national individual consumption with data on national mean occurrence obtained from national surveys, using the actual consumer body weight reported in consumption surveys.

Estimates of average intake at the national level ranged from 0.3 to 2.0 µg/kg bw per day for the general population. For consumers in the 90th to 97.5th percentile, estimates of intake ranged from 0.6 to 3.5 µg/kg bw per day, while the intake for consumers in the 99th percentile was up to 5.1 µg/kg bw per day. Based on the available data, children had intakes of acrylamide that were about 2–3 times higher than those of adults, when expressed on a body weight basis. The Committee noted that these estimates were consistent with the long-term dietary intake assessment performed by the FAO/WHO consultation (FAO/WHO, 2002), which was based on a limited data set of analytical results representing only a fraction of the diet.

In the absence of a health-based guidance value for acrylamide, the relative contribution of food commodities to the total intake is reported here. The relative contribution of each food group may be different between studies, depending on the numbers of food categories considered in the intake evaluation.

The foods that made the biggest contribution to total exposure in most countries were: potato chips (USA = "french fries"), 16–30%; potato crisps (USA = "chips"), 6–46%; coffee, 13–39%; pastry and sweet biscuits (USA = "cookies"), 10–20%; and bread and rolls/toasts, 10–30%. Other food items contributed <10% of the total exposure.

International estimates of intake were prepared by combining the international weighted means of contamination levels (see Table 8 in section 6.3) with the food consumption values reported in the GEMS/Food database. The Committee noted that these estimates are conservative as the foods considered are raw commodities, while the concentrations of acrylamide apply to specifically processed foods (i.e. the intake of all raw potatoes was combined with concentrations of contaminant in fried or baked potato products). Additionally, in regions with few or no data on concentrations of acrylamide, the use of this broad assumption may result in a mismatch between the foods considered and the data employed (e.g. cassava consumption combined with concentrations of acrylamide in processed potato products).

The intake estimates are based on an extensive database derived primarily from data from developed, industrialized nations. There are limited data for other regions. Additional data on occurrence of acrylamide in different types of human food and estimated intakes for both the average and high consumer from a range of geographical regions would enhance confidence in the final evaluation.

Taking these points into consideration, the Committee estimated the range for the international mean intakes to be 3.0–4.3 $\mu\text{g/kg bw}$ per day for the five GEMS/Food regional diets, assuming a body weight of 60 kg. Cereals and roots and tubers are the main contributors to the total exposure calculations for each regional diet. Intakes from cereals range from about 1.3 to 2.6 $\mu\text{g/kg bw}$ per day. Intakes from roots and tubers are about 0.5–2.6 $\mu\text{g/kg bw}$ per day. Other GEMS/Food groups contribute <5% to the total exposure calculations.

The Committee concluded that based on national estimates, an intake of acrylamide of 1 $\mu\text{g/kg bw}$ per day could be taken to represent the average for the general population and that an intake of 4 $\mu\text{g/kg bw}$ per day could be taken to represent consumers with a high intake. Children are also included in these estimates for average to high intake.

10.11 Dose–response analysis

The NOEL for induction of morphological changes in nerves, detected by electron microscopy, in rats given drinking-water containing acrylamide for 90 days was 0.2 mg/kg bw per day. The overall NOEL for reproductive and developmental effects and other non-neoplastic lesions was 2 mg/kg bw per day.

The Committee considered that the pivotal effects of acrylamide for the present risk assessment were its genotoxicity and carcinogenicity. The available epidemiological data, as well as data on biomarkers in humans and animals, were inadequate to establish a dose–response relationship, and therefore the assessment was performed (see section 9.2) on the basis of available studies in animals. In the dose–response analysis, eight different statistical models were fitted to the experimental data that were considered relevant for further consideration. Those resulting in acceptable fits based on biological and statistical considerations were

selected to derive the BMD and BMDL for a 10% extra risk of tumours.² This procedure results in a range of BMD and BMDL values for each end-point considered (Table 17).

Table 17. Summary of the results of dose-response modelling for induction of selected tumours in rats given drinking-water containing acrylamide

Tumour	Study			
	Johnson et al. (1986)		Friedman et al. (1985)	
	Range of BMD (mg/kg bw per day)	Range of BMDL (mg/kg bw per day)	Range of BMD (mg/kg bw per day)	Range of BMDL (mg/kg bw per day)
Total mammary tumours	0.48–0.57	0.30–0.46	1.4–1.5	0.89–1.1
Peritesticular mesothelioma	0.97	0.63–0.97	NA	NA
Thyroid follicular adenoma	NA	NA	0.88–1.2	0.63–0.93
Central nervous system tumours of glial origin	1.9–2.0	1.3–1.6	NA	NA

BMD, benchmark dose for 10% extra risk of tumours; BMDL, 95% lower confidence limit for the benchmark dose. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls; NA, not applicable

The results summarized in Table 17 show that the BMDLs are only moderately lower than the BMDs, indicating that the confidence intervals are quite narrow. The reason for the narrow confidence intervals in this case is that the uncertainty is reduced to a large extent, by imposing the constraint that the slope at zero dose should be finite. An infinite slope at dose zero is biologically implausible. When the constraint is omitted in fitting the models, the resulting BMDLs are extremely low for some of the fitted models, showing that the dose-response data contained a high degree of uncertainty regarding the shape of the dose-response curve.

The lowest range of BMDLs, i.e. 0.30–0.46 mg/kg bw per day, is found for total mammary tumours. The Committee decided to use the more conservative lower end of this range of values for the evaluation.

In order to integrate the results from all the models used for both mammary tumour data sets, a composite analysis was conducted in which the model outputs

² Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls.

were combined. This resulted in a BMD of 1.0 mg/kg bw per day and a BMDL of 0.4 mg/kg bw per day, which supports the other analysis.

11. EVALUATION

MOEs were calculated at intakes of 0.001 mg/kg bw per day, to represent the average intake of acrylamide for the general population, based on national estimates, and 0.004 mg/kg bw per day to represent the intake of acrylamide by high consumers. Comparison of these intakes with the NOEL of 0.2 mg/kg bw per day for morphological changes in nerves, detected by electron microscopy, in rats would provide MOEs of 200 and 50, respectively. Comparison of the selected intakes with the NOEL of 2.0 mg/kg bw per day for reproductive, developmental and other non-neoplastic effects in rodents would provide MOEs of 2000 and 500, respectively. Based on these MOEs, the Committee concluded that adverse effects were unlikely at the estimated average intakes, but that morphological changes in nerves could not be excluded for some individuals with a very high intake. Ongoing studies of neurotoxicity and neurodevelopmental effects in rats would more clearly define whether effects may arise in the long term, at low doses of acrylamide.

When the value of 0.001 mg/kg bw per day taken to represent the average intake of acrylamide of the general population is compared with the BMDL of 0.30 mg/kg bw per day for induction of mammary tumours in rats, the MOE is 300. For the value taken to represent consumers with a high level of intake, 0.004 mg/kg bw per day, the MOE is 75. The Committee considered these MOEs to be low for a compound that is genotoxic and carcinogenic and that this may indicate a human health concern. Therefore, appropriate efforts to reduce concentrations of acrylamide in food and beverage should be continued.

Uncertainties in the derivation of the MOEs for acrylamide arise from uncertainties and assumptions associated with the data used to derive the BMDL values and the different estimates of intake. The Committee noted that the pathways by which acrylamide is metabolized are similar in rats and humans, but that quantitative differences, such as the extent of bioactivation of acrylamide to glycidamide or detoxication of glycidamide, could result in species differences in sensitivity. Confidence in the data used to calculate the MOE for acrylamide might be enhanced by the results of currently ongoing cancer bioassays in rodents. Incorporation of additional data on the influence of dose on the conversion of acrylamide to glycidamide into a PBPK model may facilitate the extrapolation of the incidence data to humans. The intake estimates are based on an extensive database derived primarily from data from industrialized nations. There are limited data for other countries.

11.1 Recommendations

1. The Committee recommended that acrylamide be re-evaluated when the results of planned and ongoing studies of carcinogenicity and long-term studies of neurotoxicity become available.

2. The Committee recommended that work should be continued on the use of PBPK modelling to better link data on biomarkers in humans with intake assessments and toxicological effects in experimental animals.
3. The Committee recommended that appropriate efforts to reduce concentrations of acrylamide in food should continue.
4. In addition, the Committee noted that it would be useful to have data on the occurrence of acrylamide in foods as consumed in developing countries. This information would be useful in conducting intake assessments, as well as considering mitigation approaches to reduce human exposure.

12. REFERENCES

- Abernethy, D.J. & Boreiko, C.J. (1987) Acrylonitrile and acrylamide fail to transform C3H/10T1/2 cells. *Environ. Mutagen.*, **9** (Suppl. 8), 2.
- Abou-Donia, M.B., Ibrahim, S.M., Corcoran, J.J., Lack, L., Friedman, M.A. & Lapadula, D.M. (1993) Neurotoxicity of glycidamide, an acrylamide metabolite, following intraperitoneal injections in rats. *J. Toxicol. Environ. Health*, **39** (4), 447–464.
- ACGIH (1991) *Documentation of the Threshold Limit Values and Biological Exposure Indices*, 6th Ed. Cincinnati, Ohio: American Conference of Governmental Industrial Hygienists, pp. 23–25.
- Adler, I.-D. (1990) Clastogenic effects of acrylamide in different germ-cell stages of male mice. In: Allen, J., Bridges, B., Lyon, M. & Moses, M., eds., *Biology of Mammalian Germ Cell Mutagenesis*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, pp. 115–131 (Banbury Report Vol. 34).
- Adler, I.-D., Ingwersen, I., Kliesch, U. & el Tarras, A. (1988) Clastogenic effects of acrylamide in mouse bone marrow cells. *Mutat. Res.*, **206**, 379–385.
- Adler, I.-D., Zouh, R. & Schmid, E. (1993) Perturbation of cell division by acrylamide in vitro and in vivo. *Mutat. Res.*, **301**, 249–254.
- Adler, I.-D., Reitmer, P., Schmöller, R. & Schriever-Schwemmer, G. (1994) Dose response for heritable translocations induced by acrylamide in spermatids of mice. *Mutat. Res.*, **309**, 285–291.
- AFSSA (2004) *Estimation de l'exposition à l'acrylamide de la population française (adultes et enfants)*. Maisons-Alfort: Agence Française de Sécurité Sanitaire des Aliments (Note technique OCA/AT/2004-232).
- Agrawal, A.K., Squibb, R.E. & Bondy, S.C. (1981) The effects of acrylamide treatment upon the dopamine receptor. *Toxicol. Appl. Pharmacol.*, **58**, 89–99.
- Ahn, J.S. & Castle, L. (2003) Tests for the depolymerization of polyacrylamides as a potential source of acrylamide in heated foods. *J. Agric. Food Chem.*, **51**, 6715–6718.
- Ahn, J.S., Castle, L., Clarke, D.B., Lloyd, A.S., Philo, M.R. & Speck, D.R. (2002) Verification of the findings of acrylamide in heated foods. *Food Addit. Contam.*, **19**, 1116–1124.
- Aldous, C., Farr, D. & Sharma, R. (1983) Evaluation of acrylamide treatment on levels of major brain biogenic amines, their turnover rates, and metabolites. *Fundam. Appl. Toxicol.*, **3**, 182–186.
- Ali, S.F. (1983) Acrylamide-induced changes in the monamines and their acid metabolites in different regions of the rat brain. *Toxicol. Lett.*, **17**, 101–105.

- Ali, S.F., Hong, J.-S., Wilson, W.E., Uphouse, L.L. & Bondy, S.C. (1983) Effect of acrylamide on neurotransmitter metabolism and neuropeptide levels in several brain regions and upon circulating hormones. *Arch. Toxicol.*, **52**, 35–43.
- Amrein, T.M., Bachmann, S., Noti, A., Biedermann, M., Ferraz Barbosa, M., Biedermann-Brem, S., Grob, K., Keiser, A., Realini, P., Escher, F. & Amadò, R. (2003) Potential of acrylamide formation, sugars, and free asparagine in potatoes: A comparison of cultivars and farming systems. *J. Agric. Food Chem.*, **51** (18), 5556–5560.
- Amrein, T.M., Schönbachler, B., Escher, F. & Amadò, R. (2004) Acrylamide in gingerbread: critical factors for formation and possible ways for reduction. *J. Agric. Food Chem.*, **52** (13), 4282–4288.
- Andrews, F., Greenhouse, S. & Draney, D. (1987) Chemistry of acrylamide bromination for trace analysis by gas-chromatography and gas-chromatography mass-spectrometry. *J. Chromatogr.*, **399**, 269–275.
- Andrzejewski, D., Roach, J.A.G., Gay, M.L. & Musser, S.M. (2004) Analysis of coffee for the presence of acrylamide by LC-MS/MS. *J. Agric. Food Chem.*, **52**, 1996–2002.
- Anklam, E. & Wenzl, T. (2005) Acrylamide in food: A survey of two years of research activities. *J. AOAC Int.*, **88** (1), 226.
- Ashby, P., Crimes, A., Foot, R., Gelbert, J., Gondé, P., Guenther, H., Lalljie, S., Lantz, I., Lindblom, M., Martell, H., Matissek, R., Muller, D., Soons, J., Stadler, R.H., Thompson, G., Ward, R. & Wood, J. (2004) *Acrylamide Status Report December 2004. A Summary of the Efforts and Progress Achieved to Date by the European Food and Drink Industry (CIAA) in Lowering Levels of Acrylamide in Food*. Brussels: Confederation of Food and Drink Industries of the European Union.
- Backer, L.C., Dearfield, K.L., Erexson, G.L., Campbell, J.A., Westbrook-Collins, B. & Allen, J.W. (1989) The effects of acrylamide on mouse germ-line and somatic cell chromosomes. *Environ. Mol. Mutagen.*, **13**, 218–226.
- Banerjee, S. & Segal, A. (1986) In vitro transformation of C3H/10T1/2 and NIH/3T3 cells by acrylonitrile and acrylamide. *Cancer Lett.*, **32**, 293–304.
- Barber, D.S., Hunt, J.R., Ehrich, M.F., Lehning, E.J. & LoPachin, R.M. (2001) Metabolism, toxicokinetics and hemoglobin adduct formation in rats following subacute and subchronic acrylamide dosing. *Neurotoxicology*, **22**, 341–353.
- Barfknecht, T.R., Mecca, D.J. & Naismith, R.W. (1988) The genotoxic activity of acrylamide. *Environ. Mol. Mutagen.*, **11** (Suppl. 11), 9.
- Batiste-Alentorn, M., Xamena, N., Creus, A. & Marcos, R. (1991) Genotoxicity studies with the unstable zeste-white (UZ) system of *Drosophila melanogaster*. Results with ten carcinogenic compounds. *Environ. Mol. Mutagen.*, **18**, 120–125.
- Becalski, A., Lau, B.P.-Y., Lewis, D. & Seaman, S.W. (2003) Acrylamide in foods: occurrence, sources, and modeling. *J. Agric. Food Chem.*, **51** (3), 802–808.
- Becalski, A., Lau, B.P.-Y., Lewis, D., Seaman, S.W., Stephen, H., Michael, S., Manoharan, R. & Yves, L. (2004) Acrylamide in french fries: influence of free amino acids and sugars. *J. Agric. Food Chem.*, **52** (12), 3801–3806.
- Beer, M.U., Schlatter, J., Dudler, V. & Zoller, O. (2004) Fried potatoes and human cancer. *Int. J. Cancer*, **108**, 634–635.
- Bergmark, E. (1997) Haemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers, and non-smokers. *Chem. Res. Toxicol.*, **10**, 78–84. As cited in JIFSAN/NCFST (2002).

- Bergmark, E., Calleman, C.J. & Costa, L.G. (1991) Formation of hemoglobin adducts of acrylamide and its epoxide metabolite glycidamide in the rat. *Toxicol. Appl. Pharmacol.*, **111**, 352–363.
- Bergmark, E., Calleman, C.J., He, F. & Costa, L.G. (1993) Haemoglobin adducts in humans occupationally exposed to acrylamide. *Toxicol. Appl. Pharmacol.*, **120**, 45–54. As cited in European Commission (2002b) and JIFSAN/NCFST (2002).
- Besaratinia, A. & Pfeifer, G.P. (2003) Weak yet distinct mutagenicity of acrylamide in mammalian cells. *J. Natl. Cancer Inst.*, **95**, 889–896.
- Besaratinia, A. & Pfeifer, G.P. (2004) Genotoxicity of acrylamide and glycidamide. *J. Natl. Cancer Inst.*, **96**, 1023–1029.
- Biedermann, M. & Grob, K. (2003) Model studies on acrylamide formation in potato, wheat flour and corn starch; ways to reduce acrylamide contents in bakery ware. *Mitt. Lebensm. Hyg.*, **94**, 406–422.
- Biedermann, M., Biedermann-Brem, S., Noti, A. & Grob, K. (2002a) Methods for determining the potential of acrylamide formation and its elimination in raw materials for food preparation, such as potatoes. *Mitt. Lebensm. Hyg.*, **93**, 653–667.
- Biedermann, M., Biedermann-Brem, S., Mozzetti, V. & Grob, K. (2002b) Experiments on acrylamide formation and possibilities to decrease the potential of acrylamide formation in potatoes. *Mitt. Lebensm. Hyg.*, **93**, 668–687.
- Biedermann, M., Biedermann-Brem, S., Noti, A., Grob, K., Egli, P. & Mändli, H. (2002c) Two GC-MS methods for the analysis of acrylamide in foods. *Mitt. Lebensm. Hyg.*, **93**, 638–652.
- Biedermann, M., Grob, K., Gutsche, B. & Weisshaar, R. (2003) Heated foods: The component eluted next to acrylamide is 2-pyrrolidinone, not 3-butenamide. *Dtsch. Lebensm.-Rundsch.*, **99** (5), 171–175.
- Bio/dynamics, Inc. (1979) *A Fetal Toxicity Study of Acrylamide in Rats*. Conducted by Bio/dynamics, Inc., East Millstone, New Jersey. Sponsored by American Cyanamid Company, Wayne, New Jersey (EPA/OTS Document No. 878211679; NTIS/OTS0206055).
- Bishop, J.B., Morris, R.W., Seely, J.C., Hughes, L.A., Cain, K.T. & Generoso, W.M. (1997) Alterations in the reproductive patterns of female mice exposed to xenobiotics. *Fundam. Appl. Toxicol.*, **40**, 191–204.
- Boettcher, M.I., Schettgen, T., Kutting, B., Pischetsrieder, M. & Angerer, J. (2005) Mercapturic acids of acrylamide and glycidamide as biomarkers of the internal exposure to acrylamide in the general population. *Mutat. Res.*, **580**, 167–176.
- Bologna, L.S., Andrawes, F.F., Barvenik, F.W., Lentz, R.D. & Sojka, R.E. (1999) Analysis of residual acrylamide in field crops. *J. Chromatogr. Sci.*, **37**, 240–244.
- Bosetti, C., Talamini, R., Levi, F., Negri, E., Franceschi, S., Airoldi, L. & La Vecchia, C. (2003) Fried foods: a risk factor for laryngeal cancer? *Br. J. Cancer*, **87** (11), 1230–1233.
- Brown, L. & Rhead, M. (1979) Liquid chromatographic determination of acrylamide monomer in natural and polluted aqueous environments. *Analyst*, **104**, 391–399.
- Bucher, J.R., Huff, J., Haseman, J.K., Eustis, S.L., Peters, A. & Toft, J.D. (1990) Neurotoxicity and carcinogenicity of *N*-methylolacrylamide in F344 rats and B6C3F1 mice. *J. Toxicol. Environ. Health*, **31**, 161–177.
- Bull, R.J., Robinson, M., Laurie, R.D., Stoner, G.D., Greisiger, E., Meier, J.R. & Stober, J. (1984a) Carcinogenic effects of acrylamide in Sencar and A/J mice. *Cancer Res.*, **44**, 107–111.

- Bull, R.J., Robinson, M. & Stober, J.A. (1984b) Carcinogenic activity of acrylamide in the skin and lung of Swiss-ICR mice. *Cancer Lett.*, **24**, 209–212.
- Burek, J.D., Albee, R.R., Beyer, J.E., Bell, T.J., Carreon, R.M., Morden, D.C., Wade, C.E., Hermann, E.A. & Gorzinski, S.J. (1980) Subchronic toxicity of acrylamide administered to rats in the drinking water followed by up to 14 days of recovery. *J. Environ. Pathol. Toxicol.*, **4**, 157–182.
- Butterworth, B.E., Eldridge, S.R., Sprankle, C.S., Working, P.K., Bentley, K.S. & Hurtt, M.E. (1992) Tissue-specific genotoxic effects of acrylamide and acrylonitrile. *Environ. Mol. Mutagen.*, **20**, 148–155.
- CAC (2004) *Report of the Thirty-sixth Session of the Codex Committee on Food Additives and Contaminants, Rotterdam, The Netherlands, 22–26 March 2004*. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ALINORM 04/27/12; <http://www.codexalimentarius.net/web/archives.jsp?lang=en>).
- Calleman, C.J. (1996) The metabolism and pharmacokinetics of acrylamide: implications for mechanisms of toxicity and human risk assessment. *Drug Metab. Rev.*, **28**, 527–590.
- Calleman, C.J., Bergmark, E. & Costa, L.G. (1990) Acrylamide is metabolized to glycidamide in the rat: Evidence from hemoglobin adduct formation. *Chem. Res. Toxicol.*, **3**, 406–412.
- Calleman, C.J., Wu, Y., He, F., Tian, G., Bergmark, E., Zhang, S., Deng, H., Wang, Y., Crofton, K.M., Fennell, T. & Costa, L.G. (1994) Relationship between biomarkers of exposure and neurological effects in a group of workers exposed to acrylamide. *Toxicol. Appl. Pharmacol.*, **126**, 361–371. As cited in European Commission (2002b) and JIFSAN/NCFST (2002).
- Castle, L. (1993) Determination of acrylamide monomer in mushrooms grown on polyacrylamide gel. *J. Agric. Food Chem.*, **41**, 1261–1263.
- Castle, L., Campos, M.-J. & Gilbert, J. (1991) Determination of acrylamide monomer in hydroponically grown tomato fruits by capillary gas-chromatography mass-spectrometry. *J. Sci. Food Agric.*, **54**, 549–555.
- Cavalli, S., Maurer, R. & Höfler, F. (2003) Fast determination of acrylamide in food samples using accelerated solvent extraction followed by ion chromatography with UV or MS detection. *LC-GC Eur.*, **April**, 9–11.
- CERHR (2004) *NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Acrylamide*. Research Triangle Park, North Carolina: United States Department of Health and Human Services, National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR-Acrylamide-04; <http://cerhr.niehs.nih.gov>).
- Chapin, R.E., Fail, P.A., George, J.D., Grizzle, T.B., Heindel, J.J., Harry, G.J., Collins, B.J. & Teague, J. (1995) The reproductive and neural toxicities of acrylamide and three analogues in Swiss mice, evaluated using the continuous breeding protocol. *Fundam. Appl. Toxicol.*, **27**, 9–24.
- Chen, J. (2005) Submission to the 64th JECFA meeting: Dietary acrylamide intakes in China. Chinese Center for Disease Control and Prevention (2005) Submission to the 64th JECFA meeting: Chinese occurrence data on acrylamide. The Institute of Nutrition and Food Safety.
- Cihák, R. & Vontorková, M. (1988) Cytogenetic effects of acrylamide in the bone marrow of mice. *Mutat. Res.*, **209**, 91–94.
- Clarke, D.B., Kelly, J. & Wilson, L.A. (2002) Assessment of performance of laboratories in determining acrylamide in crispbread. *J. AOAC Int.*, **85**, 1370–1373.

- Collins, B.W., Howard, D.R. & Allen, J.W. (1992) Kinetochore-staining of spermatid micronuclei: Studies of mice treated with X-radiation or acrylamide. *Mutat. Res.*, **281**, 287–294.
- Costa, L.G., Deng, H., Gregotti, C., Manzo, L., Faustman, E.M., Bergmark, E. & Calleman, C. (1992) Comparative studies on the neuro- and reproductive toxicity of acrylamide and its epoxide metabolite glycidamide in the rat. *Neurotoxicology*, **13** (1), 219–224.
- Costa, L.G., Deng, H., Calleman, C.J. & Bergmark, E. (1995) Evaluation of the neurotoxicity of glycidamide, an epoxide metabolite of acrylamide: behavioral, neurochemical and morphological studies. *Toxicology*, **98** (1–3), 151–161.
- Croft, M., Tong, P., Fuentes, D. & Hambridge, T. (2004) Australian survey of acrylamide in carbohydrate-based foods. *Food Addit. Contam.*, **21** (8), 721–736.
- Crofton, K.M., Padilla, S., Tilson, H.A., Anthony, D.C., Raymer, J.H. & MacPhail, R.C. (1996) The impact of dose rate on the neurotoxicity of acrylamide: the interaction of administered dose, target tissue concentrations, tissue damage, and functional effects. *Toxicol. Appl. Pharmacol.*, **139**, 163–176.
- Cutié, S.S. & Kallos, G.J. (1986) Determination of acrylamide in sugar by thermospray liquid chromatography/mass spectrometry. *Anal. Chem.*, **58**, 2425–2428.
- Cyanamid (1969) *Chemistry of Acrylamide*. Wayne, New Jersey: American Cyanamid Co., Process Chemicals Department (Bulletin PRC 109).
- Dearfield, K.L., Douglas, G.R., Ehling, U.H., Moore, M.M., Sega, A. & Brusick, D.J. (1995) Acrylamide: a review of its genotoxicity and an assessment of heritable genetic risk. *Mutat. Res.*, **330**, 71–99.
- Deng, H., Jiao, X. & He, F. (1997) [A study on neurotoxicity of acrylamide and glycidamide.] *Zhonghua Yu Fang Yi Xue Za Zhi*, **31** (4), 202–205 (in Chinese).
- DeVries, J.W. & Post, B.E. (2004) Comment on “Soxhlet extraction of acrylamide from potato chips” by J. R. Pedersen and J. O. Olsson, *Analyst*, 2003, 128, 332. *Analyst*, **129**, 93–95.
- De Wilde, T., De Meulenaer, B., Mestdagh, F., Govaert, Y., Fraselle, S., Degroot, J.M., Vandeburie, S., Demeulemester, K., Calus, A., Ooghe, W. & van Peteghem, C. (2004) Acrylamide formation during the frying of potatoes: thorough investigations on the influence of crop and process variables. *Commun. Agric. Appl. Biol. Sci.*, **69**, 109–112.
- DiNovi, M. & Howard, D. (2004) The updated exposure assessment for acrylamide. In: *2004 Acrylamide in Food Workshop: Update — Scientific Issues, Uncertainties, and Research Strategies, 13–15 April 2004, Chicago, Illinois*. College Park, Maryland: Joint Institute for Food Safety and Applied Nutrition (<http://www.jifsan.umd.edu/acrylamide2004.htm>).
- Dixit, R., Seth, P.K. & Mukhtar, H. (1982) Metabolism of acrylamide into urinary mercapturic acid and cysteine conjugates in rats. *Drug Metab. Dispos.*, **10**, 196–197.
- Doerge, D.R., Young, J.F., McDaniel, L.P., Twaddle, N.C., Churchwell, M.I. & Beland, F.A. (2005a) Toxicokinetics of acrylamide and glycidamide in B6C3F1 mice. *Toxicol. Appl. Pharmacol.*, **202**, 258–267.
- Doerge, D.R., Young, J.F., McDaniel, L.P., Twaddle, N.C. & Churchwell, M.I. (2005b) Toxicokinetics of acrylamide and glycidamide in Fischer 344 rats. *Toxicol. Appl. Pharmacol.*, **208**, 199–209.
- Doerge, D.R., Gamboa da Costa, G., McDaniel, L.P., Twaddle, N.C. & Churchwell, M.I. (2005c) DNA adducts derived from administration of acrylamide and glycidamide to mice and rats. *Mutat. Res.*, **580**, 131–141.

- Doyle, M.E. (2003) *Reported Free Asparagine Levels in Foods*. Madison, Wisconsin: University of Wisconsin–Madison, Food Research Institute (<http://www.wisc.edu/fri/briefs/asparagine1102.pdf>).
- Dybing, E. & Sanner, T. (2003) Risk assessment of acrylamide in foods. *Toxicol. Sci.*, **75**, 7–15.
- Edwards, P.M. (1976) The insensitivity of the developing rat foetus to the toxic effects of acrylamide. *Chem.-Biol. Interact.*, **12**, 13–18.
- Ehling, U.H. & Neuhäuser-Klaus, A. (1992) Reevaluation of the induction of specific-locus mutations in spermatogonia of the mouse by acrylamide. *Mutat. Res.*, **283**, 185–191.
- El-Hadri, L. & Ghanayem, B.I. (2004) Comparative metabolism and disposition of 1-¹⁴C- and 2,3-¹⁴C-acrylamide in cytochrome P450 2E1-null (KO) and wild-type (WT) mice. *Toxicologist*, **78** (S-1), 1444.
- Elmore, J.S. & Mottram, D.S. (2002) *Compilation of Free Amino Acid Data for Various Food Raw Materials, Showing the Relative Contributions of Asparagine, Glutamine, Aspartic Acid and Glutamic Acid to the Free Amino Acid Composition*. Whiteknights, Reading: The University of Reading, School of Food Biosciences (http://www.jifsan.umd.edu/presentations/acrylamide2002/wg1_asparagine_in_foods.pdf).
- Eskin, T., Lapham, L., Maurissen, J. & Merigan, W. (1985) Acrylamide effects on the macaque visual system. II. Retinogeniculate morphology. *Invest. Ophthalmol. Vis. Sci.*, **26** (3), 317–329.
- European Commission (2002a) *European Union Risk Assessment Report Acrylamide, 2002*. Luxembourg: European Commission, European Chemicals Bureau, Institute for Health and Consumer Protection, Office for Official Publication of the European Communities (ISBN 92-894-1250-X).
- European Commission (2002b) *Opinion of the Scientific Committee on Food on New Findings Regarding the Presence of Acrylamide in Food*. Brussels: European Commission, Scientific Committee on Food (SCF/CS/CNTM/CONT/4 Final).
- European Commission (2003) *Note of the Meeting of Experts on Industrial Contaminants in Food: Acrylamide Workshop, 20–21 October 2003*. Brussels: European Commission (http://europa.eu.int/comm/food/food/chemicalsafety/contaminants/acryl_guidance.pdf).
- European Food Safety Authority (2004) *Workshop on Acrylamide Formation in Food, 17 November 2003, Brussels: Report of the Workshop*. Brussels: European Food Safety Authority, 22 pp. (http://www.efsa.eu.int/science/ahawdocuments/330/other_01_acrylamide_report_anne_x_en1.pdf).
- European Union (2004) *EU Monitoring Database of Acrylamide Levels in Food, Status June 2004*.
- FAO/WHO (2002) *Health Implications of Acrylamide in Food, Report of a Joint FAO/WHO Consultation, 25–27 June 2002*. Geneva: World Health Organization (<http://www.who.int/fsf/acrylamide/SummaryReportFinal.pdk>).
- Favor, J. & Shelby, M.D. (2005) Transmitted mutational events induced in mouse germ cells following acrylamide or glycidamide exposure. *Mutat. Res.*, **580**, 21–30.
- FEHD (2003) *Chemical Hazard Evaluation in Food*. Queensway, Hong Kong SAR: The Government of the Hong Kong Special Administrative Region, Food and Environmental Hygiene Department, December (Report No. 11) (http://www.fehd.gov.hk/safefood/report/acrylamide/ras_acrylamide.html).

- Fennell, T.R., Snyder, R.W., Krol, W.L. & Sumner, S.C.J. (2003) Comparison of the hemoglobin adducts formed by administration of *N*-methylolacrylamide and acrylamide to rats. *Toxicol. Sci.*, **71**, 164–175.
- Fennell, T.R., Sumner, S.C., Snyder, R.W., Burgess, J., Spicer, R., Bridson, W.E. & Friedman, M.A. (2005) Metabolism and hemoglobin adduct formation of acrylamide in humans. *Toxicol. Sci.*, **85**, 447–459.
- Field, E.A., Price, C.J., Sleet, R.B., Marr, M.C., Schwetz, B.A. & Morrissey, R.E. (1990) Developmental toxicity evaluation of acrylamide in rats and mice. *Fundam. Appl. Toxicol.*, **14**, 502–512.
- Fiselier, K., Gama-Baumgartner, F., Fiscalini, A., Biedermann, M., Grob, K., Imhof, D. & Beer, M. (2004) Good manufacturing practice (GMP) for french fries low in acrylamide: results of a pilot project. *Mitt. Lebensm. Hyg.*, **95** (2), 127–134.
- Fohgelberg, P., Rosen, J., Hellenas, K.E. & Abramsson-Zetterberg, L. (2005) The acrylamide intake via some common baby food for children in Sweden during their first year of life — an improved method for analysis of acrylamide. *Food Chem. Toxicol.*, **43** (6), 951–959.
- Food Standards Australia New Zealand (2004) Submission to the 64th JECFA meeting: New Zealand exposure data on acrylamide.
- Fredriksson, H., Tallving, J., Rosén, J. & Åman, P. (2004) Fermentation reduces free asparagine in dough and acrylamide in bread. *Cereal Chem.*, **81** (5), 650–653.
- Friedman, M. (2003) Chemistry, biochemistry, and safety of acrylamide, a review. *J. Agric. Food Chem.*, **51** (16), 4504–4526.
- Friedman, M.A., Dulak, L.H. & Stedham, M.A. (1995) A lifetime oncogenicity study in rats with acrylamide. *Fundam. Appl. Toxicol.*, **27**, 95–105.
- Friedman, M.A., Tyl, R.W., Marr, M.C., Myers, C.B., Gerling, F.S. & Ross, W.P. (1999) Effects of lactational administration of acrylamide on rat dams and offspring. *Reprod. Toxicol.*, **13**, 511–520.
- FSA (2004) *Results of Analysis of UK Total Diet Study (TDS) Samples for Acrylamide and Dietary Exposure Estimates, December 2004*. London: Food Standards Agency (<http://www.food.gov.uk>).
- Fullerton, P.M. & Barnes, J.M. (1966) Peripheral neuropathy in rats produced by acrylamide. *Br. J. Ind. Med.*, **23** (3), 210–221.
- Gama-Baumgartner, F., Grob, K. & Biedermann, M. (2004) Citric acid to reduce acrylamide formation in french fries and roasted potatoes? *Mitt. Lebensm. Hyg.*, **95** (1), 110–117.
- Gamboa da Costa, G., Churchwell, M.I., Hamilton, L.P., Beland, F.A., Marques, M.M. & Doerge, D.R. (2003) DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal mice. *Chem. Res. Toxicol.*, **16**, 1328–1337.
- Gertz, C., Klostermann, S. & Kochhar, S.P. (2003) Deep frying: the role of water from the food frying process and formation of acrylamide. *Oléagineux Corps Gras Lipides (OCL)*, **10** (4), 297–303.
- Ghanayem, B.I., Witt, K.L., El-Hadri, L., Hoffler, U., Kissling, G.E., Shelby, M.D. & Bishop, J.B. (2005a) Comparison of germ cell mutagenicity in male CYP2E1-null and wild-type mice treated with acrylamide: evidence supporting a glycidamide-mediated effect. *Biol. Reprod.*, **72**, 157–163.
- Ghanayem, B.I., McDaniel, L.P., Churchwell, M.I., Twaddle, N.C., Snyder, R., Fennell, R.R. and Doerge, D.R. (2005b) Role of CYP2E1 in the epoxidation of acrylamide to glycidamide and formation of DNA and hemoglobin adducts. *Toxicol. Sci.*, **88**, 311–318.

- Gilbert, S. & Maurissen, J. (1982) Assessment of the effects of acrylamide, methylmercury, and 2,5-hexanedione on motor functions in mice. *J. Toxicol. Environ. Health*, **10**, 31–41.
- Godin, A.C., Bengtsson, B., Niskanen, R., Tareke, E., Törnqvist, M. & Forslund, K. (2002) Acrylamide and N-methylolacrylamide poisoning in a herd of Charolais crossbreed cattle. *Vet. Rec.*, **151**, 724–728.
- Gold, B.G. & Schaumburg, H.H. (2000) Acrylamide. In: Spencer, P.S., Schaumburg, H.H. & Lucolph, A.C., eds., *Experimental and Clinical Neurotoxicology*, 2nd Ed. New York: Oxford University Press, pp. 123–132.
- Granada, C., Moreira, R.G. & Tichy, S.E. (2004) Reduction of acrylamide formation in potato chips by low-temperature vacuum frying. *J. Food Sci.*, **69** (8), E405–411.
- Grandby, K. & Fagt, S. (2004) Analysis of acrylamide in coffee and dietary exposure to acrylamide from coffee. *Anal. Chim. Acta*, **520**, 177–182.
- Granvogl, M., Jezussek, M., Koehler, P. & Schieberle, P.J. (2004) Quantitation of 3-aminopropionamide in potatoes — a minor but potent precursor in acrylamide formation. *J. Agric. Food Chem.*, **52** (15), 4751–4757.
- Grob, K., Biedermann, M., Biedermann-Brem, S., Noti, A., Imhof, D., Amrein, T., Pfeifferle, A. & Bazocco, D. (2003) French fries with less than 100 mg/kg acrylamide. A collaboration between cooks and analysts. *Eur. Food Res. Technol. A*, **217** (3), 185–194.
- Grob, K., Biedermann, M., Hoenicke, K. & Gatermann, R. (2004) Comment on “Soxhlet extraction of acrylamide from potato chips” by J. R. Pedersen and J. O. Olsson, *Analyst*, 2003, 128, 332. *Analyst*, **129**, 92.
- Gutsche, B., Weißhaar, R. & Buhlert, J. (2002) [Acrylamide in food — Screening results from food control in Baden-Württemberg.] *Dtsch. Lebensm.-Rundsch.*, **98**, 437–443 (in German).
- Haase, N.U. & Weber, L. (2003) Variability of sugar content in potato varieties suitable for processing. *Food Agric. Environ.*, **1**, 80–81.
- Haase, N.U., Matthäus, B. & Vosmann, K. (2003) [Acrylamide formation in foodstuff — minimising strategies for potato crisps.] *Dtsch. Lebensm.-Rundsch.*, **99** (3), 87–90 (in German).
- Habermann, C.E. (1991) Acrylamide. In: Kroschwitz, J.J. & Howe-Grant, M., eds., *Kirk-Othmer Encyclopedia of Chemical Technology*, 4th Ed. Vol. 1. New York: J. Wiley & Sons, pp. 251–266.
- Hagmar, L., Törnqvist, M., Nordander, C., Rosen, I., Bruze, M., Kautiainen, A., Magnusson, A.L., Malmberg, B., Aprea, P., Granath, F. & Axmon, A. (2001) Health effects of occupational exposure to acrylamide using hemoglobin adducts as biomarkers of internal dose. *Scand. J. Environ. Health*, **27**, 219–226.
- Hartig, L., Hummert, C., Buhlert, J., von Czapiwski, K. & Schreiber, A. (2003) Detection of acrylamide in starch-enriched foods by HPLC/MS/MS. Darmstadt: Applied Biosystems (Applied Biosystems Application Note No. 02; <http://www.appliedbiosystems.com>).
- Hashimoto, K. & Aldridge, W.N. (1970) Biochemical studies on acrylamide, a neurotoxic agent. *Biochem. Pharmacol.*, **19**, 2591–2604.
- Hashimoto, K. & Tanii, H. (1985) Mutagenicity of acrylamide and its analogues in *Salmonella typhimurium*. *Mutat. Res.*, **158**, 129–133.
- Hashimoto, K., Sakamoto, J. & Tanii, H. (1981) Neurotoxicity of acrylamide and related compounds and their effects on male gonads in mice. *Arch. Toxicol.*, **47**, 179–189.
- Hattis, D. and Shapiro, K. (1990) Analysis of dose/time/response relationships for chronic toxic effects: the case of acrylamide. *Neurotoxicology*, **11**, 219–236.

- Hersch, M., McLeod, J., Satchell, P., Early, R. & Sullivan, D. (1989) Breathing pattern, lung inflation reflex and airway tone in acrylamide neuropathy. *Resp. Physiol.*, **76**, 257–276.
- Holland, N., Ahlborn, T., Turteltaub, K., Markee, C., Moore, D., II, Wyrobek, A.J. & Smith, M.T. (1999) Acrylamide causes preimplantation abnormalities in embryos and induces chromatin adducts in male germ cells of mice. *Reprod. Toxicol.*, **13**, 167–178.
- Hoon, A.J.W., Custer, L.L., Myhr, B.C., Brusick, D., Gossen, J. & Vijg, J. (1993) Detection of chemical mutagens using Muta Mouse: a transgenic mouse model. *Mutagenesis*, **8** (1), 7–10.
- Hughes, E., Newton, D., Harling, R. & Begg, S. (1994) *Validation of Neurotoxicity Screen with Reference to Motor and Locomotor Functions*. Huntingdon, Cambridgeshire: Huntingdon Research Centre, Ltd.
- Husain, R., Dixit, R., Das, M. & Seth, P.K. (1987) Neurotoxicity of acrylamide in developing rat brain: changes in the levels of brain biogenic amines and activities of monoamine oxidase and acetylcholine esterase. *Ind. Health*, **25**, 19–28.
- IARC (1994a) Ethylene oxide. In: *Some Industrial Chemicals*. Lyon, International Agency for Research on Cancer, pp. 73–159 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 60).
- IARC (1994b) Acrylamide. In: *Some Industrial Chemicals*. Lyon, International Agency for Research on Cancer, pp. 389–433 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 60).
- IARC (1999) Acrylonitrile. In: *Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide*. Lyon, International Agency for Research on Cancer (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 71).
- Ikeda, G.J., Miller, E., Sapienza, P.P., Michel, T.C., King, M.T. & Sager, A.O. (1985) Maternal–foetal distribution studies in late pregnancy. II. Distribution of [$1\text{-}^{14}\text{C}$] acrylamide in tissues of beagle dogs and miniature pigs. *Food Chem. Toxicol.*, **23** (8), 757–776.
- Ikeda, G., Miller, E., Sapienza, P., Michel, T. & Inskeep, P. (1987) Comparative tissue distribution and excretion of [$1\text{-}^{14}\text{C}$]acrylamide in beagle dogs and miniature pigs. *Food Chem. Toxicol.*, **25** (11), 871–875.
- Inoue, K., Yoshimura, Y. & Nakazawa, H. (2003) Development of high-performance liquid chromatography – electrospray mass spectrometry with size-exclusion chromatography for determination of acrylamide in fried foods. *J. Liq. Chromatogr. Rel. Technol.*, **26**, 1877–1884.
- Institutet för Miljömedicin (1998) *Halsoriskbedömning av akrylamid och metyololakrylamid*. Stockholm: Institutet för Miljömedicin (IMM Report 2/98).
- IPCS (2005) *Principles for Modelling Dose–Response for the Risk Assessment of Chemicals*. Geneva, World Health Organization, International Programme on Chemical Safety (draft; http://www.who.int/ipcs/methods/harmonization/dose_response/en/).
- Japan Ministry of Agriculture, Forestry and Fisheries (2004) Submission to the 64th JECFA meeting: Occurrence data on acrylamide in food.
- Jezussek, M. & Schieberle, P. (2003) A new LC/MS-method for the quantitation of acrylamide based on a stable isotope dilution assay and derivatization with 2-mercaptobenzoic acid. Comparison with two GC/MS methods. *J. Agric. Food Chem.*, **51**, 7866–7871.
- JIFSAN (2004) *2004 Acrylamide in Food Workshop: Update — Scientific Issues, Uncertainties, and Research Strategies*, 13–15 April 2004, Chicago, Illinois. College

- Park, Maryland: Joint Institute for Food Safety and Applied Nutrition (<http://www.jifsan.umd.edu/acrylamide2004.htm>).
- JIFSAN/NCFST (2002) *Acrylamide in Food Workshop: Scientific Issues, Uncertainties, and Research Strategies*. Chicago, Illinois: Joint Institute for Food Safety and Applied Nutrition and National Center for Food Safety and Technology.
- Johnson, K.A., Gorzinski, S.J., Bodner, K.M., Campbell, R.A., Wolf, C.H., Friedman, M.A. & Mast, R. (1986) Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicol. Appl. Pharmacol.*, **85**, 154–168.
- Jung, M.Y., Choi, D.S. & Ju, J.W. (2003) A novel technique for limitation of acrylamide formation in fried and baked corn chips and in french fries. *J. Food Sci.*, **68** (4), 1287–1290.
- Jung, R., Engelhart, G., Herbolt, B., Jackh, R. & Muller, W. (1992) Collaborative study of mutagenicity with *Salmonella typhimurium* TA102. *Mutat. Res.*, **278**, 265–270.
- Kadry, A.M., Friedman, M.A. & Abdel-Rahman, M.S. (1999) Pharmacokinetics of acrylamide after oral administration in male rats. *Environ. Toxicol. Pharmacol.*, **7**, 127–133.
- Kaster, J.K., Kamendulis, L.M., Friedman, M.A. & Klaunig, J.E. (1998) Syrian hamster embryo (SHE) cell transformation by acrylamide and hormones. *Toxicologist*, **42** (1-S), A375.
- Khan, M.A., Davis, C.A., Foley, G.L., Friedman, M.A. & Hansen, L.G. (1999) Changes in thyroid gland morphology after acute acrylamide exposure. *Toxicol. Sci.*, **47**, 151–157.
- Kirman, C.R., Gargas, M.L., Deskin, R., Tonner-Navarro, L. & Anderson, M.E. (2003) A physiologically based pharmacokinetic model for acrylamide and its metabolite, glycidamide, in the rat. *J. Toxicol. Environ. Health A*, **66**, 253–274.
- Kita, A., Bråthen, E., Knutsen, S.H. & Wicklund, T. (2004) Effective ways of decreasing acrylamide content in potato crisps during processing. *J. Agric. Food Chem.*, **52** (23), 7011–7016.
- Kjuus, H., Goffeng, L.O., Heier, M.S., Sjöholm, H., Ovrebo, S., Skaug, V., Paulsson, B., Tornqvist, M. & Brudal, S. (2004) Effects on the peripheral nervous system of tunnel workers exposed to acrylamide and *N*-methylolacrylamide. *Scand. J. Work Environ. Health*, **30**, 21–29.
- Kjuus, H., Hansteen, I.L., Ryberg, D., Goffeng, L.O., Ovrebo, S. & Skaug, V. (2005) Chromosome aberrations in tunnel workers exposed to acrylamide and *N*-methylolacrylamide. *Scand. J. Work Environ. Health*, **31**, 300–306.
- Kligerman, A.D., Atwater, A.L. & Bryant, M.F. (1991) Cytogenetic studies of ethyl acrylate using C57BL/6 mice. *Mutagenesis*, **6** (2), 137–141.
- Knaap, A.G.A., Kramers, P.G.N., Voogd, C.E., Bergkamp, W.G., Groot, M.G., Langebroek, P.G., Mout, H.C., van der Stel, J.J. & Verharen, H.W. (1988) Mutagenic activity of acrylamide in eukaryotic systems but not in bacteria. *Mutagenesis*, **3** (3), 263–268.
- Konings, E.J.M., Baars, A.J., Van Klaveren, J.D., Spanjer, M.C., Rensen, P.M., Hiemstra, M., Van Kooij, J.A. & Peters, P.W.J. (2003) Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. *Food Chem. Toxicol.*, **41**, 1569–1579.
- Krebs, O. & Favor, J. (1997) Somatic and germ cell mutagenesis in lambda lacZ transgenic mice treated with acrylamide or ethylnitrosourea. *Mutat. Res.*, **388**, 239–248.
- Krishna, G. & Theiss, J.C. (1995) Concurrent analysis of cytogenetic damage in vivo: A multiple endpoint – multiple tissue approach. *Environ. Mol. Mutagen.*, **25**, 314–320.

- Lafferty, J.S., Kamendulis, L.M., Kaster, J.L., Jiang, J. & Klaunig, J.E. (2004) Subchronic acrylamide treatment induces a tissue-specific increase in DNA synthesis in the rat. *Toxicol. Lett.*, **154**, 95–103.
- Lähdetie, J., Suutari, A. & Sjöblom, T. (1994) The spermatid micronucleus test with the dissection technique detects the germ cell mutagenicity of acrylamide in rat meiotic cells. *Mutat. Res.*, **309**, 255–262.
- Lande, S.S., Bosch, S.J. & Howard, P.H. (1979) Degradation and leaching of acrylamide in soil. *J. Environ. Qual.*, **8**, 133–137.
- Lehning, E.J., Balaban, C.D., Ross, J.F., Reid, M.A. & LoPachin, R.M. (2002) Acrylamide neuropathy. I. Spatiotemporal characteristics of nerve cell damage in rat cerebellum. *Neurotoxicology*, **23**, 397–414.
- Leung, K.S., Lin, A., Tsang, C.K. & Yeung, S.T.K. (2003) Acrylamide in Asian foods in Hong Kong. *Food Addit. Contam.*, **20**, 1105–1113.
- Licea-Perez, H., Cheong, H.K., Yang, J.S. & Osterman-Golkar, S. (1999) Simultaneous analysis of hemoglobin adducts of acrylamide and glycidamide by gas chromatography–mass spectrometry. *Anal. Biochem.*, **274**, 59–68.
- Lijinsky, W. & Andrews, A.W. (1980) Mutagenicity of vinyl compounds in *Salmonella typhimurium*. *Teratogen. Carcinogen. Mutagen.*, **1**, 259–267.
- LoPachin, R.M. (2004) A changing view of acrylamide neurotoxicity. *Neurol. Toxicol.*, **25**, 617–630.
- LoPachin, R.M., Lehning, E.J., Opananshuk, L.A. & Jortner, G.S. (2000) Rate of neurotoxicant exposure determines morphologic manifestations of digital axonopathy. *Toxicol. Appl. Pharmacol.*, **167**, 75–86.
- LoPachin, R.M., Ross, J.F. & Lehning, E.J. (2002) Nerve terminals as the primary site of acrylamide action: a hypothesis. *Neurotoxicology*, **23**, 43–59.
- LoPachin, R.M., Balaban, C.D. & Ross, J.F. (2003) Acrylamide neuropathy revisited. *Toxicol. Appl. Pharmacol.*, **188**, 135–153.
- Madduri, V.R. & Ragaee, S. (2004) Submission to the 64th JECFA meeting: Monitoring and dietary intake assessment of acrylamide in the United Arab Emirates. United Arab Emirates University.
- Manière, I., Godard, T., Doerge, D.R., Churchwell, M.I., Guffroy, M., Laurentie, M. & Poul, J.-M. (2005) DNA damage and DNA adduct formation in rat tissues following oral administration of acrylamide. *Mutat. Res.*, **580**, 119–129.
- Manjanatha, M.G., Aidoo, A., Shelton, S.D., Bishop, M.E., McDaniel, L.P., Lyn-Cook, L.E. & Doerge, D.R. (in press) Genotoxicity of acrylamide and its metabolite glycidamide administered in drinking water to male and female Big Blue (BB) mice. *Environ. Mol. Mutagen.*
- Marlowe, C., Clark, M.J., Mast, R.W., Friedman, M.A. and Waddell, W.J. (1986) The distribution of [^{14}C]acrylamide in male and pregnant Swiss-Webster mice studied by whole-body autoradiography. *Toxicol. Appl. Pharmacol.*, **86** (3), 457–465.
- Marsh, G.M., Lucas, L.J., Youk, A.O. & Schall, L.C. (1999) Mortality patterns among workers exposed to acrylamide: 1994 follow up. *Occup. Environ. Med.*, **56**, 181–190. As cited in JIFSAN/NCFST (2002).
- Matthäus, B., Haase, N.U. & Vosmann, K. (2004) Factors affecting the concentration of acrylamide during deep-fat frying of potatoes. *Eur. J. Lipid Sci. Technol.*, **106** (11), 793–801.

- Matthys, C., Bilau, M., Govaert, Y., Moons, E., De Henauw, S. & Willems, J.L. (2005) Risk assessment of dietary acrylamide intake in Flemish adolescents. *Food Chem. Toxicol.*, **43** (2), 271–278.
- Maurissen, J., Weiss, B. & Davis, H. (1983) Somatosensory thresholds in monkeys exposed to acrylamide. *Toxicol. Appl. Pharmacol.*, **71** (2), 266–279.
- Maurissen, J., Weiss, B. & Cox, C. (1990) Vibration sensitivity recovery after a second course of acrylamide intoxication. *Fundam. Appl. Toxicol.*, **15** (1), 93–98.
- McClure, P.R., Wohlers, D.W. & DeWoskin, R.S. (2004) New data and guidelines support a revised cancer risk assessment for acrylamide. *Toxicologist*, **78** (S-1), 1788.
- McCollister, D., Oyen, F. & Rowe, V. (1964) Toxicology of acrylamide. *Toxicol. Appl. Pharmacol.*, **6**, 172–181.
- McHale, K.J., Winnik, W. & Paul, G. (2003) *Quantitation of Acrylamide in Food Samples on the FinniganTSQ Quantum Discovery by LC/APCI-MS/MS*. Thermo Electron Corporation (http://www.thermo.com/com/cda/products/product_application_details/1,1063,11684,00.html) (Application Note 319).
- Merigan, W.H., Barkdoll, E. & Maurissen, J.P. (1982) Acrylamide-induced visual impairment in primates. *Toxicol. Appl. Pharmacol.*, **62** (2), 342–345.
- Merigan, W., Barkdoll, E., Maurissen, J., Eskin, T. & Lapham, L. (1985) Acrylamide effects on the macaque visual system. I. Psychophysics and electrophysiology. *Invest. Ophthalmol. Vis. Sci.*, **26**, 30–36.
- Miller, M.J., Carter, D.E. & Sipes, I.G. (1982) Pharmacokinetics of acrylamide in Fischer-334 rats. *Toxicol. Appl. Pharmacol.*, **63**, 36–44.
- Moore, M.M., Amtower, A., Doerr, C., Brock, K.H. & Dearfield, K.L. (1987) Mutagenicity and clastogenicity of acrylamide in L5178Y mouse lymphoma cells. *Environ. Mutagen.*, **9**, 261–267.
- Mosbach-Schulz, O., Seiffert, C. & Sommerfeld, G. (2003) *Assessment of Acrylamide Intake from Foods Containing High Acrylamide Levels in Germany*. Berlin: Federal Institute for Risk Assessment (BfR) (<http://www.bfr.bund.de>).
- Mottram, D.S., Wedzicha, B.L. & Dodson, A.T. (2002) Acrylamide is formed in the Maillard reaction. *Nature*, **419**, 448–449.
- Mucci, L.A., Dickman, P.W., Steineck, G., Adami, H.O. & Augustsson, K. (2003a) Dietary acrylamide and cancer of the large bowel, kidney, and bladder: absence of an association in a population-based study in Sweden. *Br. J. Cancer*, **88**, 84–89.
- Mucci, L.A., Dickman, P.W., Steineck, G., Adami, H.O. & Augustsson, K. (2003b) Reply: Dietary acrylamide and cancer risk: additional data on coffee. *Br. J. Cancer*, **89**, 775–776.
- Mucci, L.A., Lindblad, P., Steineck, G. & Adami, H.O. (2004) Dietary acrylamide and risk of renal cell cancer. *Int. J. Cancer*, **109**, 774–776.
- Müller, W., Engelhart, G., Herbold, B., Jackh, R. & Jung, R. (1993) Evaluation of mutagenicity testing with *Salmonella typhimurium* TA102 in three different laboratories. *Environ. Health Perspect.*, **101** (Suppl. 3), 33–36.
- Murkovic, M. (2004) Acrylamide in Austrian foods. *J. Biochem. Biophys. Meth.*, **61**, 161–167.
- Nagao, T. (1994) Developmental abnormalities due to exposure of mouse paternal germ cells, preimplantation embryos, and organogenic embryos to acrylamide. *Congenit. Anom. (Kyoto)*, **34**, 35–46.

- National Institute of Public Health (2003) *Dietary Exposure Monitoring. Study of Acrylamide Exposure Sources in Foods*. Prague: National Institute of Public Health (<http://www.chpr.szu.cz>).
- Nemoto, S., Takatsuki, S., Sasaki, K. & Maitani, T. (2002) Determination of acrylamide in foods by GC/MS using C-13-labeled acrylamide as an internal standard. *J. Food Hyg. Soc. Jpn.*, **43**, 371–376.
- Neuhäuser-Klaus, A. & Schmahl, W. (1989) Mutagenic and teratogenic effects of acrylamide in the mammalian spot test. *Mutat. Res.*, **226**, 157–162.
- Newton, D., Hughes, E., Harling, R., Gopinath, C. & Beg, S. (1992) *A Neurotoxicity Screen in Rats Following Treatment with Acrylamide, Carbaryl or p,p'-DDT*. Huntingdon, Cambridgeshire: Huntingdon Research Centre Ltd.
- NICNAS (2002) *Acrylamide*. Sydney: National Industrial Chemicals Notification and Assessment Scheme (Priority Existing Chemical Assessment Report No. 23).
- Norwegian Food Control Authority (2002) *Risk Assessment of Acrylamide Intake from Foods with Special Emphasis on Cancer Risk: Report from the Scientific Committee of the Norwegian Food Control Authority*. Oslo: Norwegian Food Control Authority, 6 June (<http://snt.mattilsynet.no/nytt/tema/Akrylamid/acrylamide.pdf>).
- Noti, A., Biedermann-Brem, S., Biedermann, M., Grob, K., Albisser, P. & Realini, P. (2003) Storage of potatoes at low temperature should be avoided to prevent increased acrylamide formation during frying or roasting. *Mitt. Lebensm. Hyg.*, **94**, 167–180.
- Olsson, K., Svensson, R. & Roslund, C.A. (2004) Tuber components affecting acrylamide formation and colour in fried potato: variation by variety, year, storage temperature and storage time. *J. Sci. Food Agric.*, **84** (5), 447–458.
- Ono, H., Chuda, Y., Ohnishi-Kameyama, M., Yada, H., Ishizaka, M., Kobayashi, H. & Yoshida, M. (2003) Analysis of acrylamide by LC-MS/MS and GC-MS in processed Japanese foods. *Food Addit. Contam.*, **20**, 215–220.
- Owen, L.M., Castle, L., Kelly, J., Lloyd, A.S. & Wilson, L.A. (2005) Acrylamide analysis: Assessment of results from six rounds of proficiency testing. *J. AOAC Int.*, **88** (1), 285–291.
- Pacchierotti, F., Tiveron, C., D'Archivio, M., Bassani, B., Cordelli, E., Leter, G. & Spano, M. (1994) Acrylamide-induced chromosomal damage in male mouse germ cells detected by cytogenetic analysis of one-cell zygotes. *Mutat. Res.*, **309**, 273–284.
- Park, J., Kamendulis, L.M., Friedman, M.A. & Klaunig, J.E. (2002) Acrylamide-induced cellular transformation. *Toxicol. Sci.*, **65**, 177–183.
- Paulsson, B., Grawé, J. & Törnqvist, M. (2002) Hemoglobin adducts and micronucleus frequencies in mouse and rat after acrylamide or N-methylolacrylamide treatment. *Mutat. Res.*, **516**, 101–111.
- Paulsson, B., Kotova, N., Grawé, J., Henderson, A., Granath, F., Golding, G. & Törnqvist, M. (2003a) Induction of micronuclei in mouse and rat by glycidamide, genotoxic metabolite of acrylamide. *Mutat. Res.*, **53**: 15–24.
- Paulsson, B., Athanassiadis, I., Rydberg, P. & Törnqvist, M. (2003b) Hemoglobin adducts from glycidamide: acetonization of hydrophilic groups for reproducible gas chromatography/tandem mass spectrometric analysis. *Rapid Commun. Mass Spectrom.*, **17**, 1859–1865.
- Paulsson, B., Rannug, A., Henderson, A.P., Golding, B.T., Törnqvist, M. & Warholm, M. (2005) In vitro studies of the influence of glutathione transferases and epoxide hydrolase on the detoxification of acrylamide and glycidamide in blood. *Mutat. Res.*, **580**, 53–59.

- Pedreschi, F., Kaack, K. & Granby, K. (2004) Reduction of acrylamide formation in potato slices during frying. *Lebensm. Wiss. Technol.*, **37** (6), 679–685.
- Pelucchi, C., Franceschi, S., Levi, F., Trichopoulos, D., Bosetti, C., Negri, E. & La Vecchia, C. (2003) Fried potatoes and human cancer. *Int. J. Cancer*, **105**, 558–560.
- Pelucchi, C., La Vecchia, C., Franceschi, S. & Levi, F. (2004) Re: Fried potatoes and human cancer. *Int. J. Cancer*, **108**, 636–637.
- Peng, L., Farkas, T., Loo, L., Teuscher, J. & Kallary, K. (2003) Rapid and reproducible extraction of acrylamide in french fries using a single solid-phase sorbent. *Am. Lab.*, **35**, 10, 12, 14.
- Pittet, A., Perisset, A. & Oberson, J.-M. (2004) Trace level determination of acrylamide in cereal-based foods by gas chromatography–mass spectrometry. *J. Chromatogr. A*, **1035**, 123–130.
- Pollien, P., Lindinger, C., Yeretzyan, C. & Blank, I. (2003) Proton transfer reaction mass spectrometry, a tool for on-line monitoring of acrylamide formation in the headspace of Maillard reaction systems and processed food. *Anal. Chem.*, **75** (20), 5488–5494.
- Poole, C.F., Sye, W.F., Zlatkis, A. & Spencer, P.S. (1981) Determination of acrylamide in nerve tissue homogenates by electron-capture gas chromatography. *J. Chromatogr.*, **217**, 239–245.
- Post, E. & McLeod, J. (1977) Acrylamide autonomic neuropathy in the cat. I. Neurophysiological and histological studies. *J. Neurol. Sci.*, **33**, 353–374.
- Pournara, P., Lazar, A., Taubert, D., Tomalik-Scharte, D., Kinzig-Schippers, M., Sörgel, F., Schömig, E. and Fuhr, U. (2004) Submission to the 64th JECFA meeting: Eine Einzeldosis-Studie zur Beurteilung der Toxikokinetik von Acrylamid nach Aufnahme acrylamidhaltiger Nahrung. Köln: Universität zu Köln, Institut für Pharmakologie.
- Raymer, J.H., Sparacino, C.M., Velez, G.R., Padilla, S., Macphail, R.C. & Crofton, K.M. (1993) Determination of acrylamide in rat serum and sciatic nerve by gas chromatography–electron capture detection. *J. Chromatogr. Biomed. Appl.*, **619**, 223–234.
- Rice, J.M. (2005) The carcinogenicity of acrylamide. *Mutat. Res.*, **580**, 3–20.
- Rice, J.M. & Wilbourn, J.D. (2000) Tumors of the nervous system in carcinogenic hazard identification. *Toxicol. Pathol.*, **28**, 202–214.
- Riediker, S. & Stadler, R.H. (2003) Analysis of acrylamide in food by isotope-dilution liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *J. Chromatogr. A*, **1020**, 121–130.
- Roach, J.A.G., Andrzejewski, D., Gay, M.L., Nortrup, D. & Musser, S.M. (2003) Rugged LC-MS/MS survey analysis for acrylamide in foods. *J. Agric. Food Chem.*, **51**, 7547–7554.
- Robarge, T., Phillips, E. & Conoley, M. (2003) Analysis of acrylamide in food by GC/MS. *LC-GC North Am.*, **September**, 45.
- Robert, F., Vuataz, G., Pollien, P., Saucy, F., Alonso, M.-I., Bauwens, I. & Blank, I. (2004) Acrylamide formation from asparagine under low-moisture Maillard reaction conditions. 1. Physical and chemical aspects in crystalline model systems. *J. Sci. Food Agric.*, **52** (22), 6837–6842.
- Rosén, J. & Hellenäs, K.-E. (2002) Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry. *Analyst*, **127**, 880–882.
- Ruden, C. (2004) Acrylamide and cancer risk — expert risk assessments and the public debate. *Food Chem. Toxicol.*, **42**, 335–349.

- Russell, L.B., Hunsicker, P.R., Cacheiro, N.L.A. & Generoso, W.M. (1991) Induction of specific-locus mutations in male germ cells of the mouse by acrylamide monomer. *Mutat. Res.*, **262**, 101–107.
- Russo, A., Gabbani, G. & Simoncini, B. (1994) Weak genotoxicity of acrylamide on pre-meiotic and somatic cells of the mouse. *Mutat. Res.*, **309**, 263–272.
- Rutledge, J.C., Generoso, W.M., Shourbaji, A., Cain, K.T., Gans, M. & Oliva, J. (1992) Developmental anomalies derived from exposure of zygotes and first-cleavage embryos to mutagens. *Mutat. Res.*, **296**, 167–177.
- Rydberg, P., Eriksson, S., Tareke, E., Karlsson, P., Ehrenberg, L. & Törnqvist, M. (2003) Investigations of factors that influence the acrylamide content of heated foodstuffs. *J. Agric. Food Chem.*, **51** (24), 7012–7018.
- Sakamoto, J. & Hashimoto, K. (1986) Reproductive toxicity of acrylamide and related compounds in mice — effects on fertility and sperm morphology. *Arch. Toxicol.*, **59**, 201–205.
- Sakamoto, J., Kurosaka, Y. & Hashimoto, K. (1988) Histological changes of acrylamide-induced testicular lesions in mice. *Exp. Mol. Pathol.*, **48**, 324–334.
- Saroja, N., Gowda, L.R. & Tharanathan, R.N. (2000) Chromatographic determination of residual monomers in starch-g-polyacrylonitrile and starch-g-polyacrylate. *Chromatographia*, **51**, 345–348.
- Satchell, P. & McLeod, J. (1981) Megaesophagus due to acrylamide neuropathy. *J. Neurol. Neurosurg. Psychiatry*, **44**, 906–913.
- Scanlon, M.F. and Toft, A.D. (1996) Regulation of thyrotropin release. In: Braverman, L.E. & Utiger, R.D., eds., *Werner and Ingbar's The Thyroid*, 7th Ed. Philadelphia, Pennsylvania: Lippencott-Raven, pp. 220–240.
- Schettgen, T., Broding, H.C., Angerer, J. & Drexler, H. (2002) Haemoglobin adducts of ethylene oxide, propylene oxide, acrylonitrile and acrylamide — biomarkers in occupational and environmental medicine. *Toxicol. Lett.*, **134**, 65–70.
- Schettgen, T., Weiss, T., Drexler, H. & Angerer, J. (2003) A first approach to estimate the internal exposure to acrylamide in smoking and non-smoking adults from Germany. *Int. J. Hyg. Environ. Health*, **206**, 9–14.
- Schettgen, T., Kutting, B., Hornig, M., Beckmann, M.W., Weiss, T., Drexler, H. & Angerer, J. (2004) Trans-placental exposure of neonates to acrylamide — a pilot study. *Int. Arch. Occup. Environ. Health*, **77**, 213–216.
- Schultzová, K. & Tekel, J. (1996) Acrylamide monomer occurrence in sugar. *Dtsch. Lebensm.-Rundsch.*, **92**, 281–282.
- Schulz, M.R., Hertz-Picciotto, I., van Wijngaarden, E., Hernandez, J.C. & Ball, L.M. (2001) Dose-response relation between acrylamide and pancreatic cancer. *Occup. Environ. Med.*, **58**, 609.
- Schulze, G. & Boysen, B. (1991) A neurotoxicity screening battery for use in safety evaluation: effects of acrylamide and 3',3'-iminodipropionitrile. *Fundam. Appl. Toxicol.*, **16** (3), 602–615.
- Sega, G.A. & Generoso, E.E. (1990) Measurement of DNA breakage in specific germ-cell stages of male mice exposed to acrylamide, using an alkaline-elution procedure. *Mutat. Res.*, **242**, 79–87.
- Sega, G.A., Alcota, R.P., Tancongo, C.P. & Brimer, B.A. (1989) Acrylamide binding to the DNA and protamine of spermiogenic stages in the mouse and its relationship to genetic damage. *Mutat. Res.*, **216**, 221–230.

- Sega, G.A., Generoso, E.E. & Brimer, P.A. (1990) Acrylamide exposure induces a delayed 21 unscheduled DNA synthesis in germ cells of male mice that is correlated with the temporal pattern of adduct formation in testis DNA. *Environ. Mol. Mutagen.*, **16**, 137–142.
- Segerbäck, D., Calleman, C.J., Schroeder, J.L., Costa, L.G. & Faustman, E.M. (1995) Formation of *N*-7-(2-carbamoyl-2-hydroxyethyl) guanine in DNA of the mouse and the rat following intraperitoneal administration of [¹⁴C] acrylamide. *Carcinogenesis*, **16** (5), 1161–1165.
- Sell, M., Franke, K., Kiessling, M., Richter, H. & Reimerdes, E.H. (2004) [Less acrylamide. Evaporating water is the key factor for acrylamide formation during deep-frying.] *Lebensmitteltechnik*, **36** (5), 52–53 (in German).
- Shanker, R., Ramakrishna, C. & Seth, P.K. (1990) Microbial degradation of acrylamide monomer. *Arch. Microbiol.*, **154**, 192–198.
- Shelby, M.D., Cain, K.T., Cornett, C.V. & Generoso, W.M. (1987) Acrylamide: induction of heritable translocations in male mice. *Environ. Mutagen.*, **9**, 363–368.
- Shih, F.F., Boué, S.M., Daigle, K.W. & Shih, B.Y. (2004) Effects of flour sources on acrylamide formation and oil uptake in fried batters. *J. Am. Oil Chem. Soc.*, **81**, 265–268.
- Shiraishi, Y. (1978) Chromosome aberrations induced by monomeric acrylamide in bone marrow and germ cells of mice. *Mutat. Res.*, **57**, 313–324.
- Sickles, D.W., Stone, J.D. & Freidman, M.A. (2002) Fast axonal transport: a site of acrylamide neurotoxicity: a rebuttal. *Neurotoxicology*, **23**, 265–270.
- Skelly, N.E. & Husser, E.R. (1978) Determination of acrylamide monomer in polyacrylamide and in environmental samples by high performance liquid chromatography. *Anal. Chem.*, **50**, 1959–1962.
- Smith, E.A. & Oehme, F.W. (1993) Rapid direct analysis of acrylamide residues in polyacrylamide thickening agents. *J. Chromatogr. Sci.*, **31**, 192–195.
- Sobel, W., Bond, G.G., Parsons, T.W. & Brenner, F.E. (1986) Acrylamide cohort mortality study. *Br. J. Ind. Med.*, **43**, 785–788.
- Solomon, J.J., Fedyk, J., Mukai, F. & Segal, A. (1985) Direct alkylation of 2'-deoxy-nucleosides and DNA following in vitro reaction with acrylamide. *Cancer Res.*, **45**, 3465–3470.
- Sörgel, F., Weissenbacher, R., Kinzig-Schippers, M., Hofmann, A., Illauer, M., Skott, A. & Landersdorfer, C. (2002) Acrylamide: increased concentrations in homemade food and first evidence of its variable absorption from food, variable metabolism and placental and breast milk transfer in humans. *Chemotherapy*, **48**, 267–274.
- Spencer, P.S. & Schaumburg, H.H. (1974a) A review of acrylamide neurotoxicity. Part I. Properties, uses and human exposure. *Can. J. Neurol. Sci.*, **1** (2), 143–150.
- Spencer, P.S. & Schaumburg, H.H. (1974b) A review of acrylamide neurotoxicity. Part II. Experimental animal neurotoxicity and pathologic mechanisms. *Can. J. Neurol. Sci.*, **1** (3), 152–169.
- Spencer, P.S. & Schaumburg, H.H. (1975) Nervous system degeneration produced by acrylamide monomer. *Environ. Health Perspect.*, **11**, 129–133.
- Spencer, P.S. & Schaumburg, H.H. (1977) Ultrastructural studies on the dying-back process. III. The evolution of experimental peripheral giant axonal degeneration. *J. Neuropathol. Exp. Neurol.*, **36**, 276–299.
- Stadler, R.H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P.A., Robert, M.-C. & Riediker, S. (2002) Acrylamide from Maillard reaction products. *Nature*, **419**, 449–450.

- Stadler, R.H., Verzeegnassi, L., Varga, N., Grigorov, M., Studer, A., Riediker, S. & Schilter, B. (2003) Formation of vinylogous compounds in model Maillard reaction systems. *Chem. Res. Toxicol.*, **16** (10), 1242–1250.
- Stadler, R.H., Robert, F., Riediker, S., Varga, N., Davidek, T., Devaud, S., Goldmann, T., Hau, J. & Blank, I. (2004) In-depth mechanistic study on the formation of acrylamide and other vinylogous compounds by the Maillard reaction. *J. Agric. Food Chem.*, **52** (17), 5550–5558.
- Stegen, G., Jorissen, U., Pittet, A., Saccon, M., Steiner, W., Vincenzi, M., Winkler, M., Zapp, J. & Schlatter, C. (1997) Screening of European coffee final products for occurrence of ochratoxin A. *Food Addit. Contam.*, **14** (3), 211–216.
- Sublet, V.H., Zenick, H. & Smith, M.K. (1989) Factors associated with reduced fertility and implantation rates in females mated to acrylamide-treated rats. *Toxicology*, **55**, 53–67.
- Sumner, S.C.J., MacNeela, J.P. & Fennell, T.R. (1992) Characterization and quantitation of urinary metabolites of [1,2,3-¹³C]acrylamide in rats and mice using ¹³C nuclear magnetic resonance spectroscopy. *Chem. Res. Toxicol.*, **5**, 81–89.
- Sumner, S.C.J., Fennell, T.R., Moore, T.A., Chanas, B., Gonzalez, F. & Ghanayem, B.I. (1999) Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. *Chem. Res. Toxicol.*, **12**, 1110–1116.
- Sumner, S.C.J., Williams, C.C., Snyder, R.W., Krol, W.L., Asgharian, B. & Fennell, T.R. (2003) Acrylamide: a comparison of metabolism and hemoglobin adducts in rodents following dermal, intraperitoneal, oral, or inhalation exposure. *Toxicol. Sci.*, **75**, 260–270.
- Surdyk, N., Rosén, J., Andersson, R. & Åman, P. (2004) Effects of asparagine, fructose, and baking conditions on acrylamide content in yeast-leavened wheat bread. *J. Agric. Food Chem.*, **52** (7), 2047–2051.
- Svensson, K., Abramsson, L., Becker, W., Glynn, A., Hellenas, K.-E., Lind, Y. & Rosen, J. (2003) Dietary intake of acrylamide in Sweden. *Food Chem. Toxicol.*, **41**, 1581–1586.
- Swedish National Chemicals Inspectorate (1989) *Utvärdering av cancerframkallande ämnen I*. Sundbyberg: Swedish National Chemicals Inspectorate (KemI Report No. 11/89).
- Swiss Federal Office of Public Health (2002) *Assessment of Acrylamide Intake by Duplicate Diet Study*. Berne: Swiss Federal Office of Public Health (<http://www.bag.admin.ch/verbrau/aktuell/f/index.htm>).
- Taeymans, D., Wood, J., Ashby, P., Blank, I., Studer, A., Stadler, R.H., Gonde, P., Van Eijck, P., Lalljie, S., Lingnert, H., Lindblom, M., Matissek, R., Muller, D., Tallmadge, D., O'Brien, J., Thompson, S., Silvani, D. & Whitmore, T. (2004) A review of acrylamide: an industry perspective on research, analysis, formation and control. *Crit. Rev. Food Sci. Nutr.*, **44** (5), 323–347.
- Tanaka, M., Yoneda, Y., Terada, Y., Endo, E. & Yamada, T. (2004) Comment on “Soxhlet extraction of acrylamide from potato chips” by J. R. Pedersen and J. O. Olsson, *Analyst*, 2003, 128, 332. *Analyst*, **129**, 96–98.
- Tanii, H. & Hashimoto, K. (1983) Neurotoxicity of acrylamide and related compounds in rats. Effects on rotarod performance, morphology of nerves and neurotubulin. *Arch. Toxicol.*, **54** (3), 203–213.
- Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S. & Törnqvist, M. (2000) Acrylamide: A cooking carcinogen? *Chem. Res. Toxicol.*, **13**, 517–522.
- Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S. & Törnqvist, M. (2002) Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.*, **50**, 4998–5006.

- Tateo, F. & Bononi, M. (2003) A GC/MS method for the routine determination of acrylamide in food. *Ital. J. Food Sci.*, **15**, 149–151.
- Taubert, D., Harlfinger, S., Henkes, L., Berkels, R. & Schömig, E. (2004) Influence of processing parameters on acrylamide formation during frying of potatoes. *J. Agric. Food Chem.*, **52** (9), 2735–2739.
- Terada, H. & Tamura, Y. (2003) [Determination of acrylamide in processed foods by column-switching HPLC with UV detection.] *J. Food Hyg. Soc. Jpn.*, **44**, 303–309 (in Japanese).
- Tilson, H.A. (1981) The neurotoxicity of acrylamide: an overview. *Neurobehav. Toxicol. Teratol.*, **3**, 445–461.
- Tilson, H.A. & Cabe, P.A. (1979) The effects of acrylamide given acutely or in repeated doses on fore- and hindlimb function of rats. *Toxicol. Appl. Pharmacol.*, **47** (2), 253–260.
- Törnqvist, M., Fred, C., Haglund, J., Helleberg, H., Paulsson, B. & Rydberg, P. (2002) Protein adducts: quantitative and qualitative aspects of their formation, analysis and applications. *J. Chromatogr. B*, **778**, 279–308.
- Tripathy, N.K., Patnaik, K.K. & Nabi, M.D.J. (1991) Acrylamide is genotoxic to the somatic and germ cells of *Drosophila melanogaster*. *Mutat. Res.*, **259**, 21–27.
- Tsuda, H., Shimizu, C.S., Taketomi, M.K., Hasegawa, M.M., Hamada, A., Kawata, K.M. & Inui, N. (1993) Acrylamide; induction of DNA damage, chromosomal aberrations and cell transformation without gene mutations. *Mutagenesis*, **8** (1), 23–29.
- Twaddle, N.C., Hamilton, L.P., Gamboa da Costa, G., Churchwell, M.I., Beland, F.A. & Doerge, D.R. (2004a) Determination of acrylamide and glycidamide serum toxicokinetics in B6C3F1 mice using LC-ES/MS/MS. *Cancer Lett.*, **207**, 9–17.
- Twaddle, N.C., Churchwell, M.C., McDaniel, L.P. & Doerge, D.R. (2004b) Autoclave sterilization produces acrylamide in rodent diets: Implications for toxicity testing. *J. Agric. Food Chem.*, **52**, 4344–4349.
- Tyl, R.W. & Friedman, M.A. (2003) Effects of acrylamide on rodent reproductive performance. *Reprod. Toxicol.*, **17**, 1–13.
- Tyl, R.W., Marr, M.C., Myers, C.B., Ross, W.P. & Friedman, M.A. (2000a) Relationship between acrylamide reproductive and neurotoxicity in male rats. *Reprod. Toxicol.*, **14**, 147–157.
- Tyl, R.W., Friedman, M.A., Losco, P.E., Fisher, L.C., Johnson, K.A., Strother, D.E. & Wolf, C.H. (2000b) Rat two-generation reproduction and dominant lethal study of acrylamide in drinking water. *Reprod. Toxicol.*, **14**, 385–401.
- US EPA (1993) *Integrated Risk Information System (IRIS): Acrylamide*. Washington, D.C.: United States Environmental Protection Agency (<http://www.epa.gov/iris>).
- US EPA (2004) *Integrated Risk Information System (IRIS): Acrylamide (Draft)*. Washington, D.C.: United States Environmental Protection Agency
- US FDA (1998) *Assessment of Carcinogenic Upper-Bound Lifetime Risk Resulting from Contamination by Acrylamide of Copolymers, Retention Aids, Drainage Aids, Stabilizer or Fixing Agents in Paper and Paperboard Contacting Foods, Report of the Quantitative Risk Assessment Committee, 29 January 1998*. Washington, D.C.: United States Food and Drug Administration.
- US FDA (2005) *Exploratory Data in Acrylamide in Food Total Diet Study Results, January 2005*. Washington, D.C.: United States Food and Drug Administration.
- Valdivia, R.P., Lafuente, N.M. & Katoh, M. (1989) Acrylamide-induced chromosome-type aberrations in spermiogenic stages evaluated in the first cleavage metaphases in the mouse. *Environ. Mol. Mutagen.*, **14** (Suppl. 15), 205.

- Vass, M., Amrein, T.M., Schönbächler, B., Escher, F. & Amadó, R. (2004) Ways to reduce the acrylamide formation in cracker products, *Czech J. Food Sci.*, **22** (Special Issue), 19–21.
- Vattem, D.A. & Shetty, K. (2003) Acrylamide in food: a model for mechanism of formation and its reduction. *Innovative Food Sci. Emerg. Technol.*, **4**, 331–338.
- Vavasour, E. (2005) Submission to the 64th JECFA meeting: Dietary acrylamide intakes in Canada.
- Ver Vers, L.M. (1999) Determination of acrylamide monomer in polyacrylamide degradation studies by high performance liquid chromatography. *J. Chromatogr. Sci.*, **37**, 486–494.
- Vesper, H.W., Licea-Perez, H., Meyers, T., Ospina, M. & Meyers, G.L. (2004) Pilot study on the impact of potato chips consumption on biomarkers of acrylamide exposure. In: *American Chemical Society Spring Meeting* (Abstract AGFD 81).
- Von Tungeln, L.S., Churchwell, M.I., Doerge, D.R., McGarrity, L.L., Morris, S.M., Heflich, R.H., Gamboa da Costa, G., Marques, M. & Beland, F.A. (2005) DNA adduct formation and induction of micronuclei and mutations in B6C3F1/Tk mice treated neonatally with acrylamide or glycidamide. In: *Meeting of the American Association for Cancer Research* (Abstract 05-AB-4697).
- Walden, R., Squibb, R.E. & Schiller, C.M. (1981) Effects of prenatal and lactational exposure to acrylamide on the development of intestinal enzymes in the rat. *Toxicol. Appl. Pharmacol.*, **58**, 363–369.
- Warr, T., Parry, J., Callander, R. & Ashby, J. (1990) Methyl vinyl sulphone: a new class of Michael-type genotoxin. *Mutat. Res.*, **245**, 191–199.
- Weisshaar, R. (2004) Acrylamide in bakery products — results from model experiments. *Dtsch. Lebensm.-Rundsch.*, **100** (3), 92–97.
- Weisshaar, R. & Gutsche, B. (2002) Formation of acrylamide in heated potato products — model experiments pointing to asparagine as precursor. *Dtsch. Lebensm.-Rundsch.*, **98** (11), 397–399.
- Wenzl, T., de la Calle, M.B. & Anklam, E. (2003) Analytical methods for the determination of acrylamide in food products: a review. *Food Addit. Contam.*, **20**, 885–902.
- WHO (1995) *Reliable Evaluation of Low-Level Contamination of Food, Workshop in the Frame of GEMS/Food-EURO, Kulmbach, 26–27 May 1995*. Geneva: World Health Organization.
- WHO (2003) *GEMS/Food Diets. Revision September 2003*. Geneva: World Health Organization, Food Safety Department.
- Wise, L.D., Gordon, L.R., Soper, K.A., Duchai, D.M. & Morrissey, R.E. (1995) Developmental neurotoxicity evaluation of acrylamide in Sprague-Dawley rats. *Neurotoxicol. Teratol.*, **17**, 189–198.
- Xiao, Y. & Bates, A.D. (1994) Increased frequencies of micronuclei in early spermatids of rats following exposure of young primary spermatocytes to acrylamide. *Mutat. Res.*, **309**, 245–254.
- Yasuhara, A., Tanaka, Y., Hengel, M. & Shibamoto, T. (2003) Gas chromatographic investigation of acrylamide formation in browning model systems. *J. Agric. Food Chem.*, **51** (14), 3999–4003.
- Yaylayan, V.A., Wnorowski, A. & Locas, C.P. (2003) Why asparagine needs carbohydrates to generate acrylamide. *J. Agric. Food Chem.*, **51** (6), 1753–1757.
- Yaylayan, V.A., Locas, C.P., Wnorowski, A. & O'Brien, J. (2004) The role of creatine in the generation of *N*-methyacrylamide: a new toxicant in cooked meat. *J. Agric. Food Chem.*, **52** (17), 5559–5565.

- Zayzafoon, G. & Odeh, A. (2004) Submission to 64th JECFA meeting: Occurrence of acrylamide in food in Syria.
- Zeiger, E., Anderson, B. & Haworth, S. (1987) *Salmonella* mutagenicity tests: III. Results from the testing of 255 chemicals. *Environ. Mutagen.*, **9** (Suppl. 9), 1–110.
- Zenick, H., Hope, E. & Smith, M.K. (1986) Reproductive toxicity associated with acrylamide treatment in male and female rats. *J. Toxicol. Environ. Health*, **17**, 457–472.
- Zyzak, D.V., Sanders, R.A., Stojanovic, M., Tallmadge, D.H., Eberhart, B.L., Ewald, D.K., Gruber, D.C., Morsch, T.R., Strothers, M.A., Rizzi, G.P. & Villagran, M.D. (2003) Acrylamide formation mechanism in heated foods. *J. Agric. Food Chem.*, **51** (16), 4782–4787.

CADMIUM — IMPACT ASSESSMENT OF DIFFERENT MAXIMUM LIMITS

First draft prepared by

K. Egan,¹ T. Hambridge² and F. Kayama³

¹**Food and Drug Administration, College Park, Maryland, USA**

²**Food Standards Australia New Zealand, Canberra, Australia**

³**Jichi Medical School, Tochigi, Japan**

Explanation	157
Assessment for the sixty-first JECFA	158
Assessment for the sixty-fourth JECFA	160
New cadmium data	160
Raw data	160
Aggregated data	164
Cadmium intake estimates	164
Comparison of new and previous concentration data	165
Impact of different possible MLs on cadmium concentrations	169
Impact of different possible MLs on cadmium intakes	172
Comments	185
Data on concentrations of cadmium in food	185
Assessment of the impact of different possible MLs on mean concentrations of cadmium	186
Assessment of the impact of different possible MLs on mean intakes of cadmium	186
Evaluation	187
References	190
Appendix A: Summary of raw data on cadmium concentrations in commodities, by country and region	191
Appendix B: Weighted means for aggregated cadmium data	195

1. EXPLANATION

The dietary intake of cadmium was evaluated by the Committee at its fifty-fifth and sixty-first meetings (Annex 1, references 149 and 166). In each of these assessments, intakes of cadmium were calculated from available data on concentrations and food consumption taken from the Global Environment Monitoring System Food Contamination Monitoring and Assessment Programme (GEMS/Food) regional diets. Total intakes of cadmium estimated by the Committee at its sixty-first meeting ranged from 2.8 to 4.2 µg/kg bw per week, which equate to 40–60% of the current provisional tolerable weekly intake (PTWI) of 7 µg/kg bw per week. The seven commodity groups that contributed significantly to total intake of cadmium included rice, wheat, root vegetables, tuber vegetables, leafy vegetables, other vegetables and molluscs. These commodities accounted for 40–85% of the total intake of cadmium in the five GEMS/Food regions.

Before the sixty-first meeting of the Committee, the Codex Committee on Food Additives and Contaminants (CCFAC) at its Thirty-sixth Session (CAC,

2004a) requested that the Committee evaluate the impact of different maximum levels (MLs) for cadmium in commodities that contribute significantly to intake, but this work could not be undertaken by the Committee at that time. CCFAC subsequently requested that this analysis be completed. Specifically, the Committee was asked:

- To conduct intake and impact assessments for the seven commodity groups, taking into account three possible MLs (i.e. the draft Codex ML proposed by CCFAC and one level lower and one level higher than the proposed ML). The draft proposed Codex MLs were as follows: rice, 0.4 mg/kg; wheat, 0.2 mg/kg; potatoes, 0.1 mg/kg; stem/root vegetables, 0.1 mg/kg; leafy vegetables, 0.2 mg/kg; other vegetables, 0.05 mg/kg; and molluscs (oysters, 3 mg/kg; other molluscs, 1 mg/kg).
- To evaluate the impact of three possible MLs on concentrations and intakes in subcategories of molluscs (i.e. bivalves, scallops and cephalopods) on the basis of the data submitted.

Table 1 summarizes the possible MLs (proposed, one level lower and one level higher) for each commodity group and the specific commodities included in each group as specified by CCFAC. JECFA evaluated one additional level (0.2 mg/kg) for rice, since this had been discussed previously by CCFAC as a possible ML. This assessment took into account the potential impact of each possible ML on the distribution of concentrations of cadmium in each commodity (i.e. how eliminating samples containing cadmium at concentrations greater than the ML affected the mean value of the resulting distribution, and the proportion of samples containing cadmium at concentrations greater than the ML) and the dietary intakes of cadmium from each individual commodity (i.e. how the mean concentrations of cadmium for each ML affected mean intake of cadmium).

2. ASSESSMENT FOR THE SIXTY-FIRST JECFA

For the sixty-first JECFA, most cadmium concentration data used in the assessment were aggregated (i.e. means or medians representing a number of samples). In addition to these aggregated data, the Japanese government submitted a substantial number of data on individual samples (raw data). Mean values were calculated from the Japanese data and then included with the other aggregated data.

As recommended in the guidelines for conducting exposure assessments for contaminants in foods (CAC, 2004b), regional cadmium intakes were calculated using regional average concentration data and the GEMS/Food regional diets (WHO, 2003). The concentration data were grouped by commodity and geographic region (as per the GEMS/Food regional diets), and mean concentrations were calculated as the average of mean/median values reported for that region. Since sample sizes were not reported for all data, it was not possible to calculate weighted means. Cadmium intakes were calculated by multiplying the unweighted regional average concentration for each commodity by the amount consumed of the commodity, as specified in the GEMS/Food regional diets.

Table 1. Commodities and MLs evaluated by sixty-fourth JECFA as requested by CCFAC

One level lower (mg/kg)	Proposed ML (mg/kg)	One level higher (mg/kg)	CCFAC commodity group	Sub-categories	Codex code	Codex code description
0.3	0.4	0.5	Rice, polished		CM 0649	Rice, polished
0.1	0.2	0.3	Wheat grain		GC 0654	Wheat grain
0.05	0.1	0.2	Potato		VR 0589	Potato
0.05	0.1	0.2	Stem and root vegetables (excluding potatoes and celeriac)		VR 0075	Roots and tubers
					VS 0078	Stalk and stem vegetables
0.1	0.2	0.3	Leafy vegetables		VL 0053	Leafy vegetables including brassica leafy
0.01	0.05	0.1	Other vegetables (excluding tomatoes and mushrooms)		VA 0035	Bulb vegetables
					VB 0040	Brassica vegetables
					VC 0045	Fruiting vegetables - cucurbits
0.5	1.0	2.0	Molluscs (including cephalopods)		IM 0150	
0.5	1.0	2.0		Marine bivalves	IM 0151	Clams, cockles, mussels, oysters, scallops
0.5	1.0	2.0		Scallops	IM 1005	
1.0	2.0	3.0		Cephalopods	IM 0152	Cuttlefish, octopus, squid
2.0	3.0	4.0		Oysters	IM 1004	

Estimated total cadmium intakes ranged from 2.8 to 4.2 µg/kg bw per week, which equate to approximately 40–60% of the current PTWI of 7 µg/kg bw per week. Commodities that contributed significantly to total cadmium intake (i.e. those contributing 10% or more of the PTWI from one GEMS/Food region, or 5% or more of the PTWI for two or more regions) included the following: rice, wheat, roots and tubers, leafy and other vegetables and molluscs. On average for the five

regional diets, these commodities accounted for about 65% of total cadmium intake.

3. ASSESSMENT FOR THE SIXTY-FOURTH JECFA

The present assessment took into account the potential impact of different possible MLs on the distribution of concentrations of cadmium in each commodity (i.e. how eliminating samples containing cadmium at concentrations greater than the ML affected the mean value of the resulting distribution, and the proportion of samples containing cadmium at concentrations greater than the ML) and the dietary intakes of cadmium from each individual commodity (i.e. how the mean concentrations of cadmium for each ML affected mean intake of cadmium). Cadmium intakes were calculated for the seven commodity groups only; estimates of total cadmium intake from the previous JECFA evaluation provided a benchmark for evaluating the impact of MLs on intakes.

Conducting this assessment of possible MLs required information about the distribution of cadmium levels in each commodity group. Ideally, data reporting individual analytical results for each sample (raw data) would be used for such an assessment, rather than aggregated data. (Note that these individual samples may be either single units or composites of several units, as is often the case with Total Diet Study samples.) Since the previous JECFA assessment was based primarily on aggregated data, a data call was sent to Codex member countries requesting that any new concentration data be submitted for this evaluation.

3.1 New cadmium data

A substantial number of new data (primarily raw data) were received for this assessment. Although data on many commodities were submitted, only the data for the seven commodity groups identified by CCFAC were included in this assessment. Some countries also submitted national estimates of cadmium intake.

3.1.1 Raw data

Raw data were submitted to the JECFA Secretariat by Australia, Canada, Germany, Japan, New Zealand, Norway and the United States (Table 2). Note that many countries submitted all available cadmium data, which included many foods that were not being considered in this assessment. The information below relates only to the data relevant to the seven commodity groups specified in CCFAC's request.

Australia submitted results from their Total Diet Surveys conducted every other year between 1992 and 2000. Each Total Diet Survey data point represented a composite of three individual retail samples. In addition to the Total Diet Survey data, Australia submitted results from the Algal Biotoxin Survey (1996) and the Victorian Produce Monitoring Program (1988–1996).

Table 2. Summary of raw data submitted for the sixty-fourth JECFA

Commodity	<i>n</i>							
	Total	Australia	Canada	Germany	Japan	New Zealand	Norway	USA
<i>Grains</i>								
Rice	37 547			131	37 250			166
Wheat	940	57		209	382	2		290
<i>Vegetables</i>								
Potatoes	643	114	163	69				297
Stem/root vegetables	1570	133		223	997	10		207
Leafy vegetables	2043	177		927	673	12		254
Other vegetables	3509	100		1335	1529	47		498
<i>Molluscs</i>								
Oysters	4478	31	2192		45	11	6	2193
Scallops	74				57		4	13
Other bivalves	2471	12		103	166	11	25	2154
Cephalopods	98				98			
Molluscs, other	30				30			
<i>Grand total by country</i>		624	2355	2997	41 227	93	35	6072

The Canadian government submitted data on cadmium levels in oysters from several different monitoring studies conducted on both the west coast (British Columbia) and the east coast (Nova Scotia and New Brunswick). The largest number of samples represented a study of oysters from the west coast conducted between 2001 and 2004; this was a collaborative effort involving the Department of Fisheries and Oceans, British Columbia Ministry of Agriculture, Food and Fisheries and Simon Fraser University. Additional data were reported by the Canadian Food Inspection Agency, representing oyster samples collected on both the east and west coasts between 1989 and 2000. And finally, the Department of Fisheries and Oceans, British Columbia Ministry of Agriculture, Fisheries and Food and Environment Canada analysed samples of wild oysters from beaches in British Columbia.

The German government (Federal Office of Consumer Protection and Food Safety, Berlin) submitted data for a variety of grains, vegetables and mussels. The samples had been collected and analysed between 2001 and 2004.

The Japanese government submitted a substantial number of monitoring data on a wide range of commodities, of which the majority were rice. The collection and analysis of foods were carried out by the Ministry of Agriculture, Forestry and Fisheries of Japan.

New Zealand submitted results from two Total Diet Surveys conducted in 1997–1998 and 2003–2004. The analytical results represented both individual sample and composites of up to five units per sample, depending upon the food.

From Norway, data were submitted on cadmium levels in bivalves. These were results from a surveillance programme conducted by the National Institute of Nutrition and Seafood Research between 2000 and 2003, in which samples were collected from the entire coast of Norway. The major focus of these studies was the blue mussel, although a small number of other molluscs (oysters and scallops) were included in the studies. For mussels, each data point represented a pooled sample of 25 mussels from different locations.

The United States submitted data for molluscs from monitoring programmes of the Food and Drug Administration and the National Oceanic and Atmospheric Administration. The Food and Drug Administration submitted data on a variety of molluscs (mussels, clams, oysters and scallops); these were results of compliance monitoring programmes conducted in 1989–1999. The Food and Drug Administration also submitted cadmium data for numerous vegetables and grains; these samples were collected and analysed between 1979 and 1981. The National Oceanic and Atmospheric Administration submitted results from its 1986–1998 Mussel Watch Program; these data included results for oysters as well as mussels.

When compiling the new data, only those for raw commodities were used. All data for products that were cooked or processed (as determined by the food description or by documentation that accompanied the data) were excluded. Each data record was then assigned to one of the seven commodity groups. For

molluscs, data were further classified by subcategory (oysters, mussels, scallops, etc.) so the data could be evaluated by subcategory as requested by CCFAC.

A number of records were censored (i.e. results were reported as being below the limit of detection [LOD] or limit of quantification [LOQ] or less than a certain value). The percentage of censored records was determined for each of the seven commodity groups (Table 3). For most groups, less than 10% of the data was censored. The greatest proportion (34%) of censored data occurred in results for the group "other vegetables." As per the WHO guidelines for handling censored data (WHO, 1995), when 60% or less of the results are censored, a value of LOD/2 should be used for those results when calculating the mean. Since the LODs were not reported for all data sets, the following values were used for assigning a value to the censored results:

- LOD/2 if the LOD was reported;
- LOQ/2 if only the LOQ was reported;
- if no LOD or LOQ was reported but the results were reported as $<x$, a value of $x/2$ was assumed; or
- if none of the above was reported, one half the lowest value reported for the commodity group/country was assumed.

Table 3. Censored data

Commodity	% of censored values
Rice	8
Wheat	1
Potatoes	2
Stem/root vegetables	21
Leafy vegetables	6
Other vegetables	34
Molluscs	<1

After values were assigned to all censored results, data for each commodity group were analysed (count, mean, standard deviation, minimum and maximum values) by country, by GEMS/Food region and for all data combined. Detailed results of these analyses are included in Appendix A. For most commodities, average cadmium levels across countries were comparable. For rice, average concentrations of Japanese samples were higher than those from other countries. With regard to data for molluscs, average values for oysters were considerably higher than for other subcategories, particularly for samples from Canada and New Zealand.

3.1.2 *Aggregated data*

The major source of aggregated data was a report compiled by European Union Member States in 2004 on dietary exposure to toxic elements (EC, 2004). The specificity of the data reported by countries varied; while some reported results for individual vegetables, others reported results by broader, less specific groups (e.g. all vegetables). Data were included in this JECFA assessment only if the description was specific enough to be grouped by the commodity groups specified by CCFAC.

Other aggregated data were submitted by Spain, Sweden, Thailand and the United Kingdom. Spain provided data from samples collected between 2001 and 2003; these were mainly finfish and shellfish, although some molluscs (cephalopods and mussels) were also included. Sweden's National Food Administration reported cadmium levels in retail samples of rice collected in 2001. Data were reported by Thailand and the United Kingdom; however, due to the nature of the data, they could not be incorporated in this assessment. Thailand provided data for molluscs, but the results were reported as ranges (i.e. the number of samples falling within a certain range) rather than the mean/median value for all samples. The United Kingdom provided a report of its 2000 Total Diet Study, but the cadmium concentrations were reported for broad categories rather than for the commodities of interest in this assessment.

The selected aggregated data were grouped by commodity, and weighted means were then calculated based on sample size. Appendix B provides details of the cadmium data for each commodity and the calculation of the weighted means. The weighted means for each commodity are summarized in Table 4 below.

3.1.3 *Cadmium intake estimates*

The previous (2003) cadmium assessment addressed estimates of total intake from all commodities, but the goal of the recent assessment undertaken was quite different in scope and methodology. The focus of the 2005 assessment was to estimate the impact of three or four possible MLs on cadmium concentrations and intakes from selected commodities rather than to estimate the total average cadmium intake from all commodities. National estimates of cadmium intakes were submitted by Australia, New Zealand and the United Kingdom, but the estimates did not take into account the impact of different MLs.

The Japanese government (Ministry of Health, Labour and Welfare and the Ministry of Agriculture, Forestry and Fisheries) submitted a probabilistic intake estimate for cadmium resulting from four possible MLs for rice. Since rice is a staple in the Japanese diet and also a major source of cadmium in their diet, this model provided estimates of the greatest potential impact on total cadmium intake by the Japanese population. The model incorporated national data on food consumption and cadmium levels in foods. Consumption data were obtained from Japan's National Nutrition Survey conducted from 1995 through 2000, which included records on approximately 53 000 adults 20 years of age and older. Conversion factors were applied to multi-ingredient foods reported in the survey to

allow for estimation of consumption of commodities. Cadmium concentration data for about 130 different foods were obtained from surveys conducted by the Ministry of Agriculture, Forestry and Fisheries. Since the number of foods reported in the consumption survey was greater than the number of foods analysed for cadmium levels, concentration values for similar foods were used as surrogates when there was not a one-to-one match between consumption and concentration data. The probabilistic estimates of cadmium intake were derived from a Monte Carlo simulation using the Japanese version of Crystal Ball 2000 (Kozo Keikaku Engineering, Inc.). Lognormal distributions were assumed for food consumption amounts, and a binary distribution was assumed for frequency of consumption (eaters versus non-eaters). For foods with cadmium levels above the LOQ, lognormal distributions were assumed if more than 100 samples exceeded the LOQ; for foods with fewer than 100 samples above the LOQ, medians were used as fixed values. For samples with analytical values below the LOQ, one half the value of the LOQ was substituted when less than 60% of the samples were below the LOQ, and the LOQ value was used when more than 60% of samples were below the LOQ. Four intake scenarios were performed — one for each of four possible MLs for cadmium in rice (0.2, 0.3, 0.4, 0.5 mg/kg). For all other foods, the MLs were fixed at the level currently proposed by CCFAC.

3.2 Comparison of new and previous concentration data

The previous JECFA assessment provided an estimate of total cadmium intake based on the five GEMS/Food regional diets and regional average cadmium concentrations calculated from aggregated data. Since the current assessment is an addendum to the previous work but is based on different cadmium data, it was important to show that the new data were comparable to those used previously.

Table 4 compares the new cadmium concentration data (both raw and aggregated data) with the aggregated data used in 2003. The data are reported by GEMS/Food region, since the regional diets are the basis for estimating cadmium intakes. Most data submitted for both the 2003 and 2005 assessments were from countries in the Far Eastern and European (which includes Australia, Canada, New Zealand and the United States) regions; a limited number of data from Middle Eastern countries were available in the previous assessment.

Average concentrations of cadmium based on the new data are reported in the left section of Table 4. For rice, average concentrations of cadmium were higher in Japanese samples (0.062 mg/kg) than in samples from other countries (0.017 mg/kg). The average concentration of cadmium in wheat was 0.054 mg/kg. Average concentrations of cadmium in vegetables ranged from 0.012 to 0.040 mg/kg. For molluscs, average concentrations of cadmium derived from more than 7000 samples were as follows: oysters, 1.38 mg/kg; mussels, 0.43 mg/kg; and other bivalves or cephalopods, 0.20 mg/kg.

All three sets of cadmium values are comparable, with a few exceptions:

Table 4. Comparison of cadmium concentration data from the sixty-first and sixty-fourth JECFA meetings

Commodity	64th JECFA (2005)			64th JECFA (2005)		61st JECFA (2003) ^a					
	Raw data			Aggregated data		Aggregated data					
	European + Far Eastern GEMS/Food region			European GEMS/Food region		European GEMS/Food region		Far Eastern GEMS/Food region		Middle Eastern GEMS/Food region	
	<i>n</i>	Mean (mg/kg)	SD	<i>n</i>	Mean (wtd) (mg/kg)	partial <i>n</i>	Mean (unwtd) (mg/kg)	partial <i>n</i>	Mean (unwtd) (mg/kg)	partial <i>n</i>	Mean (unwtd) (mg/kg)
GRAINS											
Rice											
- All data combined	37 547	0.061	0.062								
- Data by region:											
Far Eastern	37 250	0.062	0.062	86	0.046	108	0.010	37 350	0.070	10	0.006
European	297	0.017	0.026								
Wheat	940	0.054	0.048	716	0.038	752	0.026	410	0.030	53	0.034
VEGETABLES											
Potatoes	643	0.037	0.029	353	0.021						
Stem/root vegetables (excluding celeriac)	1570	0.028	0.033	60	0.052	448	0.025	460	0.015	12	0.022

Table 4. (contd)

Commodity		64th JECFA (2005)			64th JECFA (2005)		61st JECFA (2003) ^a					
		Raw data			Aggregated data		Aggregated data					
							European + Far Eastern GEMS/Food region		European GEMS/Food region		European GEMS/Food region	
		<i>n</i>	Mean (mg/kg)	SD	<i>n</i>	Mean (wtg) (mg/kg)	partial <i>n</i>	Mean (unwtg) (mg/kg)	partial <i>n</i>	Mean (unwtg) (mg/kg)	partial <i>n</i>	Mean (unwtg) (mg/kg)
Leafy vegetables	2043	0.040	0.044	112	0.047	9721	0.034	706	0.025	29	0.054	
	Other vegetables except tomatoes and mushrooms	3509	0.012	0.021	108	0.049	8858	0.013	1817	0.020	143	0.024
MOLLUSCS												
All molluscs combined	7151	1.013	1.120			289	0.799	316	0.208	35	0.250	
By mollusc subcategory: ^b												
a.	All bivalves combined	7023	1.029	1.123								
Bivalves by subcategory:												
a.1.	oysters	4478	1.384	1.242	848	0.360						
a.2.	mussels	2239	0.433	0.399	2341	0.177						
a.3.	scallops	74	0.181	0.311								

Table 4. (contd)

Commodity	64th JECFA (2005)			64th JECFA (2005)		61st JECFA (2003) ^a					
	Raw data			Aggregated data		Aggregated data					
	European + Far Eastern GEMS/Food region			European GEMS/Food region		European GEMS/Food region		Far Eastern GEMS/Food region		Middle Eastern GEMS/Food region	
	<i>n</i>	Mean (mg/kg)	SD	<i>n</i>	Mean (wtd) (mg/kg)	partial <i>n</i>	Mean (unwtd) (mg/kg)	partial <i>n</i>	Mean (unwtd) (mg/kg)	partial <i>n</i>	Mean (unwtd) (mg/kg)
a.4. bivalves, other	232	0.191	0.201	100	0.102						
b. Cephalopods	98	0.172	0.262	810	0.218						
c. Molluscs, other	30	0.043	0.025	145	0.239						
All molluscs excluding oysters	2673	0.391	0.389								

SD, standard deviation; unwtd, unweighted; wtd, weighted

^a Notes regarding the 2003 data:

- Not all data submissions indicated the sample size for the reported mean/median values. The “partial *n*” indicates the total of the sample sizes that were reported.
- The means were calculated as the average of all mean/median values reported. They were not weighted by sample size.
- The data for molluscs were not specific enough to separate them by subcategories.

^b The subcategories are subsets of the data reported above for “all molluscs combined.”

- As noted above, cadmium levels in rice samples from Japan are higher than those observed in other countries.
- For molluscs as a whole (all data combined), the mean concentration is higher than the regional averages from the 2003 JECFA assessment. This is mainly due to the fact that oysters accounted for more than half of the new raw data for molluscs. The previous averages were more similar to new data for “molluscs other than oysters.”
- Slight differences are noted in the mean values for all categories of vegetables. These differences are most likely due to differences in the specific vegetables that were included in each grouping of data. For the current work, CCFAC was very specific in defining each commodity group (Table 1), whereas the previous assessment was more inclusive and was based on slightly different groupings of commodities (e.g. potatoes were included in “roots/tubers.”

Since the assessment of the impact of different MLs requires use of raw data, aggregated data were not used in the assessment. Nevertheless, they provided information to confirm that both the new and previous data sets were comparable.

3.3 *Impact of different possible MLs on cadmium concentrations*

The purpose of this task was to estimate the change in mean cadmium values if samples exceeding a given possible ML were excluded from the calculation and to estimate the proportion of samples that contained cadmium concentrations greater than the ML. For five of the commodity groups (wheat, potatoes, stem/root vegetables, leafy vegetables and other vegetables), the data from different countries were sufficiently similar to allow all data to be combined for this assessment. Owing to the substantial difference in concentrations of cadmium in rice (by region) and in molluscs (by subcategory), the potential impact of MLs was evaluated separately for subsets of these data. Two estimates of the impact of MLs on concentrations of cadmium were calculated for rice (low estimates were based on European data only, and high estimates were based on all data combined) and for molluscs (low estimates were based on data for oysters and other molluscs separately, and high estimates were based on data for all molluscs combined). Although CCFAC requested that cephalopods be evaluated at a proposed ML of 2.0 mg/kg (Table 1), the data for cephalopods were combined with the group “molluscs excluding oysters” for the assessment, since the mean cadmium level was similar to that of other subcategories in this group.

For each commodity group or subgroup, a baseline mean concentration of cadmium was calculated from all data for the group. For each of the three or four possible MLs (proposed, one level higher and one or two levels lower), the mean was recalculated after excluding values greater than the ML, and the percentage reduction from the baseline mean was calculated. The number and percentage of total data points exceeding the ML were also calculated for each ML.

Results of the assessments for all commodities are summarized in Table 5. Results for each commodity are also reported separately in the graphs that follow (see Figure 1). The greatest impacts of MLs on concentrations of cadmium in individual commodities were seen for stem/root vegetables, other vegetables and molluscs excluding oysters, with reductions in mean values of 41%, 68% and 42%, respectively, when the lowest MLs were used.

Table 5. Impact of different MLs on cadmium concentrations: Summary of assessments

	ML (mg/kg)	<i>n</i> > ML	% > ML	mean (mg/kg)	% reduction from baseline (mean)
GRAINS					
RICE - ALL DATA COMBINED					
Total <i>n</i> : 37 547					
Level relative to proposed ML:					
Two levels lower	0.2	1243	3	0.054	12
One level lower	0.3	295	1	0.059	3
Proposed	0.4	94	<1	0.060	2
One level higher	0.5	39	<1	0.061	<1
Baseline (all data)				0.061	
RICE - DATA FOR EUROPEAN REGION					
Total <i>n</i> : 297					
Level relative to proposed ML:					
Two levels lower	0.2	2	1	0.015	12
One level lower	0.3	0		0.017	0
Proposed	0.4	0		0.017	0
One level higher	0.5	0		0.017	0
Baseline (all data)				0.017	
WHEAT GRAIN					
Total <i>n</i> : 940					
Level relative to proposed ML:					
One level lower	0.1	94	10	0.042	22
Proposed	0.2	13	1	0.051	6
One level higher	0.3	4	<1	0.052	3
Baseline (all data)				0.054	

Table 5. (contd)

	ML (mg/kg)	<i>n</i> > ML	% > ML	mean (mg/kg)	% reduction from baseline (mean)
VEGETABLES					
POTATOES					
Total <i>n</i> : 643					
Level relative to proposed ML:					
One level lower	0.05	163	25	0.022	39
Proposed	0.1	16	2	0.034	8
One level higher	0.2	2	<1	0.036	3
Baseline (all data)				0.037	
STEM AND ROOT VEGETABLES (excluding potatoes and celeriac)					
Total <i>n</i> : 1570					
Level relative to proposed ML:					
One level lower	0.05	242	15	0.017	41
Proposed	0.1	57	4	0.023	16
One level higher	0.2	5	<1	0.027	3
Baseline (all data)				0.028	
LEAFY VEGETABLES					
Total <i>n</i> : 2043					
Level relative to proposed ML:					
One level lower	0.1	143	7	0.031	22
Proposed	0.2	26	1	0.037	7
One level higher	0.3	7	<1	0.039	2
Baseline (all data)				0.040	
OTHER VEGETABLES (excluding tomatoes and fungi)					
Total <i>n</i> : 3509					
Level relative to proposed ML:					
One level lower	0.01	948	27	0.004	68
Proposed	0.05	149	4	0.009	27
One level higher	0.1	36	1	0.011	9
Baseline (all data)				0.012	

Table 5. (contd)

	ML (mg/kg)	<i>n</i> > ML	% > ML	mean (mg/kg)	% reduction from baseline (mean)
MOLLUSCS					
ALL MOLLUSCS COMBINED					
Total <i>n</i> : 7151					
Level relative to proposed ML:					
One level lower	0.5	4005	56	0.253	75
Proposed	1	2415	34	0.409	60
One level higher	2	1063	15	0.638	37
Baseline (all data)				1.010	
OYSTERS					
Total <i>n</i> : 4478					
Level relative to proposed ML:					
One level lower	2	1049	23	0.839	39
Proposed	3	424	9	1.082	22
One level higher	4	190	4	1.210	13
Baseline (all data)				1.384	
MOLLUSCS EXCLUDING OYSTERS					
Total <i>n</i> : 2673					
Level relative to proposed ML:					
One level lower	0.5	681	25	0.227	42
Proposed	1	173	6	0.321	18
One level higher	2	14	1	0.378	3
Baseline (all data)				0.391	

3.4 *Impact of different possible MLs on cadmium intakes*

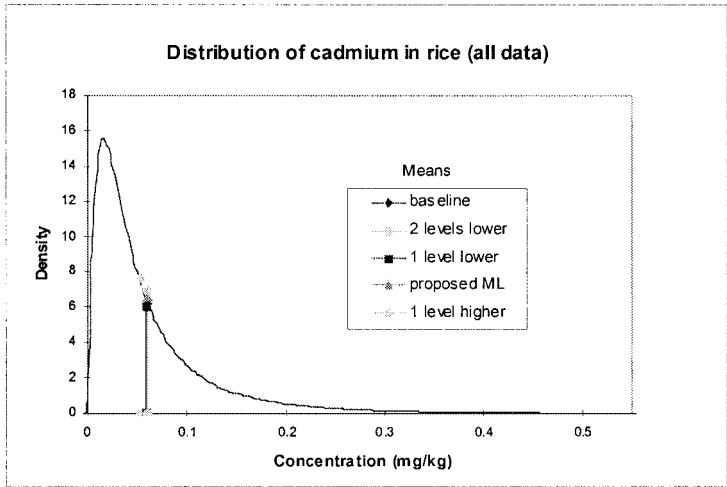
For the intake assessment completed by the Committee at its sixty-first meeting, intakes of cadmium, both by commodity and total, were calculated from the GEMS/Food regional diets and the regional average concentrations of cadmium derived from aggregated data. Total intakes ranged from 2.8 to 4.2 µg/kg bw per week, which corresponds to approximately 40–60% of the PTWI of 7 µg/kg bw per week.

Since the present assessment required the use of raw rather than aggregated data, it was necessary to recalculate the intakes using the average concentration

values derived from the new raw data. The GEMS/Food regional diets were used for the calculation, as they had been in the previous JECFA assessment. Slight differences in the intakes estimated for the previous and the current assessment resulted from differences in consumption amounts for the commodity groups. As noted previously, CCFAC was more specific in its definitions of the commodity groups for this assessment, so the consumption amounts used in each of the assessments were slightly different for some commodities (particularly for vegetables). Mean cadmium concentration values used for the two estimates were also somewhat different.

Figure 1. Impact of different possible MLs on cadmium concentrations in A) rice, B) wheat, C) potatoes, D) stem and root vegetables, E) leafy vegetables, F) other vegetables, G) oysters and H) molluscs other than oysters

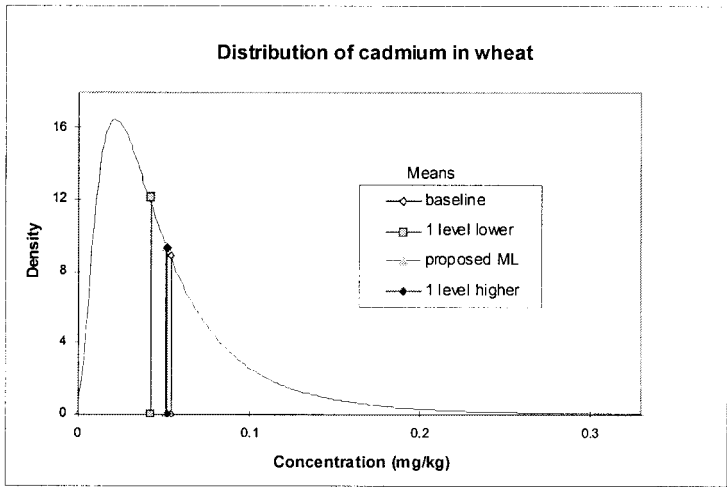
A. Impact of different MLs on cadmium concentrations in rice (all data)



Rice	Possible ML (mg/kg)	Mean (mg/kg)	Maximum value (mg/kg)	% samples > ML
Baseline (all data)		0.061	1.20	
Two levels lower	0.2	0.054		3
One level lower	0.3	0.059		1
Proposed ML	0.4	0.060		<1
One level higher	0.5	0.061		<1

Figure 1. (contd)

B. Impact of different MLs on cadmium concentrations in wheat



	Possible ML (mg/kg)	Mean (mg/kg)	Maximum value (mg/kg)	% samples > ML
Baseline (all data)		0.054	0.470	
One level lower	0.1	0.042		10
Proposed ML	0.2	0.051		1
One level higher	0.3	0.052		<1

C. Impact of different MLs on cadmium concentrations in potatoes

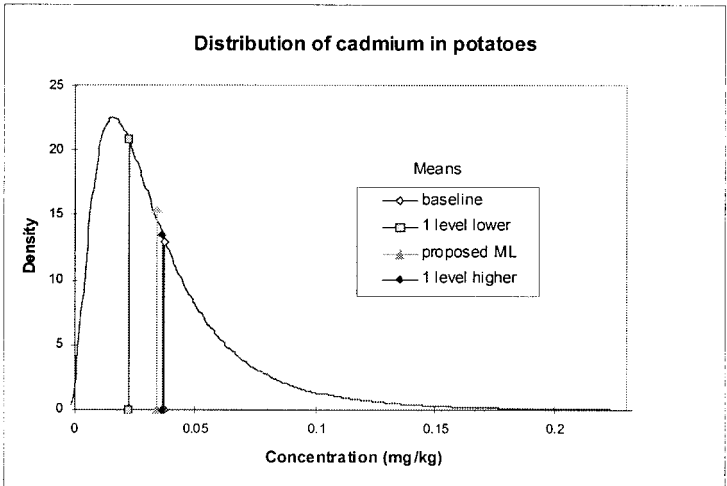
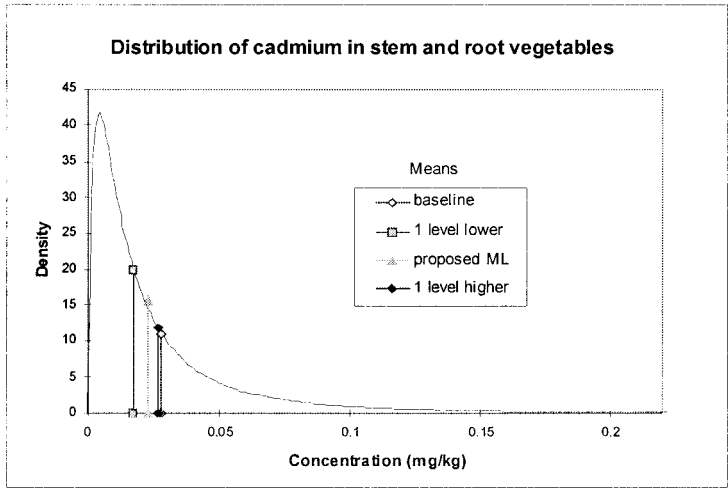


Figure 1. (contd)

	Possible ML (mg/kg)	Mean (mg/kg)	Maximum value (mg/kg)	% samples > ML
Baseline (all data)		0.037	0.220	
One level lower	0.05	0.022		25
Proposed ML	0.1	0.034		2
One level higher	0.2	0.036		<1

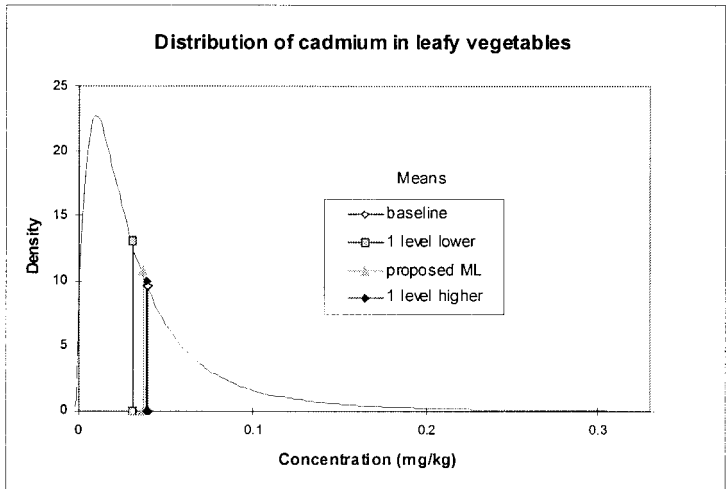
D. Impact of different MLs on cadmium concentrations in stem and root vegetables



	Possible ML (mg/kg)	Mean (mg/kg)	Maximum value (mg/kg)	% samples > ML
Baseline (all data)		0.028	0.330	
One level lower	0.05	0.017		15
Proposed ML	0.1	0.023		4
One level higher	0.2	0.027		<1

Figure 1. (contd)

E. Impact of different MLs on cadmium concentrations in leafy vegetables



	Possible ML (mg/kg)	Mean (mg/kg)	Maximum value (mg/kg)	% samples > ML
Baseline (all data)		0.040	0.490	
One level lower	0.1	0.031		7
Proposed ML	0.2	0.037		1
One level higher	0.3	0.039		<1

F. Impact of different MLs on cadmium concentrations in other vegetables

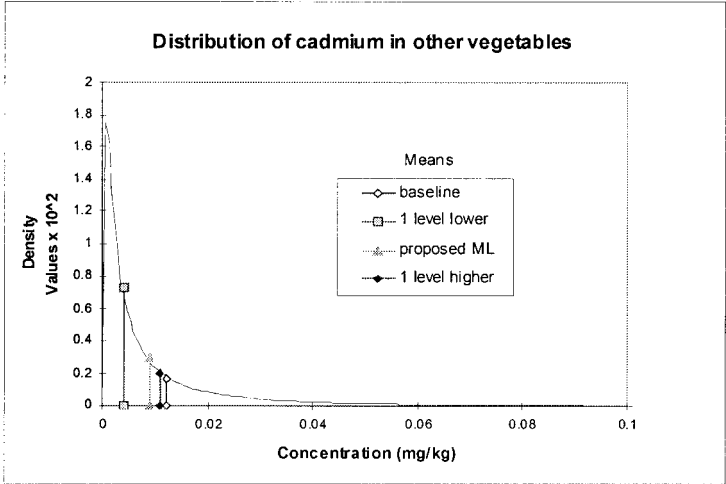
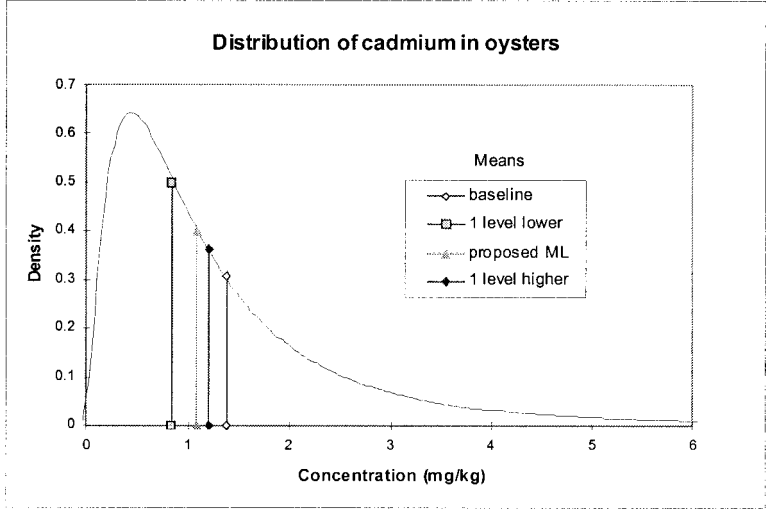


Figure 1. (contd)

	Possible ML (mg/kg)	Mean (mg/kg)	Maximum value (mg/kg)	% samples > ML
Baseline (all data)		0.012	0.240	
One level lower	0.01	0.004		27
Proposed ML	0.05	0.009		4
One level higher	0.1	0.011		1

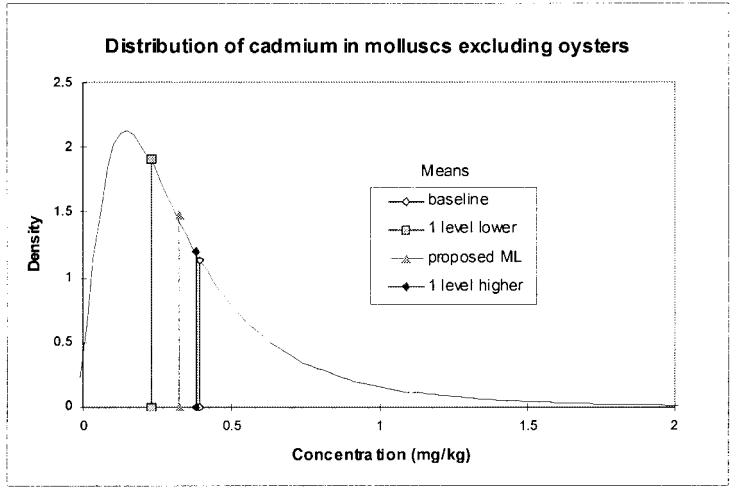
G. Impact of different MLs on cadmium concentrations in oysters



	Possible ML (mg/kg)	Mean (mg/kg)	Maximum value (mg/kg)	% samples > ML
Baseline (all data)		1.384	9.81	
One level lower	2	0.839		23
Proposed ML	3	1.082		9
One level higher	4	1.210		4

Figure 1. (contd)

H. Impact of different MLs on cadmium concentrations in molluscs other than oysters



	Possible ML (mg/kg)	Mean (mg/kg)	Maximum value (mg/kg)	% samples > ML
Baseline (all data)		0.391	9.0	
One level lower	0.5	0.227		25
Proposed ML	1	0.321		6
One level higher	2	0.378		1

An additional adjustment was made for estimating cadmium intake from molluscs. The GEMS/Food regional diets include total consumption amounts for “molluscs including cephalopods” but provide no information about consumption of mollusc subcategories. Since the new concentration data showed that cadmium levels in oysters tend to be higher than levels in other molluscs, it was preferable to calculate intake from oysters separately from other molluscs. National consumption data from Australia, France, New Zealand and the United States were used to derive average proportions of oyster and total mollusc consumption that could be applied to the regional diets (Table 6). Since mollusc consumption is highest for the GEMS/Food European region, data from these countries provided a good basis for deriving the adjustment factor. Oyster consumption was estimated to be about 16% of total mollusc consumption.

Table 7 summarizes the updated cadmium intake estimates for each commodity group. Intakes are expressed in terms of actual intake ($\mu\text{g/kg}$ bw per day) and percentage of the daily equivalent of the PTWI of $7 \mu\text{g/kg}$ bw per week (i.e. $1 \mu\text{g/kg}$ bw per day). Baseline intakes for the five GEMS/Food regions, expressed as a percentage of the daily equivalent of the PTWI, ranged from 1% to 34% for rice, 3% to 29% for wheat, 1% to 15% for potatoes, <1% to 14% for stem/root

vegetables, <1% to 3% for leafy and other vegetables, <1% to 15% for all molluscs combined and <1% to 5% for subcategories of molluscs.

Table 6. Proportional consumption of molluscs by subcategory based on national consumption data

Country	Percentage of total mollusc consumption					
	Bivalves				Cephalopods	Other molluscs
	Oysters	Scallops	Mussels	Clams		
France	24	6	70	nr	nr	nr
Australia	14	19	14	2	51	nr
New Zealand	19	7	47	3	14	9
USA	8	8	4	67	9	4
Average	16	10	34	18	19	3

nr, not reported; assumed 0 when calculating average

The impact of different possible MLs on cadmium intakes was evaluated for commodities/regions for which intakes were significant (i.e. 10% or more of the daily equivalent of the PTWI in one region or 5% or more of the daily equivalent of the PTWI in two or more regions). The remaining commodities/regions contributed minimally to total cadmium intake, and it was assumed that different MLs would have little or no impact on intakes. For rice, only the intake estimates based on all concentration data combined were assessed, since they provided a more conservative estimate. For molluscs, assessments were conducted for oysters and other molluscs only; since the majority of data for molluscs were for oysters, the combined data set was not considered representative of molluscs as a whole. Results of this assessment are reported in Table 8. Baseline intake for each commodity group, calculated from the mean of all concentration data for that commodity, is reported in terms of $\mu\text{g/kg bw}$ per day and as a percentage of the daily equivalent of the PTWI. Intakes for each scenario (total intake and reduction from baseline) are reported in terms of percentage of the daily equivalent of the PTWI. The lowest MLs generated reductions in intakes as follows, expressed here as a percentage of the PTWI: rice, 4%; wheat, 6%; potatoes, 6%; stem/root vegetables, 5%; oysters, 1%; and other molluscs, 2%. The proposed ML and one level higher had little or no impact on mean intakes of cadmium.

Results of the probabilistic intake assessment submitted by the Japanese government are presented in Tables 9 and 10. In this case, the impact on total cadmium intake was assessed for four possible MLs for rice only. Intakes were originally reported on a weekly basis but were converted for this assessment to a daily basis for comparability to the intakes calculated from the GEMS/Food regional diets. Total mean daily cadmium intake was estimated to be 45% and 48% of the daily equivalent of the PTWI at the lowest and highest possible MLs, respectively (Table 9). The lowest ML for rice (0.2 mg/kg) resulted in a slight reduction (3% of the daily equivalent of the PTWI) in total mean intake. There was

Table 7. Baseline cadmium intakes from selected commodities^a

Commodity		Middle Eastern GEMS/Food region		Far Eastern GEMS/Food region		African GEMS/ Food region		Latin American GEMS/Food region		European GEMS/Food region	
		µg/kg bw per day	% PTWI _d ^b	µg/kg bw per day	% PTWI _d	µg/kg bw per day	% PTWI _d	µg/kg bw per day	% PTWI _d	µg/kg bw per day	% PTWI _d
High estimate:	Rice - all data	0.050	5	0.341	34	0.105	11	0.088	9	0.012	1
	Rice - Japan only	0.050	5	0.346	35	0.107	11	0.089	9	0.012	1
Low estimate:	Rice - other countries	0.014	1	0.095	9	0.029	3	0.025	2	0.003	0
	Wheat	0.295	29	0.124	12	0.025	3	0.105	11	0.160	16
	Potatoes	0.036	4	0.014	1	0.013	1	0.025	3	0.148	15
	Stem and root vegetables (excluding potatoes and celeriac)	0.004	0	0.052	5	0.140	14	0.059	6	0.016	2
	Leafy vegetables	0.005	1	0.008	1	0.000	0	0.011	1	0.034	3
	Other vegetables (excluding tomatoes and mushrooms)	0.025	2	0.012	1	0.004	0	0.013	1	0.027	3
	All molluscs including cephalopods	0.000	0	0.081	8	0.008	1	0.014	1	0.154	15
Low estimate:	Oysters only	0.000	0	0.018	2	0.002	0.2	0.003	0.3	0.034	3

Table 7. (contd)

Commodity		Middle Eastern GEMS/Food region		Far Eastern GEMS/Food region		African GEMS/ Food region		Latin American GEMS/Food region		European GEMS/Food region	
		µg/kg bw per day	% PTWI _d ^b	µg/kg bw per day	% PTWI _d	µg/kg bw per day	% PTWI _d	µg/kg bw per day	% PTWI _d	µg/kg bw per day	% PTWI _d
Low estimate:	Molluscs other than oysters	0.000	0	0.026	3	0.003	0.3	0.004	0.4	0.050	5
Subtotal intake from seven commodities based on 2005 cadmium data:											
	High estimate:	0.41	41	0.63	63	0.30	30	0.31	31	0.55	55
	Low estimate:	0.38	38	0.35	35	0.22	22	0.24	24	0.47	47
Total intakes as per 2003 JECFA		0.428	40	0.617	60	0.47	50	0.378	40	0.548	50

^a Baseline intake is based on mean concentration of all data for a given commodity and GEMS/Food regional diets.

^b PTWI_d = daily equivalent of the PTWI = 1 µg/kg bw per day (derived from PTWI of 7 µg/kg bw per week).

Table 8. Impact of different possible MLs on cadmium intakes: Summary of scenarios^a

Commodity	GEMS/ Food region	GEMS/ Food regional diets – con- sump- tion (g/kg bw per day)	Cadmium intakes based on mean concentrations for baseline and each ML scenario														
			Baseline (all data)			Two levels lower			One level lower			Proposed ML			One level higher		
			Intake (µg/kg bw per day)	Intake as % PTWI _d ^b		Intake (µg/kg bw per day)	Intake as % PTWI _d	Δ ^c	Intake (µg/kg bw per day)	Intake as % PTWI _d	Δ	Intake (µg/kg bw per day)	Intake as % PTWI _d	Δ	Intake (µg/kg bw per day)	Intake as % PTWI _d	Δ
Rice (based on mean of all data combined)	Middle Eastern	0.81	0.050	5		0.044	4	–1	0.048	5	0	0.049	5	0	0.050	5	0
	Far Eastern	5.59	0.341	34		0.302	30	–4	0.330	33	–1	0.335	34	0	0.341	34	0
	African	1.72	0.105	11		0.093	9	–2	0.102	10	–1	0.103	10	–1	0.105	11	0
	Latin American	1.44	0.088	9		0.078	8	–1	0.085	9	0	0.087	9	0	0.088	9	0
Wheat	Middle Eastern	5.46	0.295	29					0.229	23	–6	0.278	28	–1	0.284	28	–1
	Far Eastern	2.30	0.124	12					0.096	10	–2	0.117	12	0	0.119	12	0
	Latin American	1.95	0.105	11					0.082	8	–3	0.099	10	–1	0.101	10	–1
	European	2.97	0.160	16					0.125	12	–4	0.151	15	–1	0.154	15	–1
Potatoes	European	4.01	0.148	15					0.088	9	–6	0.136	14	–1	0.144	14	–1

Table 8. (contd)

Commodity	GEMS/ Food region	GEMS/ Food regional diets – con- sump- tion (g/kg bw per day)	Cadmium intakes based on mean concentrations for baseline and each ML scenario													
			Baseline (all data)		Two levels lower			One level lower			Proposed ML			One level higher		
			Intake (µg/kg bw per day)	Intake as % PTWI _d ^b	Intake (µg/kg bw per day)	Intake as % PTWI _d	Δ ^c	Intake (µg/kg bw per day)	Intake as % PTWI _d	Δ	Intake (µg/kg bw per day)	Intake as % PTWI _d	Δ	Intake (µg/kg bw per day)	Intake as % PTWI _d	Δ
Stem/root vegetables (excluding potatoes, celeriac)	Far Eastern	1.85	0.052	5				0.031	3	–2	0.043	4	–1	0.050	5	0
	African	5.01	0.140	14				0.085	9	–5	0.115	12	–2	0.135	14	0
	Latin American	2.11	0.059	6				0.036	4	–2	0.049	5	–1	0.057	6	0
Molluscs																
- Oysters only	European	0.02	0.034	3				0.020	2	–1	0.026	3	0	0.029	3	0
- All other molluscs	European	0.13	0.050	5				0.029	3	–2	0.041	4	–1	0.048	5	0
- Oysters only	Far Eastern	0.01	0.018	2				0.010	1	–1	0.013	1	–1	0.015	1	–1
- All other molluscs	Far Eastern	0.07	0.026	3				0.015	2	–1	0.022	2	–1	0.026	3	0

^a Includes only commodities/regions for which baseline intake estimates = 5% or > PTWI_d.

^b PTWI_d = daily equivalent of the PTWI = 1 µg/kg bw per day (calculated from PTWI of 7 µg/kg bw per week).

^c Δ = change from baseline (% PTWI_d).

Table 9. Japanese model: impact of different possible MLs for rice on total cadmium

Intake percentile	Total cadmium intake											
	Two levels lower (0.2 mg/kg for rice)			One level lower (0.3 mg/kg for rice)			Proposed ML (0.4 mg/kg for rice)			One level higher (0.5 mg/kg for rice)		
	µg/kg bw per week	µg/kg bw per day	% PTWI/PTWI _d ^a	µg/kg bw per week	µg/kg bw per day	% PTWI/PTWI _d	µg/kg bw per week	µg/kg bw per day	% PTWI/PTWI _d	µg/kg bw per week	µg/kg bw per day	% PTWI/PTWI _d
Mean	3.18	0.45	45	3.29	0.47	47	3.33	0.48	48	3.35	0.48	48
50	2.79	0.40	40	2.85	0.41	41	2.86	0.41	41	2.86	0.41	41
75	3.85	0.55	55	3.94	0.56	56	3.97	0.57	57	3.98	0.57	57
90	5.21	0.74	74	5.45	0.78	78	5.54	0.79	79	5.57	0.80	80
95	6.27	0.90	90	6.67	0.95	95	6.85	0.98	98	6.93	0.99	99
97.5	7.38	1.05	105	8.01	1.14	114	8.32	1.19	119	8.46	1.21	121

^a PTWI_d = daily equivalent of the PTWI = 1 µg/kg bw per day, derived from a PTWI of 7 µg/kg bw per week.

no impact on total mean intake at the proposed and higher MLs. Greater reductions in total intake were seen at the upper percentiles.

Total mean intake from rice was estimated to be about 20% of the PTWI in the Japanese model (Table 10), compared with the highest estimates based on the GEMS/Food regional diets of 33–34% of the PTWI from rice. The consumption values in the GEMS/Food regional diets, which are based on Food Balance Sheet data, are generally assumed to be about 15% higher than values for actual average food consumption (WHO, 2003). Despite the difference in actual estimates of intake of cadmium from rice, both the probabilistic model and the GEMS/Food estimates demonstrated that each of the four possible MLs for rice had little or no impact on mean cadmium intake.

Table 10. Japanese model: contribution of commodities to total cadmium intake

Commodity	Cadmium intake (µg/kg bw per week) ^a	Percentage of total intake	Percentage of PTWI
Fishery products	0.45	13	6
Meat	0.09	3	1
Fruits	0.10	3	1
Vegetables	0.69	21	10
Soya beans	0.21	6	3
Wheat	0.36	11	5
Rice	1.39	42	20
Other cereals and beans	0.05	1	1

^a Results of scenario based on proposed ML for rice (0.4 mg/kg).

4. COMMENTS

4.1 Data on concentrations of cadmium in food

In order to conduct this assessment, information about the distribution of concentrations of cadmium in each commodity group (e.g. analytical results for each sample, or raw data) was required. Raw data were submitted by Australia, Canada, Germany, Japan, New Zealand, Norway and the United States. Some aggregated data were also submitted by the European Union, Spain, Sweden and Thailand.

Average concentrations of cadmium, based on the new data on individual samples, were similar to those used in the intake assessment completed by the Committee at its sixty-first meeting (Table 4). For rice, average concentrations of cadmium were higher in samples from Japan (0.062 mg/kg) than in samples from other countries (0.017 mg/kg). The average concentration of cadmium in wheat was 0.054 mg/kg. Average concentrations of cadmium in vegetables ranged from

0.012 to 0.040 mg/kg. For molluscs, average concentrations of cadmium derived from more than 7000 samples were as follows: oysters, 1.38 mg/kg; mussels, 0.43 mg/kg; and other bivalves or cephalopods, 0.20 mg/kg.

4.2 *Assessment of the impact of different possible MLs on mean concentrations of cadmium*

For five of the commodity groups (wheat, potatoes, stem/root vegetables, leafy vegetables and other vegetables), the data from different countries were sufficiently similar to allow all data to be combined for this assessment. Owing to the substantial difference in concentrations of cadmium in rice (by region) and in molluscs (by subcategory), the potential impact of MLs was evaluated separately for subsets of these data. Two estimates of the impact of MLs on concentrations of cadmium were calculated for rice (low estimates were based on European data only, and high estimates were based on all data combined) and for molluscs (low estimates were based on data for oysters and other molluscs separately, and high estimates were based on data for all molluscs combined).

For each commodity group or subgroup, a baseline mean concentration of cadmium was calculated from all data on concentrations. For each of the three or four MLs (proposed, one level higher and one level or two levels lower), the mean was recalculated after excluding values greater than that ML, and the percentage reduction from the baseline mean was calculated. The number and percentage of total data points exceeding the ML were also calculated for each ML (Table 5). The greatest impacts of MLs on concentrations of cadmium in individual commodities were seen for stem/root vegetables, other vegetables and molluscs (41%, 68%, and 42%, respectively, when the lowest MLs were used).

4.3 *Assessment of the impact of different possible MLs on mean intakes of cadmium*

For the intake assessment completed by the Committee at its sixty-first meeting (Annex 1, reference 166), intakes of cadmium, both by commodity and total, were calculated from the GEMS/Food regional diets and the regional average concentrations of cadmium derived from aggregated data. Total intakes ranged from 2.8 to 4.2 µg/kg bw per week, which corresponds to 40–60% of the PTWI of 7 µg/kg bw per week.

For the present assessment, intakes of cadmium were recalculated for the seven commodity groups on an individual basis; total intakes of cadmium calculated in the previous intake assessment were used as benchmarks (Table 7). Baseline intakes were calculated from food consumption reported in the GEMS/Food regional diets, as in the previous assessment, and values for average baseline concentrations of cadmium were derived from the new raw data. Intakes were recalculated based on the mean concentration of cadmium from each of the MLs. The impact of each ML on intake of cadmium was reported in terms of the reduction from baseline intake.

Baseline intakes for the five GEMS/Food regions, which were calculated as daily intakes and expressed as a percentage of the daily equivalent of the PTWI, ranged from 1% to 34% for rice, 3% to 29% for wheat, 1% to 15% for potatoes, <1% to 14% for stem/root vegetables, <1% to 3% for leafy vegetables, <1% to 3% for other vegetables, <1% to 3% for oysters and <1% to 5% for other molluscs (Tables 8 and 11). The lowest MLs generated reductions in intakes as follows, expressed here as a percentage of the daily equivalent of the PTWI: rice, 4%; wheat, 6%; potatoes, 6%; stem/root vegetables, 5%; oysters, 1%; and other molluscs, 2%. The proposed ML and one level higher had little or no impact on mean intakes of cadmium.

A probabilistic intake assessment for cadmium in rice using national data from Japan was submitted to the Committee. This intake assessment considered four different possible MLs and showed results similar to those based on the GEMS/Food regional diets. Total mean intake of cadmium from rice was estimated to be about 1.4 µg/kg bw per week, or 20% of the PTWI, compared with daily equivalent estimates based on the GEMS/Food diets of 33–34% of the PTWI from rice. The consumption values in the GEMS/Food diets, which are based on data from Food Balance Sheets, are generally assumed to be about 15% higher than values for actual average food consumption (WHO, 2003). Despite the difference in actual estimates of intake of cadmium from rice, both the probabilistic model and the GEMS/Food estimates demonstrated little or no impact on mean intake of cadmium from rice for the four possible MLs.

5. EVALUATION

The Committee concluded that the effect of different MLs on overall intake of cadmium would be very small. At the proposed Codex MLs, mean intake of cadmium would be reduced by approximately 1% of the PTWI. The imposition of MLs one level lower would result in potential reductions in intake of cadmium of no more than 6% (wheat grain, potatoes) of the PTWI. At the proposed Codex MLs, no more than 9% of a commodity would be violative (oysters). MLs one level below those proposed would result in approximately 25% of molluscs, potatoes and other vegetables being violative.

The use of different possible MLs to truncate the tail of the distribution of a contaminant in commodities has little impact on the intake of the contaminant from that commodity, unless a large proportion of the commodity is excluded by the ML. The Committee noted that in its previous assessment (Annex 1, reference 166), the total intake of cadmium was only 40–60% of the PTWI of 7 µg/kg bw per week; therefore, a variation of 1–6% attributable to the use of the proposed Codex MLs, and one level higher or lower, is of no significance in terms of risk to human health.

Table 11. Summary of impact of different MLs for cadmium

		Level relative to proposed ML	ML	Impact on cadmium concentration		Impact on cadmium intakes
				% reduction from baseline mean	% samples > ML	Reduction in highest intakes (as % PTWI _d) ^a
Rice - all data combined						
Baseline mean concentration (mg/kg)	0.061	Two levels lower	0.2	12	3	4
Highest baseline intake (% PTWI _d)	34	One level lower	0.3	3	1	1
		Proposed	0.4	2	<1	1
		One level higher	0.5	<1	<1	0
Wheat grain						
Baseline mean concentration (mg/kg)	0.054	One level lower	0.1	22	10	6
Highest baseline intake (% PTWI _d)	29	Proposed	0.2	6	1	1
		One level higher	0.3	3	<1	1
Potatoes						
Baseline mean concentration (mg/kg)	0.037	One level lower	0.05	39	25	6
Highest baseline intake (% PTWI _d)	15	Proposed	0.1	8	2	1
		One level higher	0.2	3	<1	1
Stem and root vegetables (excluding potatoes and celeriac)						
Baseline mean concentration (mg/kg)	0.028	One level lower	0.05	41	15	5
Highest baseline intake (% PTWI _d)	14	Proposed	0.1	16	4	2
		One level higher	0.2	3	<1	0

Table 11. (contd)

			Level relative to proposed ML	ML	Impact on cadmium concentration		Impact on cadmium intakes
					% reduction from baseline mean	% samples > ML	Reduction in highest intakes (as % PTWI _d) ^a
Leafy vegetables							
Baseline mean concentration (mg/kg)	0.040	One level lower	0.1	22	7	not evaluated	
Highest baseline intake (% PTWI _d)	<5	Proposed	0.2	7	1		
		One level higher	0.3	2	<1		
Other vegetables (excluding tomatoes and fungi)							
Baseline mean concentration (mg/kg)	0.012	One level lower	0.01	68	27	not evaluated	
Highest baseline intake (% PTWI _d)	<5	Proposed	0.05	27	4		
		One level higher	0.1	9	1		
Molluscs							
- Oysters							
Baseline mean concentration (mg/kg)	1.384	One level lower	2	39	23	1	
Highest baseline intake (% PTWI _d)	3	Proposed	3	22	9	1	
- Molluscs excluding oysters							
Baseline mean concentration (mg/kg)	0.391	One level lower	0.5	42	25	2	
Highest baseline intake (% PTWI _d)	5	Proposed	1	18	6	1	
		One level higher	2	3	1	0	

^a This represents the reduction in intake for the GEMS/Food region with the highest intake from this commodity. PTWI_d = daily equivalent of the PTWI = 1 µg/kg bw per day, derived from a PTWI of 7 µg/kg bw per week.

6. REFERENCES

- CAC (2004a) *Report of the Thirty-sixth Session of the Codex Committee on Food Additives and Contaminants, Rotterdam, The Netherlands, 22–26 March 2004*. Rome: Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ALINORM 04/27/12; <http://www.codexalimentarius.net/web/archives.jsp?lang=en>).
- CAC (2004b) Draft CCFAC policy for exposure assessment of contaminants and toxins in foods or food groups. In: *Report of the Thirty-sixth Session of the Codex Committee on Food Additives and Contaminants, Rotterdam, The Netherlands, 22–26 March 2004*. Rome: Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ALINORM 04/27/12; <http://www.codexalimentarius.net/web/archives.jsp?lang=en>).
- EC (2004) SCOOP 3.2.11. *Assessment of the Dietary Exposure to Arsenic, Cadmium, Lead and Mercury of the Population of the EU Member States*. Report of experts participating in Scientific Cooperation on Food Task 3.2.11, March 2004. Brussels: European Commission.
- WHO (1995) *Report on the Second Workshop on Reliable Evaluation of Low-level Contamination of Food, 26–27 May 1995, Kulmbach, Federal Republic of Germany*. Geneva, World Health Organization, GEMS/Food-EURO.
- WHO (2003) *GEMS/Food Regional Diets. Regional Per Capita Consumption of Raw and Semi-processed Agricultural Commodities*. Geneva: World Health Organization (http://www.who.int/foodsafety/chem/en/gems_regional_diet.pdf).

**APPENDIX A: SUMMARY OF RAW DATA ON CADMIUM CONCENTRATIONS
IN COMMODITIES, BY COUNTRY AND REGION**

Commodity		n	Cadmium concentration (mg/kg)			
			Mean	SD	Min	Max
RICE						
All data combined		37 547	0.061	0.062	<0.001	1.200
By region:	European	297	0.017	0.026	<0.001	0.226
	Far Eastern	37 250	0.062	0.062	0.005	1.200
By country:	Germany	131	0.023	0.019	0.002	0.109
	Japan	37 250	0.062	0.062	0.005	1.200
	USA	166	0.012	0.029	<0.001	0.226
WHEAT						
All data combined:		940	0.054	0.048	0.001	0.470
By region:	European	558	0.041	0.034	0.003	0.347
	Far Eastern	382	0.072	0.057	0.001	0.047
By country:	Australia	57	0.033	0.038	0.003	0.170
	Germany	209	0.041	0.032	0.005	0.347
	Japan	382	0.072	0.057	0.001	0.470
	New Zealand	2	0.027	0.002	0.025	0.028
	USA	290	0.043	0.035	0.003	0.207
POTATOES						
All data combined		643	0.037	0.029	0.001	0.220
By region:	European	574	0.038	0.031	0.001	0.220
	Far Eastern	69	0.023	0.012	0.003	0.058
By country:	Australia	114	0.083	0.027	0.055	0.220
	Germany	163	0.019	0.015	0.001	0.092
	Japan	69	0.023	0.012	0.003	0.058
	USA	297	0.031	0.020	0.002	0.182
STEM/ROOT VEGETABLES						
All data combined		1570	0.028	0.033	0.001	0.330
By region:	European	573	0.027	0.028	0.001	0.160
	Far Eastern	997	0.028	0.035	0.003	0.330
By country:	Australia	133	0.045	0.037	0.003	0.160

Appendix A (contd)

Appendix IV (contd.)

Commodity		<i>n</i>	Cadmium concentration (mg/kg)			
			Mean	SD	Min	Max
	Germany	223	0.016	0.018	0.001	0.120
	Japan	997	0.028	0.035	0.003	0.330
	New Zealand	10	0.020	0.012	0.006	0.045
	USA	207	0.028	0.025	0.002	0.132
LEAFY VEGETABLES						
All data combined		2043	0.040	0.044	0.001	0.490
By region:	European	1370	0.036	0.040	0.001	0.370
	Far Eastern	673	0.049	0.050	0.003	0.490
By country:	Australia	177	0.023	0.043	0.003	0.320
	Germany	927	0.038	0.041	0.001	0.370
	Japan	673	0.049	0.050	0.003	0.490
	New Zealand	12	0.018	0.007	0.008	0.030
	USA	254	0.042	0.036	0.001	0.195
OTHER VEGETABLES						
All data combined		3509	0.012	0.021	0.000	0.240
By region:	European	1980	0.007	0.016	0.000	0.240
	Far Eastern	1529	0.019	0.025	0.003	0.220
By country:	Australia	100	0.021	0.049	0.002	0.240
	Germany	1335	0.006	0.013	0.001	0.240
	Japan	1529	0.019	0.025	0.003	0.220
	New Zealand	47	0.003	0.004	0.000	0.022
	USA	498	0.007	0.008	0.001	0.054
MOLLUSCS, ALL						
All data combined		7151	1.013	1.120	0.000	9.810
By region:	European	6755	1.062	1.132	0.000	9.810
	Far Eastern	396	0.176	0.211	0.005	1.300
By country:	Australia	43	0.518	0.356	0.100	2.000
	Canada	2192	2.163	1.286	0.040	9.810
	Germany	103	0.159	0.123	0.000	0.839
	Japan	396	0.176	0.211	0.005	1.300
	New Zealand	22	1.757	2.225	0.078	6.700

Appendix A (contd)

Commodity		n	Cadmium concentration (mg/kg)			
			Mean	SD	Min	Max
	Norway	35	0.541	0.781	0.100	4.100
	USA	4360	0.536	0.459	0.001	9.000
MOLLUSCS - BY SUBCATEGORY						
BIVALVES, ALL (See breakout by bivalve subcategory below)						
All data combined		7023	1.029	1.123	0.000	9.810
By region:	European	6755	1.062	1.132	0.000	9.810
	Far Eastern	268	0.193	0.196	0.010	0.770
By country:	Australia	43	0.518	0.356	0.100	2.000
	Canada	2192	2.163	1.286	0.040	9.810
	Germany	103	0.159	0.123	0.000	0.839
	Japan	268	0.193	0.196	0.010	0.770
	New Zealand	22	1.757	2.225	0.078	6.700
	Norway	35	0.541	0.781	0.100	4.100
	USA	4360	0.536	0.459	0.001	9.000
CEPHALOPODS						
All data combined		98	0.172	0.262	0.005	1.300
By region:	Far Eastern	98	0.172	0.262	0.005	1.300
By country:	Japan	98	0.172	0.262	0.005	1.300
MOLLUSCS, OTHER						
All data combined		30	0.043	0.025	0.005	0.100
By region:	Far Eastern	30	0.043	0.025	0.005	0.100
By country:	Japan	30	0.043	0.025	0.005	0.100
SUBCATEGORIES - BIVALVES						
OYSTERS						
All data combined		4478	1.384	1.242	0.001	9.810
By region:	European	4433	1.395	1.244	0.001	9.810
	Far Eastern	45	0.301	0.155	0.100	0.680
By country:	Australia	31	0.531	0.364	0.160	2.000
	Canada	2192	2.163	1.286	0.040	9.810
	Japan	45	0.301	0.155	0.100	0.680
	New Zealand	11	3.272	2.309	0.265	6.700

Appendix A (contd)

Commodity		n	Cadmium concentration (mg/kg)			
			Mean	SD	Min	Max
	Norway	6	0.772	0.326	0.480	1.350
	USA	2193	0.633	0.497	0.001	6.752
SCALLOPS						
All data combined		74	0.181	0.311	0.010	2.100
By region:	European	17	0.401	0.565	0.062	2.100
	Far Eastern	57	0.115	0.125	0.010	0.560
By country:	Japan	57	0.115	0.125	0.010	0.560
	Norway	4	0.950	0.889	0.200	2.100
	USA	13	0.232	0.312	0.062	1.220
MUSSELS						
All data combined		2239	0.433	0.399	0.000	9.000
By region:	European	2239	0.433	0.399	0.000	9.000
	Australia	7	0.600	0.294	0.200	1.100
By country:	Germany	103	0.159	0.123	0.000	0.839
	New Zealand	11	0.242	0.128	0.078	0.455
	Norway	25	0.420	0.829	0.100	4.100
	USA	2093	0.447	0.397	0.001	9.000
OTHER BIVALVES						
All data combined		232	0.191	0.201	0.008	1.000
By region:	European	66	0.194	0.168	0.008	1.000
	Far Eastern	166	0.190	0.213	0.020	0.770
By country:	Australia	5	0.320	0.383	0.100	1.000
	Japan	166	0.190	0.213	0.020	0.770
	USA	61	0.183	0.139	0.008	0.723

Max, maximum; Min, minimum; SD, standard deviation

APPENDIX B: WEIGHTED MEANS FOR AGGREGATED CADMIUM DATA

Data source	Country	GEMS region	Group #	Commodity	n	Cadmium concentration (mg/kg)				Year
						Mean	Mean * n	Min	Max	
EU SCOOP report	Italy	EUR	1	rice	42	0.070	2.940		0.360	1997–2002
memo to FAO	Sweden	EUR	1	rice, polished	44	0.024	1.056	<0.001	0.088	2001
				sum:	86		3.996			
				weighted mean:		0.046				
EU SCOOP report	Ireland	EUR	2	flour	2	0.050	0.100	0.050	0.050	1999
EU SCOOP report	Netherlands	EUR	2	flour	8	0.030	0.240	0.018	0.038	1997
EU SCOOP report	Netherlands	EUR	2	flour	70	0.038	2.660	0.009	0.084	2000
EU SCOOP report	Netherlands	EUR	2	flour	176	0.045	7.920	0.010	0.130	2001
EU SCOOP report	Netherlands	EUR	2	flour	5	0.021	0.105	0.009	0.050	2002
EU SCOOP report	Finland	EUR	2	wheat	36	0.036	1.296	0.009	0.094	1991
EU SCOOP report	Italy	EUR	2	wheat flour	2	0.030	0.060	0.020	0.040	1997–1998
EU SCOOP report	Italy	EUR	2	wheat, durum grain	239	0.037	8.843	0.009	0.076	1996
EU SCOOP report	Italy	EUR	2	wheat, soft grain	178	0.035	6.230	0.011	0.081	1996
				sum:	716		27.454			
				weighted mean:		0.038				
EU SCOOP report	Finland	EUR	3	potato	100	0.010	1.000	0.003	0.043	2000

Appendix B (contd)

Data source	Country	GEMS region	Group #	Commodity	n	Cadmium concentration (mg/kg)				Year
						Mean	Mean * n	Min	Max	
EU SCOOP report	Ireland	EUR	3	potatoes	13	0.069	0.892	0.015	0.160	2000–2001
EU SCOOP report	Netherlands	EUR	3	potatoes	69	0.022	1.518	0.007	0.082	1997
EU SCOOP report	Netherlands	EUR	3	potatoes	7	0.018	0.126	0.007	0.033	2002
EU SCOOP report	Portugal	EUR	3	potatoes	53	0.038	2.019	<0.007	<0.1	1998–2000
EU SCOOP report	Sweden	EUR	3	potatoes	75	0.010	0.750	0.001	0.028	2000
EU SCOOP report	United Kingdom	EUR	3	potatoes	20	0.026	0.512	0.007	0.129	1997
				sum:	353		6.817			
				weighted mean:		0.021				
EU SCOOP report	Netherlands	EUR	4	beetroot, red	1	0.080	0.080			1998
EU SCOOP report	Netherlands	EUR	4	beetroot, red	1	0.006	0.006			2002
EU SCOOP report	Finland	EUR	4	carrot	33	0.031	1.023	0.005	0.150	1993
EU SCOOP report	Netherlands	EUR	4	carrot	1	0.029	0.029			2001
EU SCOOP report	Netherlands	EUR	4	carrot	1	0.083	0.083			2002
EU SCOOP report	Netherlands	EUR	4	carrot	1	0.020	0.020			1997
EU SCOOP report	Netherlands	EUR	4	carrot	1	0.013	0.013			1999
EU SCOOP report	Netherlands	EUR	4	carrot	3	0.019	0.057	0.005	0.011	2002
EU SCOOP report	Ireland	EUR	4	carrots	3	0.120	0.360	0.060	0.179	2000–2001
EU SCOOP report	Italy	EUR	4	celery	5	0.090	0.450	0.060	0.190	1997–1998

Appendix B (contd)

Data source	Country	GEMS region	Group #	Commodity	n	Cadmium concentration (mg/kg)				Year
						Mean	Mean * n	Min	Max	
EU SCOOP report	Netherlands	EUR	4	celery	1	0.010	0.010			1997
EU SCOOP report	Netherlands	EUR	4	celery	1	0.510	0.510			1998
EU SCOOP report	Ireland	EUR	4	parsnips	2	0.075	0.150	0.050	0.100	2000–2001
EU SCOOP report	Netherlands	EUR	4	radish	2	0.025	0.050	0.020	0.030	1997
EU SCOOP report	Ireland	EUR	4	rhubarb	3	0.087	0.261	0.022	0.200	2000–2001
EU SCOOP report	Netherlands	EUR	4	tuber	1	0.010	0.010			1997
				sum:	60		3.112			
				weighted mean:		0.052				
EU SCOOP report	Netherlands	EUR	5	chard	1	0.380	0.380			1998
EU SCOOP report	Netherlands	EUR	5	chicory	5	0.005	0.025	0.005	0.005	1997
EU SCOOP report	Finland	EUR	5	Chinese cabbage	30	0.005	0.150	0.001	0.032	1993
EU SCOOP report	Italy	EUR	5	endive	5	0.030	0.150	<0.01	0.050	1997–1998
EU SCOOP report	Netherlands	EUR	5	endive	1	0.025	0.025			1999
EU SCOOP report	Netherlands	EUR	5	endive	1	0.042	0.042			2001
EU SCOOP report	Netherlands	EUR	5	endive	4	0.034	0.136	0.021	0.046	2002
EU SCOOP report	Finland	EUR	5	lettuce	28	0.013	0.364	0.005	0.068	1993
EU SCOOP report	Ireland	EUR	5	lettuce	9	0.151	1.363	0.026	0.440	2000–2001

Appendix B (contd)

Data source	Country	GEMS region	Group #	Commodity	n	Cadmium concentration (mg/kg)				Year
						Mean	Mean * n	Min	Max	
EU SCOOP report	Italy	EUR	5	lettuce	13	0.030	0.390	0.010	0.060	1997–1998
EU SCOOP report	Netherlands	EUR	5	lettuce	9	0.036	0.324	0.016	0.087	2002
EU SCOOP report	Netherlands	EUR	5	lettuce, crinkly	1	0.069	0.069			2002
EU SCOOP report	Ireland	EUR	5	spinach	2	0.550	1.100	0.330	0.770	2000–2001
EU SCOOP report	Italy	EUR	5	spinach	5	0.160	0.800	0.090	0.290	1997–1998
EU SCOOP report	Netherlands	EUR	5	spinach	4	0.047	0.188	0.013	0.061	2002
				sum:	118		5.506			
				weighted mean:		0.047				
EU SCOOP report	Ireland	EUR	6	cabbage	13	0.086	1.121	0.020	0.179	2000–2001
EU SCOOP report	Netherlands	EUR	6	cabbage, white	2	0.005	0.010	0.005	0.005	1997
EU SCOOP report	Netherlands	EUR	6	cabbage, white	1	0.040	0.040			1998
EU SCOOP report	Netherlands	EUR	6	cabbage, white	3	0.004	0.012	0.004	0.005	2002
EU SCOOP report	Netherlands	EUR	6	cauliflower	1	0.010	0.010			1997
EU SCOOP report	Netherlands	EUR	6	cauliflower	1	0.260	0.260			1998
EU SCOOP report	Netherlands	EUR	6	cauliflower	1	0.013	0.013			1999
EU SCOOP report	Netherlands	EUR	6	cauliflower	15	0.020	0.300	0.005	0.032	2001
EU SCOOP report	Netherlands	EUR	6	cauliflower	4	0.025	0.100	0.005	0.053	2002
EU SCOOP report	Netherlands	EUR	6	cucumber	1	0.002	0.002			2002

Appendix B (contd)

Data source	Country	GEMS region	Group #	Commodity	n	Cadmium concentration (mg/kg)				Year
						Mean	Mean * n	Min	Max	
sent to FAO	New Zealand	EUR	6	cucumber	2	0.004	0.008	<0.0006	<0.0008	1997–1998
EU SCOOP report	Netherlands	EUR	6	leek	15	0.068	1.020	0.009	0.430	2002
EU SCOOP report	Netherlands	EUR	6	onion	1	0.002	0.002			2002
EU SCOOP report	Netherlands	EUR	6	onion	1	0.010	0.010			1997
EU SCOOP report	Netherlands	EUR	6	onion	1	0.040	0.040			1998
EU SCOOP report	Netherlands	EUR	6	onion	4	0.065	0.260	0.005	0.005	2002
EU SCOOP report	Ireland	EUR	6	onion, spring	1	0.090	0.090	0.090	0.090	2000–2001
EU SCOOP report	Ireland	EUR	6	onions	10	0.129	1.288	0.020	0.660	2000–2001
EU SCOOP report	Italy	EUR	6	pepper, guinea	13	0.030	0.390	0.020	0.050	1997–1998
EU SCOOP report	Netherlands	EUR	6	peppers	20	0.015	0.300	0.010	0.020	2002
sent to FAO	New Zealand	EUR	6	pumpkin	2	0.007	0.014	0.005	0.009	1997–1998
				sum:	112		5.290			
				weighted mean:		0.047				
EU SCOOP report	France	EUR	7	molluscs	145	0.239	34.655	<0.0005	3.450	2000
EU SCOOP report	Portugal	EUR	7A	bivalve molluscs	34	0.104	3.546	<0.01	0.380	1998–2002
EU SCOOP report	Greece	EUR	7A	bivalve molluscs, fresh	14	0.186	2.604	0.022	0.456	2000–2002
EU SCOOP report	Netherlands	EUR	7A4	cockle	15	0.024	0.360	0.005	0.080	1999

Appendix B (contd)

Data source	Country	GEMS region	Group #	Commodity	n	Cadmium concentration (mg/kg)				Year
						Mean	Mean * n	Min	Max	
EU SCOOP report	United Kingdom	EUR	7A4	cockles, winkles	7	0.130	0.910	0.030	0.230	1996–1997
EU SCOOP report	Italy	EUR	7A	molluscs, bivalve	30	0.091	2.730	0.366		2001
letter to JECFA	Spain	EUR	7A3	mussel, blue	25	0.141	3.525		0.270	2002
letter to JECFA	Spain	EUR	7A3	mussel, blue	6	0.128	0.768		0.150	2002
letter to JECFA	Spain	EUR	7A3	mussel, blue	2	0.055	0.110		0.060	2002
letter to JECFA	Spain	EUR	7A3	mussel, blue	12	0.209	2.508		0.296	nr
EU SCOOP report	Finland	EUR	7A3	mussels	5	0.077	0.385	0.054	0.091	2002
EU SCOOP report	Greece	EUR	7A3	mussels	57	0.696	39.655	0.048	2.450	1999–2002
EU SCOOP report	Ireland	EUR	7A3	mussels	2150	0.166	357.330	0.050	0.440	1996–2001
EU SCOOP report	Italy	EUR	7A3	mussels	30	0.100	3.000			1995
EU SCOOP report	Netherlands	EUR	7A3	mussels	1	0.793	0.793			1998
EU SCOOP report	Netherlands	EUR	7A3	mussels	47	0.079	3.713	0.040	0.310	1999
sent to FAO	New Zealand	EUR	7A3	mussels	6	0.282	1.694	0.046	0.521	1997–1998
letter to JECFA	Spain	EUR	7A1	oyster	11	0.197	2.167		0.257	2003
EU SCOOP report	Greece	EUR	7A1	oysters	1	0.492	0.492	0.492	0.492	2002
EU SCOOP report	Ireland	EUR	7A1	oysters	1	0.247	0.247	0.247	0.247	1996–2001
EU SCOOP report	Netherlands	EUR	7A1	oysters	11	0.131	1.441	0.060	0.620	1999

Appendix B (contd)

Data source	Country	GEMS region	Group #	Commodity	n	Cadmium concentration (mg/kg)				Year
						Mean	Mean * n	Min	Max	
EU SCOOP report	Ireland	EUR	7A1	oysters, Pacific	549	0.333	182.543	0.098	0.631	1996–2001
EU SCOOP report	Ireland	EUR	7A1	oysters, flat	275	0.429	118.003	0.260	0.590	1996–2001
				sum:	3289		728.523			
				all bivalves - weighted mean:		0.222				
				sum:	2341		413.481			
				mussels only – weighted mean:		0.177				
				sum:	848		304.892			
				oysters only – weighted mean:		0.360				
EU SCOOP report	Greece	EUR	7B	cephalopods	2	0.002	0.004	<0.002	0.004	2001
EU SCOOP report	Italy	EUR	7B	cephalopods	42	0.002	0.088	0.198		2001
EU SCOOP report	Greece	EUR	7B	cuttlefish	10	0.436	4.360	0.050	1.300	1999–2000
EU SCOOP report	Greece	EUR	7B	cuttlefish	10		0.000			
EU SCOOP report	Netherlands	EUR	7B	cuttlefish	1	2.520	2.520			1998
EU SCOOP report	Netherlands	EUR	7B	cuttlefish	19	0.153	2.907	0.020	0.260	1999
EU SCOOP report	Netherlands	EUR	7B	cuttlefish	6	0.585	3.510	0.090	1.591	2001
letter to JECFA	Spain	EUR	7B	cuttlefish	4	0.048	0.192		0.071	2002

Appendix B (contd)

Data source	Country	GEMS region	Group #	Commodity	n	Cadmium concentration (mg/kg)				Year
						Mean	Mean * n	Min	Max	
letter to JECFA	Spain	EUR	7B	cuttlefish	10	0.021	0.210		0.080	2002
letter to JECFA	Spain	EUR	7B	cuttlefish	8	0.033	0.264		0.070	2002
letter to JECFA	Spain	EUR	7B	cuttlefish	7	0.003	0.021		0.004	2001
letter to JECFA	Spain	EUR	7B	cuttlefish	6	0.142	0.852		0.336	2002
letter to JECFA	Spain	EUR	7B	cuttlefish	16	0.069	1.104		0.124	2002
letter to JECFA	Spain	EUR	7B	cuttlefish	10	0.303	3.030		0.594	2003
EU SCOOP report	Greece	EUR	7B	octopus	37	0.156	5.757	<0.002	1.300	1999–2001
letter to JECFA	Spain	EUR	7B	octopus	8	nr			0.002	2001
letter to JECFA	Spain	EUR	7B	octopus	8	0.273	2.184		0.715	2001
letter to JECFA	Spain	EUR	7B	octopus	8	0.004	0.032		0.015	2001
letter to JECFA	Spain	EUR	7B	octopus	7	0.145	1.015		0.252	2002
letter to JECFA	Spain	EUR	7B	octopus	8	0.043	0.344		0.075	2002
letter to JECFA	Spain	EUR	7B	octopus	4	0.156	0.624		0.425	nr
EU SCOOP report	Italy	EUR	7B	octopus, spider	300	0.400	120.000	0.140	1.040	1996
EU SCOOP report	Greece	EUR	7B	squid	2	0.386	0.772	0.058	0.715	2001
letter to JECFA	Spain	EUR	7B	squid	5	0.010	0.050		0.016	2001
letter to JECFA	Spain	EUR	7B	squid	4	0.144	0.576		0.206	2003
letter to JECFA	Spain	EUR	7B	squid	28	0.142	3.976		0.336	2003

Appendix B (contd)

Data source	Country	GEMS region	Group #	Commodity	n	Cadmium concentration (mg/kg)				Year
						Mean	Mean * n	Min	Max	
letter to JECFA	Spain	EUR	7B	squid	8	0.073	0.584		0.200	2002
letter to JECFA	Spain	EUR	7B	squid	7	0.004	0.028		0.008	2001
letter to JECFA	Spain	EUR	7B	squid	8	0.017	0.136		0.053	2001
letter to JECFA	Spain	EUR	7B	squid	5	1.290	6.450		2.180	nr
EU SCOOP report	Italy	EUR	7B	squid, broadtail	212	0.070	14.840	0.040	0.100	1997
				sum:	810		176.431			
				weighted mean:		0.218				

EU, European Union; EUR, European region; FAO, Food and Agriculture Organization of the United Nations; JECFA, Joint FAO/WHO Expert Committee on Food Additives; Max, maximum; Min, minimum; nr, not reported; SCOOP, Scientific Cooperation on Food

ETHYL CARBAMATE

First draft prepared by

**E. Vavasour,¹ A.G. Renwick,² B. Engeli,³ S. Barlow,⁴
L. Castle,⁵ M. DiNovi,⁶ W. Slob,⁷ J. Schlatter³ and M. Bolger⁶**

¹ Food Directorate, Health Canada, Ottawa, Ontario, Canada

² University of Southampton, Southampton, United Kingdom

³ Swiss Federal Office of Public Health, Zurich, Switzerland

⁴ Brighton, East Sussex, United Kingdom

⁵ Central Science Laboratory, Sand Hutton, York, United Kingdom

**⁶ Center for Food Safety and Applied Nutrition, Food and Drug
Administration, College Park, Maryland, USA**

**⁷ National Institute of Public Health and the Environment (RIVM), Bilthoven,
The Netherlands**

Explanation.....	206
Biological data.....	207
Biochemical aspects	207
Absorption, distribution and excretion	207
Biotransformation	209
Effects on enzymes and other biochemical parameters.....	214
Toxicological studies	215
Acute toxicity	215
Short-term studies of toxicity.....	215
Long-term studies of toxicity and carcinogenicity	221
Genotoxicity	237
Reproductive toxicity	253
Special studies	265
Observations in humans.....	274
Biomarkers of exposure or effects.....	274
Clinical observations	274
Epidemiological studies.....	274
Analytical methods	275
Chemistry.....	275
Description of analytical methods.....	275
Beverages	275
Solid and semi-solid foods	276
Derivatization approaches.....	276
Identification aspects.....	276
Quantification aspects.....	277
General conclusions.....	277
Sampling protocols.....	277
Effects of processing	277

Levels and patterns of contamination of food commodities	278
Dietary intake assessment	279
Methods	279
Estimates of dietary intake	279
National estimates.....	279
International estimates	284
Special considerations — alcohol consumption	284
Prevention and control	286
Formation from cyanide.....	286
Stone-fruit brandies.....	286
Whiskey	286
Formation from urea.....	287
Wine (including rice wine, sake).....	287
Formation from citrulline and other <i>N</i> -carbaryl compounds	287
Formation from exogenous substances	288
Diethylpyrocarbonate	288
Azodicarbonamide	288
Dose-response analysis and estimation of carcinogenic/toxic risk	289
Contribution of above data to assessment of risk.....	289
Pivotal data from biochemical and toxicological studies.....	289
Pivotal data from human clinical/epidemiological studies.....	290
Biomarker studies	290
General modelling considerations	290
Selection of data	290
Measure of exposure	291
Measure of response	291
Selection of mathematical model	292
Comments.....	294
Absorption, distribution, metabolism and excretion	294
Toxicological data	295
Observations in humans.....	297
Analytical methods	297
Levels and pattern of food contamination.....	297
Prevention and control	297
Dietary intake assessment	298
Dose-response analysis	299
Evaluation.....	299
References	300

1. EXPLANATION

Ethyl carbamate (urethane), the ethyl ester of carbamic acid, has not been evaluated previously by the Committee. Although past industrial, medical and veterinary uses of ethyl carbamate have been reported, information available to the Committee at its present meeting suggested that the major route of exposure to ethyl carbamate in the human population is through consumption of fermented foods and beverages in which it may be present — for example, as a

consequence of its unintentional formation during the fermentation process or during storage. Ethyl carbamate can be formed in fermented foods and beverages, such as spirits, wine, beer, bread, soya sauce and yoghurt.

There is an extensive literature (dating from the 1940s to the present day) on the genotoxicity and carcinogenicity of ethyl carbamate. Major reviews of the available data pertaining to carcinogenicity have been performed, most recently in 1989 by the California Department of Health Services (Salmon et al., 1991). Ethyl carbamate was designated as "possibly carcinogenic to humans" (Group 2B) by IARC (1974) and is listed as "reasonably anticipated to be a human carcinogen" in the *Report on Carcinogens* of the United States National Toxicology Program (NTP, 2005).

At its present meeting, the Committee evaluated the results of studies assessing the genotoxic and carcinogenic potential of ethyl carbamate, particularly from those studies that had become available since the review in 1989, as well as data on metabolism and disposition, short-term toxicity, reproductive and developmental toxicity, perinatal carcinogenicity and immunotoxicity. Analytical methods, occurrence and intake were also considered.

The request for evaluation of ethyl carbamate originated from the Codex Committee on Food Additives and Contaminants (CCFAC) at its Thirty-fifth Session (CAC, 2003).

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

In mice, about 90% of an intraperitoneal injection of [carboxy- ^{14}C]ethyl carbamate or [methylene- ^{14}C]ethyl carbamate was completely degraded to carbon dioxide, water and ammonia within 24 h, as detected by exhaled radiolabelled carbon dioxide. About 4–10% of the label was excreted in the urine, less than 1% was eliminated in the faeces and 1–10% was retained in the body. The authors concluded that the retained radioactivity was probably attributable to reincorporation of the hydrolysis products $^{14}\text{CO}_2$ and [^{14}C]ethanol into endogenous metabolites (Bryan et al., 1949; Skipper et al., 1951).

[Carbonyl- ^{14}C]ethyl carbamate (specific activity 540 MBq/mmol) was administered orally or intravenously to groups of three male Fischer 344/N rats and male B6C3F1 mice at doses ranging from 0.475 to 475 mg/kg bw (0.740 to 740 kBq/kg bw). Following oral administration, ethyl carbamate was completely absorbed from the gastrointestinal tract of both species at all doses studied. Only the parent compound was present in blood, lung, skin, liver, kidney, muscle and bile of treated rats and mice. There was a mono-exponential decrease in the concentrations of radioactivity in blood and other tissues (except fat) between 15 min and 24 h following intravenous dosing of rats and mice with 47.5 mg/kg bw. After intravenous administration of ethyl carbamate at 4.75 mg/kg bw to mice and rats, approximately 98% was exhaled as carbon dioxide within 8 or 12 h, respectively.

Following oral or intravenous administration to rats or mice, only 2–8%, 0.3–1.0% and 0.3–2% of all doses studied were excreted in urine, in faeces and as volatile organics, respectively. A similar distribution was obtained in a multiple dosing experiment in which rats were treated orally with 47.5 mg/kg bw per day for 9 days. The pattern of elimination of a single dose of ethyl carbamate was not greatly altered by co-administration with tri-*o*-cresyl phosphate or paraoxon (inhibitors of esterases) or by co-administration with SKF525A or piperonyl butoxide (inhibitors of cytochrome P450 [CYP] isoenzymes). Several metabolites were detected in urine and faeces of rats and mice but were not further identified. The authors concluded that the metabolites in faeces were of microbial origin, because a high-performance liquid chromatographic analysis of bile indicated that the radioactivity present was attributable to ethyl carbamate. There was evidence of saturation of metabolism and elimination at doses greater than 47.5 mg/kg bw in mice and greater than 4.75 mg/kg bw in rats (Nomeir et al., 1989).

Plasma kinetic analyses were conducted in groups of 10 male and 10 female B6C3F1 mice, 6 weeks of age, receiving ethyl carbamate (purity $\geq 99\%$) at 0, 110, 330, 1100, 3300 or 10 000 mg/l per day in drinking-water or in 5% ethanol *ad libitum*, 7 days a week for 13 weeks (equivalent to 0, 17, 50, 165, 500 and 1500 mg/kg bw per day, assuming a drinking-water consumption of 150 ml/kg bw). The average half-life for elimination of ethyl carbamate from the plasma by male mice was 0.77 h (range 0.6–0.9 h) for both drinking-water and 5% ethanol. The data for the female mice were too variable to conduct a kinetic analysis. In nearly all plasma samples, the ethanol concentration was below the limit of detection (NTP, 1996).

A single dose of [ethyl-1- ^{14}C]ethyl carbamate at 100 mg/kg bw (93 kBq/25 g bw) was administered either orally in water or dermally in acetone to male SENCAR and BALB/c mice. Radioactivity was measured in skin, liver, lung and stomach after 1–48 h. The peak concentration occurred at 1 h (first sampling), regardless of strain or route of administration. In the first hour, absorption of ethyl carbamate was greater in SENCAR than in BALB/c mice by both routes (Fossa et al., 1985).

Paper chromatographic analysis of plasma collected 2.5–12 h after Swiss mice and rabbits were given a single injection of [methyl- ^{14}C]ethyl carbamate or [carbonyl- ^{14}C]ethyl carbamate at 1.5 g/kg bw and of mouse lung, liver and skin revealed a single peak corresponding to free ethyl carbamate (Kaye, 1960, 1968).

Ethyl carbamate was rapidly and evenly distributed throughout the body in rats following subcutaneous injection of 500 or 1000 mg/kg bw. Only 4% of the dose was excreted in urine, the major part as metabolites. The metabolism was slower in tumour-bearing rats than in normal rats (Boylard & Rhoden, 1949).

A subcutaneous injection of [carbonyl- ^{14}C]ethyl carbamate at 0.57 mg/kg bw (7.4 MBq/kg bw) resulted in the immediate appearance of radioactivity in approximately equal concentrations in all the organs, tissues and body fluids of both pregnant ICR/JCL mice and their fetuses. Labelled ethyl carbamate was catabolized rapidly within 6 h, as indicated by a decrease of radioactivity in blood. Ethyl

carbamate crossed the placental barrier freely when administered subcutaneously to pregnant female ICR/JCL mice (Nomura et al., 1973).

The radiolabel from [carbonyl- ^{14}C]ethyl carbamate (500 mg/kg bw; 7.4 MBq/kg bw) given by subcutaneous injection daily for 6 days was uniformly distributed in all the major organs of ICR/JCL mice and decreased rapidly in all organs, with half-lives of about 18 days when given within 1 h of birth and of 1–2 days when given to young and adult male and female mice and to pregnant mice (Nomura, 1976).

When ethyl carbamate at 1000 mg/kg bw was injected subcutaneously into lactating ICR/JCL mice, pulmonary tumours were induced in more than 50% of sucklings. This indicates a transmission of ethyl carbamate into mother's milk (Nomura & Okamoto, 1972; Nomura, 1976). For details, see section 2.2.5 on transplacental carcinogenicity. About 10% of the maximum concentrations of radioactivity in maternal blood and tissues were detected in the milk and in the stomach contents of newborns after [^{14}C]ethyl carbamate at 0.57 mg/kg bw (7.4 MBq/kg bw) was injected subcutaneously into lactating female ICR/JCL mice (Nomura et al., 1973). The tissue concentrations of radioactivity in various organs of suckling pups that were nursed by lactating dams given [carbonyl- ^{14}C]ethyl carbamate at 0.5 mg/kg bw subcutaneously for 6 days were 2–3% of the maternal concentrations (Nomura, 1976).

The rate of catabolism of intraperitoneally administered [carbonyl- ^{14}C]ethyl carbamate (500 mg/kg bw) in newborn SWR mice (less than 24 h old) is about 0.1 times the rate found in adults. The catabolic rate was shown to increase slowly for the first 10 days after birth and most sharply between days 15 and 20. The time required for elimination of 50% of the initial dose from blood decreased from 38 h for 1-day-old mice to 5.6 h for 20-day-old mice (Cividalli et al., 1965).

2.1.2 Biotransformation

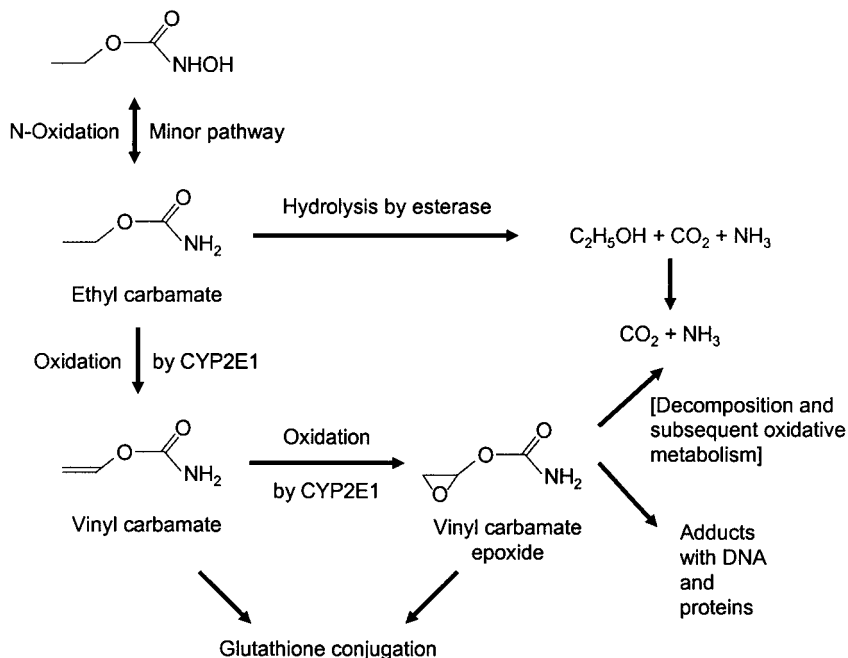
(a) *Metabolic processes involved in the overall elimination of ethyl carbamate*

There has been considerable debate over the years on the pathways of metabolism of ethyl carbamate and the enzymes and metabolite(s) responsible for DNA binding and carcinogenicity. More recent studies have also investigated the metabolic basis for the saturable elimination of ethyl carbamate and its interaction with ethanol. Pathways of metabolism of potential importance include *N*-hydroxylation, hydrolysis to ethanol and ammonia and side-chain oxidation to vinyl carbamate; carbon dioxide is the final metabolic product of hydrolysis and side-chain oxidation (Figure 1) (Hoffler et al., 2003).

Early studies indicated that ethyl carbamate was metabolized in rats, rabbits and humans by *N*-hydroxylation, based on studies in which 16 female rats received five daily intraperitoneal doses of 1 g ethyl carbamate/kg bw (total dose, 16.6 g), two rabbits received six daily intraperitoneal doses of 1 g ethyl carbamate/kg bw (total dose, 27.5 g) and two male patients received two daily oral doses of 2 g ethyl carbamate per adult (total dose, 8 g). Small amounts of ethyl

carbamate, *N*-hydroxyethyl carbamate, ethylmercapturic acid, ethylmercapturic acid sulfoxide and *N*-acetyl-*S*-carbethoxycysteine were found in urine of all species (Boyland & Nery, 1965). There was an incomplete description of the dosing regimen and lack of information about animal strains. *N*-Hydroxyethyl carbamate and acetyl-*N*-hydroxyethyl carbamate were reported subsequently in urine from patients with multiple myeloma who were treated with ethyl carbamate in conjunction with an alkylating agent (Boyland, 1968), but again there was little information about dose and route of administration of the ethyl carbamate. Ethyl carbamate and *N*-hydroxyethyl carbamate were reported to be metabolically interconvertible in mice in vivo (Nery, 1968). However, *N*-hydroxyethyl carbamate was not detected in later studies as a urinary metabolite of ethyl carbamate in rats (Nomeir et al., 1989) or as a plasma metabolite in mice (O'Flaherty & Sichak, 1983). *N*-Hydroxyethyl carbamate is rapidly reduced to ethyl carbamate by aldehyde oxidase in vitro (Sugihara et al., 1983), and the formation of ethyl carbamate is considered to be responsible for the carcinogenicity of the *N*-hydroxy compound in C57BL/6 mice (Boiato et al., 1966; Mirvish, 1966).

Figure 1. Pathways of biotransformation of ethyl carbamate



2-Hydroxyethyl carbamate, vinyl carbamate and ethyl *N*-hydroxycarbamate were detected when ethyl carbamate was incubated in vitro with NADPH and human liver microsomes (Guengerich & Kim, 1991). 2-Hydroxyethyl carbamate

shows very weak carcinogenic activity in mice compared with ethyl carbamate (Mirvish et al., 1994). In contrast, vinyl carbamate is believed to be responsible for the mutagenicity and carcinogenicity of ethyl carbamate (Dahl et al., 1978; Csukas et al., 1981) and also the immunosuppression reported in BALB/c mice (Cha et al., 2000).

Until recently, simple hydrolysis of the carbamate moiety was considered to be the major metabolic pathway that was responsible for elimination from the body. Hydrolysis by cytosolic carboxylesterases (Yamamoto et al., 1990) and oxidation by microsomal CYP2E1 (Guengerich et al., 1991) were considered to be competing pathways (Figure 1), both for the overall elimination of ethyl carbamate from the body and within potential target organs for carcinogenesis.

The rate of elimination of [ethyl-1-¹⁴C]ethyl carbamate (11.1 mg/kg bw) in male A/Jax mice in vivo was reduced by prior treatment with the esterase inhibitors paraoxon (1 mg/kg bw) and carbaryl (50 mg/kg bw), indicating that hydrolysis is an important route of elimination. In addition, these treatments were associated with a reduction in covalent binding to hepatic protein, indicating that hydrolysis is linked to bioactivation (Yamamoto et al., 1990). These observations appear inconsistent with the results of other studies (see below), which indicate that bioactivation is via CYP2E1-mediated oxidation, because CYP2E1-mediated oxidation would be expected to increase if the competing hydrolysis reaction were inhibited.

The rate of elimination of [carbonyl-¹⁴C]ethyl carbamate by CYP2E1-null mice given 10 mg/kg bw or 100 mg/kg bw orally was considerably slower than in wild-type mice, with estimated half-lives of 22 and 0.8 h, respectively. The tissue levels of radioactivity at 24 h after the dose were about 20–100 times higher in the CYP2E1-null mice. The data indicated that about 96% of the elimination of ethyl carbamate was mediated by CYP2E1. Carbon dioxide was the main excretory product in both knockout and conventional mice, accounting for about 40% and over 90% of the dose by 24 h, respectively. There was only a slight increase in renal elimination of ethyl carbamate in the knockout mice (Hoffler et al., 2003).

The in vivo effects of inducers and inhibitors of esterases and CYP2E1 on the conversion of [carbonyl-¹⁴C]ethyl carbamate to carbon dioxide have been studied in male SD rats after a single intraperitoneal dose of 2.5 mg/kg bw. Phenobarbital and β -naphthoflavone, which are inducers of cytochrome P450, and paraoxon, which is an inhibitor of esterases, did not alter the rate or extent of labelled carbon dioxide production, which represented about 50–60% of the dose within 6 h. Diethyldithiocarbamate, which is an inhibitor of CYP2E1, reduced labelled carbon dioxide production to less than 2%. These data indicate that CYP2E1 is responsible for about 95% of the total elimination of ethyl carbamate in rats. A single ethanol dose of 4 ml/kg bw, given 1 h before the radiolabelled ethyl carbamate, reduced ¹⁴CO₂ elimination to 3.5% within 6 h, but chronic treatment with ethanol given *ad libitum* at 10% in the drinking-water did not affect ¹⁴CO₂ elimination (Carlson, 1994). Prior treatment of mice and rats with pyridine, which is an inducer of CYP2E1, increased the conversion of ethyl carbamate to carbon dioxide in vitro (Page & Carlson, 1994) but paradoxically inhibited the extent of ¹⁴CO₂ elimination

in vivo (Carlson, 1994); the authors suggested that this difference was due to the presence of pyridine, which would act as a competitive inhibitor in the in vivo study, but not in the in vitro system.

The elimination of ethyl carbamate by male A/Jax mice given 11.1 mg/kg bw is reduced markedly by compounds that are metabolized by CYP2E1, such as ethanol (see below) and other short-chain aliphatic compounds (e.g. acetaldehyde, acetone and 2-propanol), and by conditions such as food deprivation and streptozotocin-induced diabetes, which increase the blood levels of acetone (Kurata et al., 1990, 1991a).

The biotransformation of ethyl carbamate exhibits two toxicologically important characteristics that affect how rapidly it can be eliminated: dose dependency or non-linear kinetics at the doses studied in animals, and an interaction with ethanol.

(b) Biotransformation and bioactivation at sites of carcinogenesis

The in vitro metabolism of ethyl carbamate (2 mg/ml microsomal suspension) to *N*-hydroxyethyl carbamate, *N*-hydroxyvinyl carbamate and the epoxide of ethyl carbamate was detected using microsomes from the lungs of Wistar rats, but not when incubated with microsomes from liver, brain or kidneys (Gupta & Dani, 1989). The authors stated that these data may explain the organ-specific carcinogenicity of ethyl carbamate.

Incubation of mouse lung microsomes with ethyl carbamate resulted in covalent protein binding and inhibition of CYP2E1 activity. A monoclonal antibody against CYP2E1 reduced the ethyl carbamate-induced loss of CYP2E1 activity and the extent of covalent binding to microsomes, while inhibitors of esterase activity enhanced the covalent binding to proteins and the loss of CYP2E1 activity (Forkert & Lee, 1997). Studies with human lung microsomes showed that oxidation of vinyl carbamate by CYP2E1 resulted in inactivation of the CYP2E1 enzyme, presumably by formation of the reactive epoxide metabolite (Forkert et al., 2001). Prior incubation with phenylmethylsulfonyl fluoride, an inhibitor of carboxylesterase, enhanced the inactivation of CYP2E1 that was produced on incubation with vinyl carbamate (Forkert et al., 2001). Therefore, both hydrolysis and oxidation may be of importance in this potential site of carcinogenicity. These data indicate that similar metabolic processes occur in the lungs of both mice and humans.

The reactive metabolite of ethyl carbamate, vinyl carbamate, undergoes further metabolic activation mediated by CYP2E1 in mouse lung (Lee & Forkert, 1999) and is inactivated by hydrolysis (Lee & Forkert, 1999) and by conjugation with glutathione (Kemper et al., 1995). Administration of 0.2% *N*-acetylcysteine in the diet of Swiss albino mice for 2 weeks before and 4 months after a single intraperitoneal injection of ethyl carbamate (10 mg/kg bw) significantly reduced the numbers of animals with lung tumours and the numbers of tumours per animal (De Flora et al., 1986).

(c) *Saturation of the metabolism of ethyl carbamate*

In 6-month-old Swiss mice, a constant rate of ethyl carbamate catabolism with a V_{\max} of 0.086 mg/ml per hour was observed after a single intraperitoneal injection of 0.75 mg [carbonyl- ^{14}C]ethyl carbamate. A V_{\max} of 0.061 mg/ml per hour was found in 2-week-old Swiss mice (Kaye, 1960).

There was evidence of saturation of metabolism and elimination at intravenous doses greater than 47.5 mg/kg bw in mice and greater than 4.75 mg/kg bw in rats (Nomeir et al., 1989).

Outbred Swiss mice injected intraperitoneally with single ethyl carbamate doses of 400–1800 mg/kg bw showed a saturated elimination at all tested dose levels. The authors calculated a V_{\max} of 0.087 ± 0.006 mg/ml per hour (or 70 mg/kg bw per hour) (O'Flaherty & Sichak, 1983). The K_m value was thought to be an order of magnitude lower than the concentrations measured in the study.

(d) *Interaction with ethanol*

Ethanol (2 g/kg bw) given by gavage delayed the elimination of a single ethyl carbamate dose of 37.5 mg/kg bw in female NMRI mice and increased the area under the curve (AUC) in plasma about 3-fold (Kristiansen et al., 1994).

A single ethanol dose of 4 ml/kg bw given by gavage 1 h before [carbonyl- ^{14}C]ethyl carbamate reduced $^{14}\text{CO}_2$ elimination from about 50–60% of the dose within 6 h to 3.5%; in contrast, chronic treatment with ethanol given *ad libitum* at 10% in the drinking-water did not affect $^{14}\text{CO}_2$ elimination (Carlson, 1994).

A single dose of [ethyl-1- ^{14}C]ethyl carbamate (220 kBq/24 g of mouse, equivalent to 9.3 MBq/kg bw) in 12% ethanol (5 g ethanol/kg bw) administered orally to male A/Jax mice strongly decreased the amount of radioactivity in tissues 1 h after administration compared with the high accumulation seen in the whole-body autoradiogram when ethyl carbamate was administered in water (Waddell et al., 1987).

[Ethyl-1- ^{14}C]ethyl carbamate (11.15 mg/kg bw, 370 kBq/20 g of mouse, equivalent to 18.5 MBq/kg bw) dissolved in water or ethanol (5 g ethanol/kg bw) was orally administered to male A/Jax mice. Radioactivity in blood decreased rapidly, with complete elimination within 3 h after administration of the aqueous solution. In contrast, radioactivity in the blood of mice receiving ethyl carbamate together with ethanol was constant for 8 h after administration and declined rapidly thereafter. There was a very high rate of $^{14}\text{CO}_2$ expiration following ethyl carbamate administration in water, but much slower elimination when given with ethanol. There was extensive covalent binding to liver proteins within 3 h when ethyl carbamate was given in water, but 3-fold lower binding was found at 10 h when ethyl carbamate was given with ethanol; the full time course for binding was not defined following either treatment. The authors concluded that blood ethanol concentrations above 0.15% (150 mg/dl) inhibited the initial metabolism of ethyl carbamate and prevented the formation of active metabolites, although the data

presented indicated reduced and delayed formation of the active metabolite rather than the prevention of its formation (Yamamoto et al., 1988).

The suggestion that co-administration with ethanol delays rather than prevents the toxicity of ethyl carbamate is supported by data on the formation of micronuclei in treated mice. An initial study on the induction of micronuclei in the bone marrow erythrocytes of CD-1 mice showed that a single intraperitoneal injection of 2.5 g/kg bw or more of ethanol given with ethyl carbamate (1000 mg/kg bw) caused a dose-dependent reduction in the induction of micronuclei at 24 h after the dose (Choy et al., 1995). A subsequent study in which micronuclei were measured for up to 96 h showed that an intraperitoneal injection of 2.5 g ethanol/kg bw given concurrently with 1000 mg ethyl carbamate/kg bw to male CD-1 mice delayed the appearance of an increased frequency of micronucleated polychromatic erythrocytes, but did not significantly alter the total amount, as indicated by the area under the frequency–time curve (Choy et al., 1996). The lack of an effect of ethanol on the total formation of micronuclei is consistent with CYP2E1 being the main enzyme responsible for both the bioactivation of the ethyl carbamate and also its systemic elimination.

CYP2E1 is inducible by prior treatment with ethanol, and this interaction has been the focus of both metabolism studies and studies on toxicity and carcinogenicity. A decrease in the AUC of ethyl carbamate in the blood of mice given 11.125 mg/kg bw was produced by giving 10% ethanol in drinking-water between 48 and 12 h prior to the oral dose of ethyl carbamate. In contrast, there was no change in AUC when mice were treated with 5% ethanol in drinking-water for 7 days followed by a 24-h washout or with 5 g ethanol/kg bw 48 and 24 h before the ethyl carbamate (Kurata et al., 1991b). These data indicate that the increased hepatic CYP2E1 produced by ethanol treatment is short-lived.

2.1.3 Effects on enzymes and other biochemical parameters

Groups of four male and four female B6C3F1 mice received ethyl carbamate at 0, 10, 30 or 90 mg/l in drinking-water *ad libitum* in the presence of 0, 2.5 or 5% ethanol for 4 weeks. Liver and lung samples were collected for measurement of glutathione, CYP2E1 and DNA adduct formation. Mean ethyl carbamate concentrations of 0.4–2.8 mg/l in serum were detected in mice exposed to 30 or 90 mg/l and 5% ethanol; the ethyl carbamate concentration in all other samples was at or below the detection limit (0.01 mg/l). Ethanol was not detected in any of the samples. The percentage of hepatocytes in the G0 phase was decreased and the percentage in the G1 phase was increased in females exposed to ethyl carbamate at 30 or 90 mg/l; this effect was independent of the ethanol concentration. The percentage of proliferating cell nuclear antigen labelling was decreased in the lungs of mice exposed to ethyl carbamate at 30 or 90 mg/l, and the effect was independent of the ethanol concentration. Increasing the concentration of ethanol caused an exposure-related increase in CYP2E1 activity and an exposure-related decrease in glutathione content in the liver of females. These parameters in females exposed to 2.5% or 5% ethanol were significantly different from the controls; the changes were independent of the ethyl carbamate concentration. Ethenodeoxyadenosine (etheno-dA) adduct concentrations in hepatic DNA were

significantly increased by exposure to ethyl carbamate and decreased by exposure to ethanol, but neither ethyl carbamate nor ethanol affected etheno-dA or ethenodeoxycytidine (etheno-dC) adduct concentrations in lung DNA (NTP, 2004).

2.2 Toxicological studies

2.2.1 Acute toxicity

The results of studies of the acute toxicity of ethyl carbamate are summarized in Table 1.

Table 1. Acute toxicity of ethyl carbamate

Species (strain)	Sex	Route	LD ₅₀ (mg/kg bw)	Reference
Mice (Copenhagen GN strain)	Female	Oral	2500	Osswald (1959)
Mice (B6C3F1)	Male	Intraperitoneal	1539	Salamone et al. (1981)
Mice (ICR/JCL)	Female	Subcutaneous	1750	Nomura & Okamoto (1972)
Mice (NMR1)	Male	Either intramuscular or intraperitoneal (not further specified)	1400	Schönenberger et al. (1974)
Rat (Sprague-Dawley)	Male	Oral (gavage)	1809	Pereira et al. (1991)
Rat (Wistar, pregnant)	Female	Intraperitoneal	1500	Chaube & Murphy (1966)
Rat (BD II)	Male	Either intramuscular or intraperitoneal (not further specified)	1400	Schönenberger et al. (1974)

2.2.2 Short-term studies of toxicity

(a) Mice

(i) Oral route

Groups of 18 Swiss-Webster mice (sex not stated) received ethyl carbamate at 0 or 1000 mg/l in drinking-water (equivalent to 150 mg/kg bw per day, assuming a drinking-water consumption of 150 ml/kg bw) for 10 weeks. No information on body weight or water consumption was provided. All treated animals had small lung tumours and hyperplasia of bronchiolar epithelium (Kauffman, 1971).

Groups of 21 Swiss-Webster mice (sex not stated) received ethyl carbamate at 0 or 1000 mg/l in drinking-water (equivalent to 150 mg/kg bw per day) for 10 weeks; [^3H]thymidine was injected, and groups of three animals were killed 30 min to 7 days after treatment. All treated animals had small lung tumours of type 2 alveolar epithelial cells and hyperplasia of bronchiolar epithelium. They also showed a significantly prolonged cell cycle time and a 5-fold increase in labelling index of alveolar epithelial cells compared with control animals (Kauffman, 1972).

Groups of 20 male 8-week-old heterozygous p53 knockout mice received ethyl carbamate at 0, 1, 10 or 100 mg/kg bw per day orally by gavage for 6 months. Physical condition, body weights and food consumption were assessed at regular intervals throughout the study. All animals were necropsied, and weights of adrenal gland, brain, heart, kidney, liver, testis and thymus were weighed at final sacrifice. Samples of 46 types of tissues and all gross lesions detected at necropsy were subjected to histopathological evaluation. The highest dose tested (100 mg/kg bw per day) had a significant effect on body weight that was evident after approximately 4 months of treatment and increased in severity until the end of the study. Mortality was also increased at the highest dose, such that 17 of 20 mice had died by the end of the study compared with 2 in the mid-dose group and 1 in controls. The probable cause of death in the high-dose group was internal haemorrhage secondary to vascular tumours. There was a dose-dependent increase in vascular tumours, with an incidence of 0/20, 3/20 and 20/20 in the three treatment groups, respectively, compared with 0/20 in the control group. Animals receiving 1 mg/kg bw per day showed no signs of toxicity and no tumours. At 100 mg/kg bw per day, haemangioma of the liver and heart (14/20), haemangiosarcoma of the liver and spleen (8/20), hepatocellular carcinoma (4/20), lung adenoma (5/20) and malignant lymphoma originating in the thymus (3/20) were observed (corresponding tumour incidences of the 10 mg/kg bw per day group were 0–1/20 animals). A high proportion of mice administered 10 or 100 mg/kg bw per day showed red spots (8/20 or 20/20) and non-proliferative vascular changes (i.e. angiectasis; 8/20 or 9/20) in the liver. In the high-dose group, there was also zonal necrosis of hepatocytes in 14 of 20 animals, enlarged spleen in 10 of 20 animals and bilateral retinal atrophy in 12 of 13 animals (the authors do not explain why the eyes of only 13 animals were examined). It should be noted that while this strain has a low incidence of spontaneous tumours until 9–12 months of age, the rate of tumour development accelerates after 12 months, and osteosarcomas, malignant lymphomas and haemangiosarcomas are typically detected in 50% of animals after 18 months (Carmichael et al., 2000).

Interaction with ethanol

Groups of 25 female Han/NMRI mice received daily ethyl carbamate doses of 0, 18, 36, 90 or 180 mg/kg bw either in water or in 20% ethanol by gavage for 8 weeks. This inbred strain exhibits a high genetic homogeneity and is particularly susceptible to lung carcinogenicity. No clinical signs of toxicity were observed during the treatment period. After a further 8 weeks without treatment, the animals were sacrificed and lung adenomas were counted. Ethyl carbamate increased the incidence and number of lung adenomas per mouse dose-dependently in all dose

groups. When ethyl carbamate was administered in tap water, there were 24% (control), 65%, 96%, 100% and 100% of animals with an average of 0.3 (control), 2, 7, 32 or 81 lung adenomas per mouse in the groups receiving 0, 18, 36, 90 or 180 mg/kg bw per day, respectively. Tumour incidence or multiplicity, however, was not significantly different in respective groups receiving ethyl carbamate in 20% ethanol. The authors concluded that ethanol had no effect on ethyl carbamate-induced tumorigenesis (Altmann et al., 1991).

Groups of 15 female mice of strain A/Ph, 6.5 weeks of age, received ethyl carbamate at 0, 200, 500 or 1000 mg/l in drinking-water (equivalent to 0, 35, 90.3 and 173 mg/kg bw per day) containing 0, 5, 10 and 20% (v/v) ethanol. Body weight, food consumption and water consumption were recorded during the study. Animals were killed after 12 weeks of treatment. Gross necropsy was conducted on all animals, the livers were weighed, and livers, lungs and any other tissues showing pathological changes were preserved for subsequent histopathological examination. Body weights at the end of the study were significantly reduced from controls in the group receiving the highest dose of ethyl carbamate in drinking-water. The highest dose of ethanol was also associated with lower body weights at the end of the study compared with respective groups receiving ethyl carbamate in drinking-water, except at the highest dose of ethyl carbamate. Food intake was slightly reduced in all groups dosed with combinations of ethyl carbamate and ethanol and in the group receiving 20% ethanol alone. No effects of treatment on relative liver weights were noted. The background incidences of lung adenoma were high (40–67%) in all groups of the control mice (receiving 0–20% ethanol in drinking-water). Tumour incidences in groups receiving ethyl carbamate at any dose were 100%, with the exception of the low-dose group receiving 10% ethanol, in which the incidence was 80%. No tumours other than the pulmonary adenomas were detected. Ethyl carbamate treatment increased the number of lung adenomas per mouse in a dose-dependent manner both alone and in combination with ethanol. The highest number of tumours per mouse was observed when ethyl carbamate was administered in drinking-water; at the highest ethyl carbamate dose of 1000 mg/l, 70.9 ± 15.5 tumours per mouse were observed, compared with 0.4 ± 0.7 tumours per mouse in the respective control group. Ethanol treatment reduced this effect of ethyl carbamate on tumour multiplicity in a dose-dependent manner that attained statistical significance ($P < 0.001$) at 10% and 20%, but not 5%, ethanol in drinking-water (Kristiansen et al., 1990).

Groups of 10 male and 10 female B6C3F1 mice, 6 weeks of age, received ethyl carbamate (purity $\geq 99\%$) at 0, 110, 330, 1100, 3300 or 10 000 mg/l in drinking-water or in 5% ethanol *ad libitum*, 7 days a week, for 13 weeks (equivalent to 0, 17, 50, 165, 500 or 1500 mg/kg bw per day, assuming a drinking-water consumption of 150 ml/kg bw). The authors noted that there was excessive spillage of drinking-water. Body weights and clinical observations were recorded throughout the study. Sperm motility and vaginal cytology evaluations were conducted at the end of the study. Plasma was analysed for ethyl carbamate and ethanol concentrations at the end of 13 weeks, 0, 1, 2, 3 and 5 h after dosed water was removed. Complete necropsy was performed on all animals, and organ weights for heart, right kidney, liver, lungs, right testis and thymus were taken.

Complete histopathological evaluations of 42 tissues and organs, plus any tissues showing gross lesions, were conducted on all mice in the control and top three dose groups in the drinking-water study and in the control and top two dose groups in the 5% ethanol study. In the lower dose groups, bone marrow, heart, kidneys, liver, lungs, mandibular and mesenteric lymph nodes, spleen, thymus, ovaries, pancreatic islets, testes and all tissues showing gross lesions were evaluated in all mice. Clinical signs of toxicity were generally restricted to the two highest dose groups, regardless of whether ethyl carbamate was administered with or without ethanol. All mice administered ethyl carbamate at 10 000 mg/l in either vehicle died during or before week 3 of the study. All mice that received ethyl carbamate at 3300 mg/l in drinking-water died during or before week 5, whereas all but one male and four females receiving ethyl carbamate at 3300 mg/l in 5% ethanol survived to the end of the study. Body weight gain was reduced in all groups receiving ethyl carbamate at or above 1100 mg/l. The absolute organ weights were generally decreased and the relative organ weights were generally increased in male and female mice receiving ethyl carbamate at 1100 mg/l in drinking-water or 1100 and 3300 mg/l in 5% ethanol. Because of the 20–35% body weight decrements in these groups compared with their respective controls and the lack of a dose–response relationship for organ weights in the lower dose groups, it was not possible to ascertain whether any of these observations were related to the effects of treatment or secondary to body weight decrements. Even with the early deaths of mice in the two highest drinking-water dose groups, treatment-related increases were noted in the incidence and severity of inflammation of the lung, bronchiole hyperplasia, renal nephropathy (longer latency in females), loss of normal cytoplasmic vacuolation of hepatocytes in the periportal area, cardiac haemorrhage and mineralization, lymphoid depletion of the spleen, mesenteric and mandibular lymph nodes and thymus, cellular depletion of the bone marrow, ovarian follicle degeneration and seminiferous tubule degeneration (high dose only) involving these doses, and in some cases the next lower dose (1100 mg/l). Histopathological changes in the testis and ovary were considered secondary to the debilitated condition of the mice, as these were noted only in the mice that died early. Effects that were not dose-related at the two highest dose groups included hyperplasia of the alveolar epithelium, which was evident in the 1100 mg/l females but was not dose-related in males, cardiomyopathy and ovarian atrophy. The same spectrum of lesions was observed when ethyl carbamate was offered in 5% ethanol, although the effect was less pronounced, and some lesions (degeneration of seminiferous tubules, alteration of cytoplasmic vacuolation of hepatocytes and cardiomyopathy, all in males) were absent. Lung adenomas occurred in four males and one female of the ethyl carbamate groups treated with ethanol, but not in a dose-related manner, compared with one ethyl carbamate-treated male in the water group. Spermatozoal motility was slightly, but significantly, reduced (<2%) in mice receiving ethyl carbamate at 1100 mg/l in drinking-water or 330 and 1100 mg/l in 5% ethanol and drastically reduced (63%) in the 3300 mg/l in ethanol group. Estrous cycling effectively ceased in females receiving ethyl carbamate at 1100 mg/l in drinking-water or 3300 mg/l in ethanol. Females receiving ethyl carbamate at 1100 mg/l in ethanol had a longer estrous cycle length than control mice; this appeared to be a less severe manifestation of the

effects on estrous cycling observed in the 3300 mg/l group. The no-observed-effect level (NOEL) for this study was 330 mg/l in drinking-water (NTP, 1996).

Groups of 18–21 male weanling C3H/HeJ mice were given ethyl carbamate at 0, 10 or 20 mg/kg bw per day in tap water, 12% ethanol or commercial wine (red or white Concord wine or Riesling wine), *ad libitum* for 41 weeks. At sacrifice, liver and lung tissues were collected from all animals for histopathological examination. Survival at the end of week 41 was significantly decreased in the group drinking tap water containing ethyl carbamate at 20 mg/kg bw per day compared with all other groups. The mean body weight gain of mice drinking wine was lower than that of mice drinking tap water for corresponding ethyl carbamate treatment groups. Nevertheless, as the ethyl carbamate concentration in each liquid was adjusted according to body weight on a weekly basis, the mean ethyl carbamate intake was very similar within each level of intake, no matter what drinking fluid was provided. There was an increased incidence of benign lung tumours (both Clara cell adenoma and alveolar adenoma) in mice treated with ethyl carbamate in any vehicle. No malignant tumours were detected in any group. When ethyl carbamate at 0, 10 or 20 mg/kg bw per day was administered in water, incidences of Clara cell adenoma were 0%, 52% and 57%, and incidences of alveolar adenoma were 0, 19% and 48%, respectively. The various alcoholic treatments resulted in a dose-related, but lower, incidence of both tumour types that was particularly evident at the lower dose of ethyl carbamate. The incidence of spontaneously occurring hepatocellular adenoma of 22.2% in control animals was increased to 57.1% and 46.6%, respectively, in the 10 and 20 mg/kg bw per day treatment groups in tap water. A low incidence of hepatocellular carcinoma (5% and 15%, respectively) was also observed in these treated groups only. Treatment with the Concord wines had the effect of lowering the incidence of hepatocellular adenoma in the control and low-dose, but not the high-dose, groups. No dose effect of ethyl carbamate on this parameter was noted for the ethanol and Riesling wine treatments. The incidence of hepatic haemangioendothelioma and haemangiosarcoma, which was absent in controls, also showed a dose-related trend with ethyl carbamate treatment that was most pronounced when ethyl carbamate was administered in tap water (Stoewsand et al., 1991).

(b) *Rats*

(i) *Oral route*

Interaction with ethanol

Groups of 10 male and 10 female F344/N rats, 6 weeks of age, received ethyl carbamate (purity $\geq 99\%$) at 0, 110, 330, 1100, 3300 or 10 000 mg/l in drinking-water or 5% ethanol *ad libitum* for 13 weeks. Satellite groups of 10 male and 10 female rats per dose were designated for clinical pathology testing. Administered in drinking-water, these concentrations were equivalent to ethyl carbamate doses of 0, 8/11, 23/33, 78/114, 287/332 or 622/525 mg/kg bw per day for male/female rats, respectively. Co-administered with ethanol, they were equivalent to ethyl carbamate doses of 0, 9/10, 27/28, 87/79, 221/201 or 545/473 mg/kg bw per day for male/female rats, respectively. Body weights and clinical observations were

recorded throughout the study. Haematology and clinical chemistry parameters were determined from blood samples taken at days 3 and 23 (days 15/16 for the 10 000 mg/l dose group) from the clinical study pathology groups and from the baseline study rats during week 13. Sperm motility and vaginal cytology evaluations were conducted on rats at the end of the study. Complete necropsy was performed on all animals, and organ weights for heart, right kidney, liver, lungs, right testis and thymus were taken. Complete histopathological evaluations of 42 tissues and organs, plus any tissues showing gross lesions, were conducted on all mice in the control and top two dose groups in the drinking-water study and in the control and top two dose groups in the 5% ethanol study. In the lower dose groups, bone marrow, heart, kidneys, liver, lungs, mandibular and mesenteric lymph nodes, spleen, thymus and all tissues showing gross lesions were evaluated in all rats. Mortality was increased and body weight gain drastically reduced in both sexes of the 10 000 mg/l group receiving ethyl carbamate in water. Most deaths occurred during the final week of the study. Body weight gain in the 3300 mg/l males and 1100 and 3300 mg/l females was also reduced, but to a lesser extent, reaching a body weight decrement of 10% in the female rats at 3300 mg/l. When administered in 5% ethanol, none of the females receiving 10 000 mg/l survived, and a body weight decrement of 9% was observed at 3300 mg/l; the deaths occurred early in the study, during weeks 3–6. Mortality was slightly increased and body weight gain was reduced in the males receiving ethyl carbamate at 10 000 mg/l in 5% ethanol. Water consumption by rats receiving ethyl carbamate in drinking-water was reduced at the 10 000 mg/l concentration. In rats receiving ethyl carbamate in 5% ethanol, water consumption was also reduced at the 3300 mg/l dose in males and 1100 and 3300 mg/l doses in females, such that these groups received a lower dose of ethyl carbamate on a body weight basis than did the respective groups receiving ethyl carbamate in drinking-water. Leukopenia and lymphopenia were observed in rats receiving ethyl carbamate in either drinking-water or ethanol and occurred in males receiving 330 mg/l or greater and females receiving 110 mg/l or greater. There were no other effects on haematology or clinical chemistry parameters considered biologically relevant. Dose-related differences between ethyl carbamate-treated groups and their respective controls were observed in most organ weights both as absolute (primarily decreases) and relative to body weight (primarily decreases) measurements. These differences were likely due to the lower body weights of the higher treated groups at the end of the study. Lymphoid depletion of the spleen, mandibular and mesenteric lymph nodes and thymus, which most severely affected the spleen, was noted in both sexes at ethyl carbamate concentrations of 1100 mg/l or higher when administered in drinking-water and 3300 mg/l or higher when administered in 5% ethanol. Other treatment-related effects consisted of fatty changes in the centrilobular area of the liver, affecting male rats at the 10 000 mg/l concentration in both drinking-water and 5% ethanol; bone marrow cellular depletion at the 10 000 mg/l concentration in both drinking-water and 5% ethanol, affecting both sexes, with a more pronounced effect in females (one female at 3300 mg/l in drinking-water was also affected); an increase in the severity of renal nephropathy and cardiomyopathy, which occurred at a 100% spontaneous rate in males; and an increase in both incidence and severity of these lesions in females (statistically significant at 1100 mg/l and higher for renal nephropathy and 330 mg/l

and higher for cardiomyopathy). Ethanol treatment did not alter the response in males; in females, it elevated the spontaneous rate of renal nephropathy and eliminated the cardiomyopathy response. Sperm motility was slightly (<2%) but significantly reduced in rats receiving ethyl carbamate at 1100 or 3300 mg/l in either vehicle; sperm counts were also significantly reduced in all but the 1100 mg/l in ethanol group. There was a slight lengthening of the estrous cycle in females receiving ethyl carbamate at 3300 mg/l that was significant only for the ethanol-treated group. A NOEL could not be established for this study due to the observation of decreased serum lymphocyte and leukocyte counts in females down to the lowest drinking-water concentration of ethyl carbamate tested, 110 mg/l (NTP, 1996).

2.2.3 Long-term studies of toxicity and carcinogenicity

(a) Mice

(i) Oral route

Groups of 24 male and 24 female 1-week-old mice of two different hybrid strains ((C57BL/6 × C3H/Anf)F1, designated "strain X"; (C57BL/6 × AKR)F1, designated "strain Y") received ethyl carbamate at 158 mg/kg bw per day by gavage from days 7 to 28 of age, then 600 mg/kg in the diet *ad libitum* (equivalent to 90 mg/kg bw per day) for the rest of their lives (approximately 18 months in total). This treatment was the maximum tolerated dose of ethyl carbamate in a series of separate studies that resulted in zero mortality after 19 days. Four untreated control groups and a gelatin control group treated with gelatin gavage during days 7–28 of age received untreated diet. At sacrifice, gross necropsy was performed on all animals, and histological examination of major tissues (not specified) and grossly visible lesions was made. Statistical analysis was performed for four tumour groupings: hepatomas, pulmonary tumours, lymphomas and total mice with tumours. There was a statistically increased incidence of hepatomas and pulmonary tumours in both strains compared with controls and of lymphomas in strain Y compared with controls (see Table 2). The pulmonary tumours consisted primarily of adenomas; most tumours of the lymphoid organs were Type B reticulum cell sarcomas. Metastasizing hepatic cell tumours were rare. The authors also noted the occurrence of a substantial number of adenomas of the Harderian gland affecting 24 treated mice (strain and sex not stated). Tumours of other organs were rare (Innes et al., 1969).

Groups of 25 male and 25 female 7-week-old Swiss mice received ethyl carbamate at 0 or 1000 mg/kg in their diet (equivalent to 0 or 150 mg/kg bw per day) for 116 weeks. Body weights were assessed at irregular intervals. Surviving animals and, where possible, animals dying on test were examined macroscopically, and 13 types of tissues (from liver, kidneys, heart, lungs, spleen, pancreas, adrenals, gastrointestinal tract, urinary bladder, prostate, testes, ovaries and uterus) were preserved for histopathological evaluation. No differences in growth were observed in the treated mice compared with controls. The average life span of the treated animals was 40 weeks for both sexes, compared with 75 weeks in the control group. Multiple lung tumours (more than 20 lung tumours per animal)

Table 2. Tumour incidence at selected sites

Strain ^a	Treatment group	Number of mice at term		Total mice necropsied		Mice with hepatomas (%)		Mice with pulmonary tumours (%)		Mice with lymphomas (%)		Total mice with tumours (%)	
		M	F	M	F	M	F	M	F	M	F	M	F
X	Control	73	83	79	87	8 (10)	0 (0)	5 (6)	3 (3)	5 (6)	4 (5)	22 (28)	8 (9)
	Ethyl carbamate	9	13	20	23	8 (40)	12 (52)	6 (30)	6 (26)	1 (5)	3 (13)	13 (65)	19 (83)
Y	Control	89	75	90	82	5 (6)	1 (1)	10 (11)	3 (4)	1 (1)	4 (5)	16 (18)	7 (9)
	Ethyl carbamate	15	18	22	19	14 (64)	5 (26)	15 (68)	17 (89)	6 (27)	1 (5)	20 (91)	18 (95)

From Innes et al. (1969)

^a Hybrid mice (C57BL/6 × C3H/Anf)F1 were designated as "strain X" and hybrid mice (C57BL/6 × AKR)F1 as "strain Y." The last mice of the ethyl carbamate groups died at an age of 69–74 weeks, whereas control mice died at 78–88 weeks (depending on strain and sex).

developed in 46 of 48 mice given ethyl carbamate, compared with an average of 2.5 per animal in 10 of 49 control animals. The incidences of mammary adenomas and adenocarcinomas and of lymphomas were reduced in the treated mice compared with controls, likely as a result of the reduction in their life span due to the presence of lung tumours (Van Esch & Kroes, 1972).

CF-1 mice received ethyl carbamate at 100 mg/l in drinking-water (equivalent to 15 mg/kg bw per day starting at 6–7 weeks of age for their life span over two generations) (48 male and 40 female parents; 61 males and 38 females of the F1 generation). A group of approximately 60 mice per sex per generation received untreated drinking-water. An autopsy was carried out on all mice, and histological examination was made of the lungs, heart, thymus, liver, kidney, spleen, gonads and brain, together with all organs showing gross abnormalities. Survival of the ethyl carbamate-treated groups was similar to that of controls. The incidence of lung tumours was 40/48 (83%) in male parents, 28/40 (70%) in female parents, 56/61 (92%) in male F1 and 31/38 (82%) in female F1, compared with 42%, 23%, 31% and 40% of control animals, respectively. Mammary tumours were observed in 10% of treated females (4/40 parents and 4/38 F1) compared with 5% and 0% in controls (3/56 parents, 0/55 F1). There was no increase in any other tumour incidences as a result of treatment with ethyl carbamate (Tomatis et al., 1972).

Ethyl carbamate was administered in 20 ml of drinking-water to groups of 40 male and 40 female 8-week-old NMRI mice at doses of 0, 0.1, 0.5, 2.5 or 12.5 mg/kg bw per day for their lifetime (maximum of 660–760 days). On Mondays and Fridays, the concentration was doubled, while on Saturdays and Sundays, tap water only was given *ad libitum*. All animals were autopsied, and organs with macroscopic findings were examined histologically. Median survival times fell between 500 and 600 days. The tumours showing a clear dose dependence were tumours of the lung (mainly adenomas and adenocarcinomas) and haemangio-endotheliomas (mainly in the liver) in both sexes and mammary gland carcinomas in females. At 12.5 mg/kg bw per day, males and females showed incidences of pulmonary adenocarcinomas of 5/32 and 10/33, respectively, and at 2.5 mg/kg bw per day, only males had an increase in malignant lung tumour incidence (4/28), while there were no malignant lung tumours in the control groups (Schmähl et al., 1977; specific tumour data reanalysed in Schlatter & Lutz, 1990).

Ethyl carbamate was administered in the drinking-water to groups of 50 6-week-old male B6C3F1 mice at 0, 0.6, 3, 6, 60 or 600 mg/l (equivalent to 0, 0.10, 0.58, 1.0, 10 and 100 mg/kg bw per day, estimated by measured water consumption) for 70 weeks. Water consumption and individual body weights were recorded throughout the study. At the end of treatment, all major organs were weighed and microscopically examined. All mice of the 600 mg/l group died by the 46th treatment week, while mortality in all other groups was not significantly changed compared with the control group. Mice of the 60 and 600 mg/l groups had incidences of alveolar/bronchiolar adenoma (68% and 95.5%, respectively) that were statistically significantly increased compared with controls (18%); the incidence of this tumour in the lower dose groups was similar to or lower than that of controls (8–15%). In addition, mice of the 600 mg/l group had increased incidences of alveolar/bronchiolar carcinoma (13.6%; none in all other groups,

including controls). The second most common tumour types related to treatment were haemangioma (0%, 0%, 0%, 0%, 4% and 46% in order of increasing dose) and angiosarcoma (0%, 0%, 0%, 4%, 4% and 25% in order of increasing dose) of the liver; the incidence in both cases was statistically significantly increased at the highest dose. Peliosis in liver, a non-neoplastic lesion involving dilated sinusoids, possibly related to haemangioma, was noted at an incidence of 14% and 9.1% in the two top doses, respectively, and in none of the lower dose groups or controls. Haemangioma in the heart was detected in the highest dose group (9.1%) and in none of the other groups, including controls. Single incidences of haemangiomas of the spleen and pancreas were noted without relationship to dose. Alveolar/bronchiolar adenoma or carcinoma of the lung and haemangioma and angiosarcoma of the liver revealed a clear dose-response relationship (Inai et al., 1991).

Interaction with ethanol

Groups of 48 male and 48 female B6C3F1 mice, 5 weeks of age, received ethyl carbamate (purity $\geq 99\%$) at concentrations of 0, 10, 30 or 90 mg/l in the presence of 0%, 2.5% or 5% ethanol *ad libitum* for 2 years. Two sets of satellite groups of four mice per sex per group, designated for studies on cell proliferation and apoptosis and on pharmacokinetic considerations, received the same doses for 4 weeks. Doses were equivalent to 0, 1.2/0.9, 3.3/2.8 and 10.1/8.2 mg/kg bw per day in males/females when ethyl carbamate was administered in tap water (calculated as the average mean over the whole life span). Water consumption was constant at about 4.5 g/day per animal over the whole life span, but body weight almost doubled from week 1 to week 13 compared with a slower rate of increase over weeks 14–104. Therefore, ethyl carbamate intake on a mg/kg bw per day basis was much higher in young mice (see Table 3 for details). Ethyl carbamate intake was slightly reduced with increasing ethanol concentration in males only. Clinical observations, body weights, water consumption and food consumption were recorded throughout the study. Complete necropsies were performed on all mice. The weights of liver and lung were taken, and all organs and tissues were examined for grossly visible lesions. Histopathological examination was made of all major organs from all mice in the baseline studies and included assessment of 56 organs and tissues and any grossly visible lesions. Significant treatment-related decreases in survival were observed in both sexes as a function of ethyl carbamate concentration, for each exposure concentration of ethanol. There was no consistent effect of increasing ethanol concentrations on survival at a particular dose of ethyl carbamate. The decrease in survival due to ethyl carbamate was consistently statistically significant at 90 mg/l in both male and female mice, significant in three of six groups receiving 30 mg/l and marginally significant in one female group receiving 10 mg/l. The mean body weights of both sexes of mice receiving ethyl carbamate at 90 mg/l were lower than respective controls at any concentration of ethanol; ethyl carbamate, but not ethanol, caused a treatment-related decrease in the terminal body weights of mice. Increasing concentrations of ethyl carbamate did not affect water or feed consumption for a particular concentration of ethanol throughout the study, while increasing ethanol concentrations reduced both water and feed consumption for a particular concentration of ethyl carbamate. The effect on water consumption was more

Table 3. Tumour incidences at selected organ sites from a 2-year carcinogenicity study in B6C3F1 mice administered ethyl carbamate at 0, 10, 30 or 90 mg/l in drinking-water containing 0%, 2.5% or 5% ethanol

	Ethyl carbamate concentration in drinking-water (mg/l)							
	Male B6C3F1 mice				Female B6C3F1 mice			
	0	10	30	90	0	10	30	90
Equivalent to ethyl carbamate dose (mg/kg bw per day) calculated from mean water consumption and mean body weight over mean life span (and mean weeks 1–13)								
0% ethanol	0	1.2 (1.5)	3.3 (4.7)	10.1 (13.1)	0	0.9 (1.4)	2.8 (4.3)	8.2 (12.9)
2.5% ethanol	0	1.0 (1.4)	3.0 (4.2)	8.8 (12.4)	0	0.9 (1.5)	2.7 (4.2)	7.9 (12.8)
5% ethanol	0	0.9 (1.3)	2.7 (3.9)	8.5 (11.6)	0	0.9 (1.5)	2.7 (4.3)	8.1 (12.8)
Incidences of non-neoplastic lesions								
Liver, eosinophilic foci								
0% ethanol	6/46	7/47	19/46**	28/44**	3/48	14/47**	32/47**	20/47**
2.5% ethanol	6/47	3/48	17/46**	22/48**	2/47	20/47**	21/47**	28/46**
5% ethanol	10/48	9/46	18/48*	25/48**	2/48	26/47**	25/48**	21/48**
Liver, angiectasis								
0% ethanol	0/46	4/47*	6/46**	17/44**	0/48	3/47*	10/47**	24/47**
2.5% ethanol	0/47	0/48	7/46**	16/48**	2/47	5/47	7/47*	20/46**
5% ethanol	1/48	1/46	8/48*	19/48**	2/48	1/47	5/48	22/48**
Heart, hyperplasia of endothelium								
0% ethanol	0/48	0/48	4/47**	9/48**	1/48	0/48	3/48	6/48**
2.5% ethanol	0/48	1/48	4/47*	9/48**	0/47	0/47	3/48*	8/48**

Table 3. (contd)

	Ethyl carbamate concentration in drinking-water (mg/l)							
	Male B6C3F1 mice				Female B6C3F1 mice			
	0	10	30	90	0	10	30	90
5% ethanol	0/47	0/48	0/48	2/48*	0/47	1/48	3/48*	14/47**
Heart, angiectasis								
0% ethanol	0/48	1/48	2/47	11/48**	0/48	0/48	0/48	1/48
2.5% ethanol	0/48	0/48	7/47**	13/48**	0/47	0/47	1/48	4/48**
5% ethanol	0/47	0/48	1/48	5/48**	0/47	0/48	3/48*	4/47*
Uterus, angiectasis								
0% ethanol					0/48	4/47*	6/48**	7/46**
2.5% ethanol					1/47	2/47	7/48**	9/48**
5% ethanol					4/48	2/48	8/47	6/45
Incidences of neoplastic lesions								
Liver, hepatocellular adenoma								
0% ethanol	7/46	13/47	17/46*	17/44**	5/48	10/47	19/47**	18/47**
2.5% ethanol	12/47	15/48	16/46	24/48**	6/47	5/47	15/47**	23/46**
5% ethanol	19/48	9/46	16/48	12/48	3/48	6/47	16/48**	16/48**
Liver, hepatocellular carcinoma								
0% ethanol	7/46	6/47	9/46	9/44	No data available			

Table 3. (contd)

	Ethyl carbamate concentration in drinking-water (mg/l)							
	Male B6C3F1 mice				Female B6C3F1 mice			
	0	10	30	90	0	10	30	90
2.5% ethanol	6/47	5/48	5/46	4/48	11/47	0/47	3/47	1/46
5% ethanol	7/48	9/46	2/48	9/48	No data available			
Liver, hepatocellular adenoma or carcinoma								
0% ethanol	12/46	18/47	24/46*	23/44**	5/48	11/47	20/47**	19/47**
2.5% ethanol	16/47	19/48	17/46	24/48*	7/47	5/47	16/47**	23/46**
5% ethanol	25/48	16/46	17/48	18/48	3/48	7/47	16/48**	17/48**
Lung, alveolar/bronchiolar adenoma								
0% ethanol ^a	4/48	17/48**	22/47**	34/48**	4/48	6/48	17/48**	29/47**
2.5% ethanol	10/48	16/48	19/47**	35/48**	5/47	10/47	16/48**	28/48**
5% ethanol	6/48	8/48	9/48	33/48**	5/48	10/48	18/48**	30/48**
Lung, alveolar/bronchiolar carcinoma								
0% ethanol ^b	1/48	1/48	9/47**	9/48**	2/48	4/48	13/48**	19/47**
2.5% ethanol	2/48	3/48	8/47*	24/48**	0/47	2/47	6/48**	23/48**
5% ethanol	5/48	4/48	5/48	17/48**	1/48	7/48*	9/48**	23/48**
Lung, alveolar/bronchiolar adenoma or carcinoma								
0% ethanol ^c	5/48	18/48**	29/47**	37/48**	6/48	8/48	28/48**	39/47**
2.5% ethanol	11/48	19/48	24/47**	43/48**	5/47	11/47	21/48**	38/48**

Table 3. (contd)

	Ethyl carbamate concentration in drinking-water (mg/l)							
	Male B6C3F1 mice				Female B6C3F1 mice			
	0	10	30	90	0	10	30	90
5% ethanol	11/48	11/48	14/48	40/48**	5/48	17/48**	24/48**	37/48**
Harderian gland, adenoma								
0% ethanol	3/47	11/47*	22/47**	28/47**	3/48	10/48*	8/48**	21/48**
2.5% ethanol	6/48	14/48*	21/47**	27/48**	2/47	3/47	9/46**	19/47**
5% ethanol	5/47	12/48*	15/48**	26/45**	4/48	7/48	6/46	20/46**
Harderian gland, carcinoma								
0% ethanol	0/47	1/47	7/47*	16/47**	0/48	1/48	11/48**	11/48**
2.5% ethanol	0/48	0/48	1/47	16/48**	1/47	3/47	6/46*	16/47**
5% ethanol	0/47	2/48	2/48	10/45**	1/48	11/48**	7/46*	10/46**
Harderian gland, adenoma or carcinoma								
0% ethanol	3/47	12/47**	30/47**	38/47**	3/48	11/48*	19/48**	30/48**
2.5% ethanol	6/48	14/48*	21/47**	38/48**	3/47	5/47	15/46**	35/47**
5% ethanol	5/47	14/48**	17/48**	35/45**	5/48	18/48**	13/46*	29/46**
Forestomach, squamous cell papilloma or squamous cell carcinoma								
0% ethanol	0/46	2/47	3/44	5/45*	2/48	4/46	3/46	4/46
2.5% ethanol	1/47	4/47	1/46	4/46	0/47	4/46	0/47	1/46
5% ethanol	2/48	3/47	4/45	6/48	2/48	3/47	2/48	1/45

Table 3. (contd)

	Ethyl carbamate concentration in drinking-water (mg/l)							
	Male B6C3F1 mice				Female B6C3F1 mice			
	0	10	30	90	0	10	30	90
Skin, squamous cell papilloma or squamous cell carcinoma								
0% ethanol	0/47	1/48	3/47	6/48**	No data available			
2.5% ethanol	0/48	1/48	4/46*	7/47**	No data available			
5% ethanol	0/48	2/47	0/48	7/45**	No data available			
Mammary gland, adenoacanthoma								
0% ethanol					0/47	1/46	1/46	11/48**
2.5% ethanol					0/47	0/45	2/48	3/47
5% ethanol					0/47	0/48	1/48	9/45**
Mammary gland, adenocarcinoma								
0% ethanol					4/47	3/46	3/46	11/48**
2.5% ethanol					4/47	3/45	11/48*	14/47**
5% ethanol					3/47	4/48	6/48	15/45**
Mammary gland, adenoacanthoma or adenocarcinoma								
0% ethanol					4/47	4/46	4/46	22/48**
2.5% ethanol					4/47	3/45	12/48**	16/47**
5% ethanol					3/47	4/48	7/48	23/45**

Table 3. (contd)

	Ethyl carbamate concentration in drinking-water (mg/l)							
	Male B6C3F1 mice				Female B6C3F1 mice			
	0	10	30	90	0	10	30	90
Ovary, benign granulosa cell tumour								
0% ethanol					0/48	0/46	2/46	3/39*
2.5% ethanol					0/47	0/46	3/47	3/48
5% ethanol					0/46	0/47	5/46*	3/45
Ovary, malignant granulosa cell tumour								
0% ethanol					0/48	0/46	0/46	3/39*
2.5% ethanol					0/47	0/46	0/47	0/48
5% ethanol					0/46	0/47	1/46	0/45
Ovary, benign or malignant granulosa cell tumour								
0% ethanol					0/48	0/46	2/46	5/39**
2.5% ethanol					0/47	0/46	3/47	3/48
5% ethanol					0/46	0/47	6/46*	3/45
Uterus, haemangiosarcoma								
0% ethanol					0/48	0/47	0/48	2/46
2.5% ethanol					0/47	0/47	1/48	1/48
5% ethanol					0/48	2/48	1/47	1/45

Table 3. (contd)

	Ethyl carbamate concentration in drinking-water (mg/l)							
	Male B6C3F1 mice				Female B6C3F1 mice			
	0	10	30	90	0	10	30	90
All organs, haemangiosarcoma								
0% ethanol	4/48	4/48	7/47	19/48**	0/48	0/48	4/48*	11/48**
2.5% ethanol	3/48	5/48	9/47*	21/48**	1/47	2/47	1/48	17/48**
5% ethanol	3/48	3/48	5/48	16/48**	0/48	3/48	1/48	13/48**
Liver, haemangiosarcoma								
0% ethanol	1/46	2/47	5/46	13/44**	0/48	0/47	1/47	7/47**
2.5% ethanol	3/47	4/48	3/46	11/48*	1/47	2/47	0/47	7/46**
5% ethanol	2/48	2/46	4/48	13/48**	0/48	0/47	0/48	6/48**
Heart, haemangiosarcoma								
0% ethanol	0/48	0/48	1/47	5/48*	0/48	0/48	1/48	0/48
2.5% ethanol	0/48	0/48	2/47	4/48*	0/47	0/47	0/48	3/48*
5% ethanol	0/47	0/48	1/48	4/48*	0/47	0/48	0/48	6/47**
Spleen, haemangiosarcoma								
0% ethanol	2/44	2/46	2/45	5/42	0/48	0/45	1/47	4/46*
2.5% ethanol	0/46	0/46	1/46	3/46	0/47	0/46	0/46	3/46
5% ethanol	0/48	1/46	0/46	1/45	0/48	1/47	0/48	1/45

Table 3. (contd)

	Ethyl carbamate concentration in drinking-water (mg/l)							
	Male B6C3F1 mice				Female B6C3F1 mice			
	0	10	30	90	0	10	30	90
Skin, haemangiosarcoma								
0% ethanol	1/47	0/48	0/47	2/48	0/48	0/48	0/46	2/48
2.5% ethanol	0/48	0/48	4/46	1/47	0/47	0/47	0/48	2/47
5% ethanol	0/48	0/47	0/48	1/45	0/48	0/48	0/48	0/47
All organs, benign tumours								
0% ethanol ^d	19/48	29/48**	41/47**	45/48**	22/48	26/48	38/48**	42/48**
2.5% ethanol	27/48	33/48	37/47**	46/48**	21/47	21/47	31/48**	39/48**
5% ethanol	30/48	29/48	32/48	40/48**	22/48	26/48	37/48**	41/48**
All organs, malignant tumours								
0% ethanol	27/48	30/48	37/47	43/48**	26/48	20/48	37/48*	46/48**
2.5% ethanol	31/48	32/48	33/47	45/48**	14/47	22/47*	38/48**	45/48**
5% ethanol	31/48	30/48	37/48*	45/48**	17/48	35/48**	37/48**	46/48**
All organs, benign and malignant tumours								
0% ethanol ^d	33/48	39/48*	46/47**	47/48**	37/48	35/48	45/48*	47/48**
2.5% ethanol	41/48	45/48	42/47	47/48	29/47	33/47	45/48**	48/48**
5% ethanol	42/48	41/48	44/48	46/48*	32/48	43/48**	47/48**	47/48**

Table 3. (contd)

* Significantly different ($P \leq 0.05$) from the control group by the Poly-3 (neoplasms) or Williams' (non-neoplastic lesions) test

** Significantly different ($P \leq 0.01$) from the control group by the Poly-3 (neoplasms) or Williams' (non-neoplastic lesions) test

Remarks: The Poly-3 method, used for calculating the significance levels of neoplastic effects, is a survival-adjusted (age-adjusted) test that accounts for differential mortality in animals that do not reach terminal sacrifice by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study.

^a Historical incidence for males: 71/473 (15%), range 8–27%; for females: 22/515 (4.3%), range 2–6%.

^b Historical incidence for males: 11/473 (2.3%), range 0–8%; for females: 3/515 (0.6%), range 0–4%.

^c Historical incidence for males: 82/473 (17.3%), range 11–31%; for females: 25/515 (4.9%), range 2–11%.

^d Including uncertain neoplasms.

marked in male mice than in female mice. In males, in the absence of ethanol, there were dose-dependently increased incidences of hepatocellular adenoma/carcinoma, alveolar/bronchiolar adenoma/carcinoma, Harderian gland adenoma/carcinoma, squamous cell papilloma/carcinoma of the forestomach and the skin, and haemangiosarcoma, primarily of the liver and the heart (see Table 3). In females, in the absence of ethanol, there were dose-dependently increased incidences of hepatocellular adenoma/carcinoma, alveolar/bronchiolar adenoma/carcinoma, Harderian gland adenoma/carcinoma, mammary gland adenocanthoma/adenocarcinoma, ovarian granulosa cell tumour (benign and malignant) and haemangiosarcoma, primarily of the liver and spleen. The authors noted that the occurrences of haemangiosarcoma of the spleen in males and of the uterus and skin in females were not significantly increased, but may have been exposure-related as well. When 0%, 2.5% or 5% ethanol was administered in the absence of ethyl carbamate, incidences of hepatocellular adenoma or carcinoma (combined) were significantly increased in male mice (12/46, 16/47, 25/48); in females, however, ethanol in the absence of ethyl carbamate caused no increased tumour incidences. The co-administration of ethyl carbamate and ethanol resulted in marginal changes in the incidences of some neoplasms that were attributed to ethyl carbamate alone. In males, increasing the ethanol concentration caused a decreasing trend in the incidence of alveolar/bronchiolar and Harderian gland adenoma or carcinoma responses to ethyl carbamate. In females, increasing the ethanol concentration increased slightly the incidence of haemangiosarcoma of the heart and alveolar/bronchiolar adenoma or carcinoma responses to ethyl carbamate. The authors concluded that there was clear evidence of carcinogenic activity of ethyl carbamate in both male and female mice and that, overall, the findings were insufficient to establish a definitive effect of ethanol on the carcinogenicity of ethyl carbamate (NTP, 2004; Beland et al., 2005).

Groups of four male and four female mice were given ethyl carbamate and ethanol in a 4-week mechanistic study conducted by the NTP (2004) that used the same dosing protocol as in the main 2-year carcinogenicity study. Ethyl carbamate concentrations of 10, 30 and 90 mg/l in drinking-water resulted in average daily intakes of approximately 35, 110 and 315 µg ethyl carbamate in males and 30, 80 and 245 µg in females, approximately equivalent to 1.4, 4.2 and 12.6 mg/kg bw per day in either sex. Ethanol concentrations of 2.5% or 5% in drinking-water resulted in average daily intakes of approximately 85 and 170 mg ethanol in males and 70 and 130 mg in females. At the end of the 4-week treatment period, measurements were made of glutathione, total cytochrome P450 and CYP2E1, cell cycle distribution and apoptosis in the liver, ethyl carbamate and ethanol concentrations in serum, proliferating cell nuclear antigen (PCNA) labelling in the lungs and etheno-DNA adducts in liver and lungs. Low serum concentrations of ethyl carbamate were present at all dose levels of ethyl carbamate without co-administered ethanol, with higher concentrations found in mice treated also with 5% ethanol. The hepatic concentrations of glutathione decreased slightly, whereas CYP2E1 increased in both sexes of mice given 5% ethanol, but these changes were not affected by ethyl carbamate treatment. The percentage of hepatocytes in the G0 phase was decreased, and the percentage in the G1 phase was increased in female but not in male mice treated with ethyl carbamate at 30 or 90 mg/l; these

changes were not affected by co-administration with ethanol. Apoptosis was increased by ethyl carbamate in males but not in females, while ethanol had little or no effect. The percentage of PCNA labelling was decreased in the lungs of both male and female mice exposed to ethyl carbamate at 30 or 90 mg/l, and the effect was independent of the ethanol administration. Based on pooled data for males and females, the concentrations of etheno-dA adducts in hepatic DNA were significantly increased by exposure to ethyl carbamate and decreased by exposure to ethanol, with no apparent interaction between the two treatments.

(b) *Rats*

Ethyl carbamate was administered in 20 ml of drinking-water to groups of 40 male and 40 female 8-week-old Sprague-Dawley rats at doses of 0, 0.1, 0.5, 2.5 or 12.5 mg/kg bw per day for their lifetime (maximum of 670–730 days). On Mondays and Fridays, the concentration was doubled, and on Saturdays and Sundays, tap water only was given *ad libitum*. All animals were autopsied, and organs with macroscopic findings were examined histologically. About 50% of animals from all groups (control group included) died between 350 and 450 days after start of treatment, which was, according to the authors, probably due to a viral infection in the case of most of them. The median survival time was between 410 and 430 days. Mortality due to malignant tumours increased steadily with increasing dose, beginning with 0.5 mg/kg bw per day. In the two higher dose groups, more females than males developed malignant tumours, although females in these groups survived longer than males. Female rats showed increased tumour incidences in the mammary glands at 12.5 mg/kg bw per day (9/38) compared with the controls (0/36). There was no organ-specific increase in tumour incidence in male rats (Schmähl et al., 1977; data reanalysed by Schlatter & Lutz, 1990). It is not completely clear from all available data which animals were excluded from the tumour evaluation, as half of the rats died early in the study but were partly included in the evaluation.

(c) *Hamsters*

A group of 10 male and 10 female Syrian golden hamsters received ethyl carbamate at 2000 mg/l in drinking-water (equivalent to 200 mg/kg bw per day, assuming a drinking-water consumption of 100 ml/kg bw) starting at 8–10 weeks of age, for their lifetime. A group of 14 female and 49 male Syrian golden hamsters untreated for 80 weeks served as the controls. Necropsies were performed on all dead animals. Survival of the treated hamsters was greater in males than in females (the last male died at week 76, the last female at week 55). There was an increased incidence of melanotic tumours in males (7/10; mean latent period of 56 weeks) compared with females (1/10) and control animals (1/49 males, 0/14 females). The authors noted that the sex difference could be due to the significantly shorter life span of the females. There was also an increased incidence of papillomas of the forestomach (6/10 males, 4/10 females, none in control animals). In addition, single incidences of malignant lymphoma and bronchial adenoma in the treated male group and of thyroid adenoma and haemangiosarcoma of the liver in the treated female group were noted (Pietra & Shubik, 1960).

No melanotic tumours were seen when 10 male and 10 female Syrian golden hamsters received 6 drops of 20% ethyl carbamate in acetone (30 mg ethyl carbamate) twice weekly on their skin for their lifetime. Two of 10 females (no males, no control animals) had papillomas of the forestomach (Pietra & Shubik, 1960).

Fifty-two male and 48 female 5-week-old Syrian golden hamsters received ethyl carbamate at 1000 mg/l in drinking-water (equivalent to 100 mg/kg bw per day) for their lifetime. One hundred males and 100 females were left untreated for the control group. The treated and control animals were checked at weekly intervals. Complete necropsies were performed on all animals, and histological evaluation was made of the liver, kidney, spleen, at least four lobes of the lungs and those organs showing gross pathological changes for each hamster. Survival of the treated males and females was similar, with the last male dying prior to 110 weeks and the last female prior to 100 weeks. Mortality was increased and body weight gain decreased in the treated group compared with untreated controls. There was a significant increase in the incidence of dermal melanocytomas (50% of males and 52% of females; 1% and 0% control), papillomas of the forestomach (69% of males and 72% of females; 6% and 2% control), carcinomas of the forestomach (34% of males and 14% of females; 0% control) and adenomatous polyps of the caecum (7% of males and 14% of females; 0% control). Incidences of lung tumours, adrenal cortical tumours and thyroid tumours were also slightly increased (Toth & Boreisha, 1969).

(d) *Primates*

Thirty-two monkeys from a mixed species colony received oral ethyl carbamate doses of 250 mg/kg bw per day in sterile water 5 days a week for 5 years (60 months). This study was part of a larger study initiated in 1961, in which a variety of substances were tested in cynomolgus, rhesus and African green monkeys over a period of 30 years. For most compounds, exposure was initiated shortly after birth, after weaning (approximately 6 months of age) or at 1 year of age (specific time of initiation for ethyl carbamate not indicated). At least five monkeys in the colony received sterile water only as a control group for treatment with ethyl carbamate. Some treated monkeys also received 7–10 weekly courses of whole-body radiation (0.5 Gy per course). During their lifetime, a routine physical examination was conducted every 6 months, and clinical chemistry examinations were performed every 3 or 6 months. Complete necropsies were performed on all animals at their deaths. Six of the 32 animals (19%) had malignant tumours at their deaths. These included adenocarcinomas of lung, pancreas, bile ducts and small intestine, hepatocellular adenoma and carcinoma, haemangiosarcoma of the liver, ependymoma, pheochromocytoma, endocervical adenofibroma and squamous papilloma of the pouch. The average cumulative dose for the tumour-bearing monkeys was 235 g (215–339 g), and the average latent period was 186 months (145–267 months). The autopsy records of 373 breeders and control animals showed incidences of malignant tumours of 1.5% for cynomolgus, 2.8% for rhesus and 8% for African green monkeys. No information was given about the relative species composition of monkey(s) used in the study with ethyl carbamate. Only two of six monkeys with malignant tumours had been irradiated, indicating

that ethyl carbamate is carcinogenic regardless of irradiation (Thorgeirsson et al., 1994).

2.2.4 Genotoxicity

The results of genotoxicity assays with ethyl carbamate are summarized in Table 4.

(a) *Interaction with ethanol*

B6C3F1 mice were administered ethyl carbamate at 111–3300 mg/l in drinking-water with and without 5% ethanol *ad libitum* for 90 days. A micronucleus test with peripheral blood was positive in both cases, regardless of whether or not ethanol was added to the drinking-water (NTP, 1996).

When ethyl carbamate at 1000 mg/kg bw was administered intraperitoneally with or without ethanol at 2500 mg/kg bw to CD-1 mice, micronucleus tests with bone marrow or peripheral blood were positive. Ethanol induced an increase in micronucleated polychromatic erythrocyte (PCE) frequency in bone marrow from 0.19% in control to 8.63% at 24 h and to 6.98% at 48 h. When ethyl carbamate was co-administered with ethanol, the micronucleated PCE frequency was suppressed to 0.49% at 24 h, but increased to 7.35% at 48 h. In the peripheral blood micronucleus assay, the peak micronucleus PCE frequency was 11.6% at 52 h without and 11.2% at 64 h with ethanol. Thus, ethanol delays the occurrence, but not the magnitude, of ethyl carbamate genotoxicity (Choy et al., 1996).

(b) *In vivo germ cell genotoxicity: Induction of tumours and malformations in the offspring of mice treated with ethyl carbamate before mating*

A number of studies have been conducted to investigate whether treatment of males or females with single or repeated doses of ethyl carbamate prior to mating influences the incidence of tumours and malformations in their offspring.

Male NIH Swiss mice 6 weeks of age were given a single intraperitoneal injection of ethyl carbamate at a dose of 1500 mg/kg bw 2 weeks before mating (i.e. post-meiotic spermatozoa were exposed), and each was placed with five untreated females for mating. Pregnant females were allowed to litter out, and the offspring were kept until natural death or killed when moribund. There was no effect of treatment on fertility, fecundity or offspring survival to weaning. Forty-three and 44 litters were produced from 12 and 11 fathers, and 35 and 36 litters were weaned from treated and control fathers, respectively. Twelve litters from each group were killed at 6 weeks of age for biochemical measurements, and males from several litters of each group were killed at 12 or 15 months for study of lung tumours. This left 78 and 71 female offspring and 54 and 48 male offspring from 20 and 14 litters of treated and control males, respectively, for the lifetime study. Offspring from treated fathers showed small, but significant, increases in the incidence of tumours of the adrenal gland and glandular stomach (males only). Tumours of the thyroid gland, Harderian gland, lymphoid tissues (females only),

Table 4. Results of genotoxicity assays with ethyl carbamate

Test system	Test object	Concentration	Results	Reference
<i>In vitro</i>				
Bacteria				
Bacterial mutation assay	<i>Salmonella typhimurium</i> TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, TA1538	≤10 mg/plate	Negative ^a except positive for TA1535 with hamster liver S9	Zeiger et al. (1992); NTP (1996)
Bacterial mutation assay	<i>S. typhimurium</i> TA98	≤0.5 mg/plate	Negative ^a	Bruce & Heddle (1979)
Bacterial mutation assay	<i>S. typhimurium</i> TA1538, TA98, TA100	0.01–10 mg/plate	Positive ^b	Richold & Jones (1981)
Bacterial mutation assay	<i>S. typhimurium</i> TA1535, TA1537	0.01–10 mg/plate	Negative ^a	Richold & Jones (1981)
Bacterial mutation assay	<i>S. typhimurium</i> TA98, TA100; <i>Escherichia coli</i> WP2 uvrA	0.0005–0.5 mg/plate	Positive ^b	Venitt & Crofton-Sleigh (1981)
Bacterial mutation assay	<i>S. typhimurium</i> TA98, TA100, TA102	5–25 mg/plate	Negative ^a except positive for TA100 with S9	Hübner et al. (1997)
Bacterial mutation assay	<i>S. typhimurium</i> TA1535, TA100, TA98	0.05–0.4 mg/plate	Negative ^a	Dahl et al. (1978)
Forward mutations to 8-azaguanine resistance	<i>S. typhimurium</i> TM677	≤1 mg/ml	Positive ^b	Skopek et al. (1981)

Table 4. (contd)

Test system	Test object	Concentration	Results	Reference
Yeast				
Mitotic gene conversion	<i>Saccharomyces cerevisiae</i> strain D4	0.0003–0.333 mg/plate	Negative ^a	Jagannath et al. (1981)
Mitotic gene conversion	<i>S. cerevisiae</i> strain D7, trp5 locus	4.8 mg/ml	Positive ^b Negative ^c	Zimmermann & Scheel (1981)
Mitotic gene conversion	<i>S. cerevisiae</i> strain JD1, trp5 and his4 locus	0.15–0.3 mg/ml	Positive ^a	Sharp & Parry (1981)
Mitotic recombination	<i>S. cerevisiae</i> strain YB110	25–100 mg/ml	Positive ^c	Hübner et al. (1997)
Mitotic chromosome loss	<i>S. cerevisiae</i> strain D61.M	12.4–25 mg/ml	Negative ^c Positive in combination with propionitrile only	Zimmermann & Mohr (1992)
Mammalian cells				
Forward mutations	L5178Y mouse lymphoma cells, TK locus	0.047–3 mg/ml	Negative ^a	Jotz & Mitchell (1981)
Forward mutations	L5178Y mouse lymphoma cells, TK locus	1.2–16 mg/ml	Negative ^b (less than 50% cell survival for 12 mg/ml or more)	Amacher & Turner (1982)
Forward mutations	Human lymphoblastoid cell line TK6, TK and HGPRT locus	1–9 mg/ml	Negative ^a	Hübner et al. (1997)
Gene mutations (6-thioguanine)	Chinese hamster V-79 cells	5–20 mg/ml	Negative ^a	Allen et al. (1982b)

Table 4. (contd)

Test system	Test object	Concentration	Results	Reference
Sister chromatid exchange	Mouse bone marrow cell culture	≤5 mg/ml	Negative ^c	Roberts & Allen (1980)
Sister chromatid exchange	Rat cell line originated from ascites hepatoma AH66-B	0.009–0.09 mg/ml	Positive ^c	Abe & Sasaki (1982)
Sister chromatid exchange	Rat cell line originated from oesophageal tumour R1	0.009–0.09 mg/ml	Negative ^c	Abe & Sasaki (1982)
Sister chromatid exchange	Chinese hamster cells (Don)	0.9–7.2 mg/ml	Positive ^c	Abe & Sasaki (1977)
Sister chromatid exchange	Chinese hamster V-79 cells	5–20 mg/ml	Negative ^a	Allen et al. (1982a, 1982b)
Sister chromatid exchange	Chinese hamster ovary cells	≤5 mg/ml	Equivocal ^c (1 trial negative, 1 positive) Positive ^b	Galloway et al. (1987)
Sister chromatid exchange	Chinese hamster ovary cells	0.063–1 mg/ml	Negative ^a	Evans & Mitchell (1981)
Sister chromatid exchange	Chinese hamster ovary cells	0.001–0.1 mg/ml	Negative ^a	Perry & Thomson (1981)
Sister chromatid exchange	Human peripheral blood lymphocytes	0.18–0.71 mg/ml	Negative ^c	Rencüzogullari & Topaktas (1998)
Sister chromatid exchange	Human lymphocytes	0.09–0.9 mg/ml	Positive ^c	Csukas et al. (1979)
Sister chromatid exchange	Human peripheral blood lymphocytes	0.9 mg/ml	Positive ^c	Csukas et al. (1981)

Table 4. (contd)

Test system	Test object	Concentration	Results	Reference
Sister chromatid exchange	Human blood lymphocytes	0.9 mg/ml	Positive ^c Negative ^b	Solymosy (1981)
Sister chromatid exchange	Human Bloom syndrome B-lymphoblastoid cell lines	0.032 mg/ml	Positive ^a	Shiraishi (1986)
Unscheduled DNA synthesis	Human fibroblasts	0.02 mg/ml	Positive ^b	Agrelo & Severin (1981)
Chromosomal aberrations	ICR mouse whole embryo culture	0.001–8.9 mg/ml for 12–24 h (during 9.5–10.5 days or 11.75–12.25 days of embryonic age)	Positive ^c (chromosome breaks in both embryonic and yolk sac cells at concentrations lower than the threshold value for retardation of embryonic growth: in lung and liver cells at 0.09 mg/ml, in heart and intestinal cells at 0.89–8.9 mg/ml)	Itoh & Matsumoto (1984)
Chromosomal aberrations	Chinese hamster ovary cells	1.6–5 mg/ml	Negative ^a	Galloway et al. (1987)
Chromosomal aberrations	Human peripheral lymphocytes	0.18.2–0.71 mg/ml	Positive ^c	Rencüzogullari & Topaktas (2000)
<i>In vivo</i>				
<i>Drosophila</i>				
Sex-linked recessive lethal mutations	<i>Drosophila melanogaster</i> male germ cells	12 000 mg/kg in feed	Positive	Foureman et al. (1994)

Table 4. (contd)

Test system	Test object	Concentration	Results	Reference
Sex-linked recessive lethal mutations	<i>D. melanogaster</i> male germ cells	Ethyl carbamate gas	Positive	Nomura (1979)
Sex-linked recessive lethal mutations	<i>D. melanogaster</i> male germ cells	Injection	Positive	Vogt (1948)
Reciprocal translocations	<i>D. melanogaster</i> male germ cells	11 000 mg/kg in feed	Positive	Foureman et al. (1994)
Reciprocal translocations	<i>D. melanogaster</i>	Ethyl carbamate gas	Negative	Nomura (1979)
Somatic mutations and recombination (wing spots)	<i>D. melanogaster</i>	0.5–2.7 mg/ml	Positive	Froelich & Wuergler (1990)
Somatic mutations and recombination (wing spots)	<i>D. melanogaster</i>	0.9–1.8 mg/ml	Positive	Graf & van Schaik (1992); Graf et al. (1998)
Somatic mutations and recombination (wing spots)	<i>D. melanogaster</i>	0.9–1.8 mg/ml	Positive	Abraham & Graf (1996)
Mammals				
Micronucleus test mouse	Female C57BL/6N mouse peripheral blood	0, 5000, 10 000 or 15 000 mg/l (equivalent to 0, 750, 1500 and 2250 mg/kg bw per day) in drinking-water for 12 weeks	Positive	Director et al. (1998)

Table 4. (contd)

Test system	Test object	Concentration	Results	Reference
Micronucleus test mouse	B6C3F1 mouse peripheral blood and bone marrow	750–2000 mg/l in drinking-water (equivalent to 113–300 mg/kg bw per day) for 45 and 90 days	Positive	MacGregor et al. (1990); Witt et al. (2000)
Micronucleus test mouse	C57BL/6 × C3H/He mouse bone marrow	200–5000 mg/kg bw per day intraperitoneally on 5 consecutive days	Positive	Bruce & Heddle (1979)
Micronucleus test mouse	B6C3F1 mouse bone marrow	20–60% of LD ₅₀ , intra-peritoneally (LD ₅₀ not stated)	Positive	Salamone et al. (1981)
Micronucleus test mouse	CBA mouse bone marrow	900 mg/kg bw intraperitoneally or orally by gavage (single dose)	Positive	Ashby et al. (1990)
Micronucleus test mouse	CD-1 mouse bone marrow	300–1200 mg/kg bw per day for 3 days		Holmstrom (1990)
		- intraperitoneally	Positive (females more susceptible than males)	
		- orally	Negative	
Micronucleus test mouse	CRH and CD-1 mouse bone marrow	600–1250 mg/kg bw per day orally for 1–3 days	Positive	Westmoreland et al. (1991)
Micronucleus test mouse	BDF1 mouse bone marrow	500–1000 mg/kg bw intra-peritoneally (single dose)	Positive (young and male animals more susceptible)	Balansky et al. (1992)
Micronucleus test mouse	BALB/c mouse bone marrow	0, 0.5, 1 or 1.5 mg/kg bw subcutaneously (single dose)	Positive	Aldovini & Ronchese (1983)

Table 4. (contd)

Test system	Test object	Concentration	Results	Reference
Micronucleus test mouse	BALB/c mouse peripheral blood	3000 mg/l in drinking-water for 3 weeks (equivalent to 450 mg/kg bw per day)	Positive	Balansky et al. (1992)
Micronucleus test mouse	Fetal BDF1 mouse liver and peripheral blood	1000 mg/kg bw intraperitoneally on day 17 of gestation of pregnant females	Positive	Balansky et al. (1992)
Micronucleus test mouse	NMRI mouse bone marrow	180 or 356 mg/kg bw per day intraperitoneally for 2 days	Positive	Wild (1978)
Micronucleus test mouse	CD-1 mouse bone marrow	200–800 mg/kg bw per day intraperitoneally for 2 days	Positive	Tsushima & Matter (1981)
Micronucleus test mouse	Male CD-1 and BDF1 mouse bone marrow and peripheral blood	62–1000 mg/kg bw intraperitoneally (single dose)	Positive	Kishi et al. (1992)
Micronucleus test mouse	Mouse bone marrow (strain and sex not stated)	25–1000 mg/kg bw intraperitoneally (single dose)	Positive	Adler et al. (1996)
Micronucleus test mouse	Female Swiss albino mouse adult bone marrow and fetal liver	990 mg/kg bw intraperitoneally (= 0.75 LD ₅₀) (single dose, administered on gestation days 17–19)	Positive	Sanderson & Clark (1993)
Micronucleus test mouse	HRA/Skh hairless mouse skin	2–2000 mg/kg bw dermally	Positive	He & Baker (1991)
Micronucleus test mouse	CD-1 mouse peripheral blood	750–1000 mg/kg bw intraperitoneally (single dose)	Positive 48 h after dosing (24 h and 72 h negative)	Hynes et al. (2002)
Micronucleus test mouse	B6C3F1 mouse peripheral blood	500 mg/kg bw intraperitoneally (single dose)	Positive 48 and 72 h after dosing	Kim et al. (1999)

Table 4. (contd)

Test system	Test object	Concentration	Results	Reference
Micronucleus test mouse	lacZ ⁻ transgenic mouse	900 mg/kg bw intraperitoneally (single dose)	Positive	Williams et al. (1998)
Micronucleus test mouse, + ethanol	B6C3F1 mouse peripheral blood	110–3300 mg/l in drinking-water for 90 days with and without 5% ethanol (equivalent to 17–500 mg/kg bw per day)	Positive (same result with as without ethanol)	NTP (1996)
Micronucleus test mouse, + ethanol	CD-1 mouse bone marrow and peripheral blood	1000 mg/kg bw intraperitoneally with and without ethanol at 2500 mg/kg bw intraperitoneally (single dose)	Positive (ethanol delayed ethyl carbamate genotoxicity)	Choy et al. (1996)
Micronucleus test mouse, + ethanol	CD-1 mouse bone marrow	1000 mg/kg bw intraperitoneally with and without ethanol at 625–3500 mg/kg bw intraperitoneally (single dose)	Positive (ethanol inhibited ethyl carbamate genotoxicity 24 h post-treatment)	Choy et al. (1995)
Micronucleus test rat	PVG rat bone marrow	600–1250 mg/kg bw orally (single dose)	Positive	Westmoreland et al. (1991)
Micronucleus test rat	Wistar Han rat peripheral blood	750–1000 mg/kg bw intraperitoneally (single dose)	Positive 48 and 72 h after dosing (24 h negative)	Hynes et al. (2002)
Micronucleus test rat	Splenectomized male Fischer 344 rat peripheral blood and bone marrow	300 mg/kg bw per day, 5 intraperitoneal injections per week for 10 weeks	Positive	Schlegel & MacGregor (1984)
Micronucleus test rat	Male Sprague-Dawley rat bone marrow	1000–2500 mg/kg bw intraperitoneally (single dose)	Negative	Trzos et al. (1978)

Table 4. (contd)

Test system	Test object	Concentration	Results	Reference
Sister chromatid exchange	Male AKR, BALB/c, C3Hf, C57BL/6J and DBA/2 mouse bone marrow	150 or 300 mg/kg bw intra-peritoneally (single dose)	Positive	Dragani et al. (1983)
Sister chromatid exchange	A/J, C3HeB/FeJ and C57BL/6J mouse lung cells	300 or 1000 mg/kg bw intra-peritoneally (single dose)	Positive	Allen et al. (1986)
Sister chromatid exchange	Strain A and C57BL mouse bone marrow	400 mg/kg bw intraperitoneally (single dose)	Positive	Allen et al. (1982a, 1982b)
Sister chromatid exchange	CBA/J mouse bone marrow and liver cells	50–400 mg/kg bw intraperitoneally (single dose)	Positive	Roberts & Allen (1980)
Sister chromatid exchange	CBA/J mouse spermatogonial cells	50–200 mg/kg bw intraperitoneally (single dose)	Negative	Roberts & Allen (1980)
		400–1000 mg/kg bw intra-peritoneally (single dose)	Positive	
Sister chromatid exchange	Mouse bone marrow and alveolar macrophages	293.7 mg/kg bw intraperitoneally (single dose)	Positive (no evidence of repair)	Conner & Cheng (1983)
Sister chromatid exchange	BD2F1 mouse bone marrow, alveolar macrophages, peripheral blood lymphocytes	293.7 mg/kg bw intraperitoneally (single dose and 10 times every other day)	Positive	Goon & Conner (1984)
Sister chromatid exchange	BALB/c and BALB/Mo mouse lymphocytes	1000 mg/kg bw intraperitoneally (single dose)	Positive	Majone et al. (1983)
Sister chromatid exchange	C57BL/6J and DBA/2 mouse bone marrow	300 mg/kg bw intraperitoneally (single dose)	Positive	Sozzi et al. (1985)
Sister chromatid exchange	Swiss Webster mouse maternal bone marrow and fetal liver	89–294 mg/kg bw intraperitoneally on days 13–17 of gestation (single dose)	Positive (depending on gestation day)	Neeper-Bradley & Conner (1989)

Table 4. (contd)

Test system	Test object	Concentration	Results	Reference
Sister chromatid exchange	Adenoma-susceptible ICR/JCL and -resistant C57BL/6J and DBA/2J mouse maternal bone marrow and fetal liver of their respective ICR/JCL, BDF1 and DBF1 fetuses	89–294 mg/kg bw intraperitoneally on day 13 or 14 of gestation (single dose)	Positive	Neeper-Bradley & Conner (1990)
Sister chromatid exchange	Swiss Webster, ICR/JCL and C57BL/6J mouse bone marrow (non-gravid females)	89–294 mg/kg bw intraperitoneally (single dose)	Positive (no significant strain differences)	Neeper-Bradley & Conner (1992)
Sister chromatid exchange	Strain A and C57BL/6 mouse, Chinese hamster, Syrian golden hamster and rat (strain not further specified) bone marrow	400 mg/kg bw intraperitoneally (single dose)	Positive	Sharief et al. (1984)
Sister chromatid exchange	ddY and C57BL/6 mouse lymphocytes	900 mg/kg bw intraperitoneally (single dose)	Positive	Endo & Watanabe (1988)
Sister chromatid exchange	BD2F1 mouse lymphocytes	294 mg/kg bw per day intraperitoneally for 1–6 days and 12 intraperitoneal injections of 196 mg/kg bw per day (3 times weekly)	Positive	Neft et al. (1985)
Sister chromatid exchange	Male BDF1 mouse alveolar macrophages, bone marrow and regenerating liver cells	12 intraperitoneal injections of 196 mg/kg bw per day, 3 times weekly	Positive	Conner (1986)

Table 4. (contd)

Test system	Test object	Concentration	Results	Reference
Sister chromatid exchange	Male BDF1 mouse (hepatectomized and intact, 2- and 4-month-old) regenerating liver cells, alveolar macrophages, bone marrow	0.1 mg/l inhalative for 4 h or 193 mg/kg bw intravenously or intraperitoneally (same dose as inhalative)	Positive (response lower in bone marrow than in regenerating liver and alveolar macrophages)	Cheng et al. (1981a)
Sister chromatid exchange	C57BL/6J × DBA/2J F1 (= BD2F1) mouse (hepatectomized and intact) alveolar macrophages, bone marrow and regenerating liver cells	12 (3 times weekly) serial intraperitoneal injections of 196 mg/kg bw per day or single intraperitoneal injection of 0, 0.05, 0.1, 0.2 or 0.4 mg/kg bw	Positive (no evidence of cytotoxicity; significantly higher sister chromatid exchange levels in regenerating liver cells and macrophages than in bone marrow)	Cheng et al. (1981b)
Sister chromatid exchange	Male Swiss albino mouse bone marrow and skin cells	300–4000 mg/kg bw topical (single dose)	Positive	Barale et al. (1992)
Sister chromatid exchange	Chinese hamster and golden hamster V-79 cells	400 mg/kg bw intraperitoneally (single dose)	Positive	Allen et al. (1982a)
Chromosomal aberrations	Male Carworth CF-1 mouse bone marrow	1000 mg/kg bw per day intraperitoneally for 1 or 2 days (single dose)	Positive (acentric fragments, chromatid breaks and chromatid gaps)	Dean (1969)
Chromosomal aberrations	Male Long-Evans rat bone marrow	800 mg/kg bw intravenously (single dose)	Positive (maximum 20% of cells with chromosomal aberrations, but lack of negative control)	Sugiyama et al. (1981)

Table 4. (contd)

Test system	Test object	Concentration	Results	Reference
Chromosomal aberrations	H-2 congenic strains of male mice with B10 and A backgrounds bone marrow and spleen cells	500 or 1000 mg/kg bw sub-cutaneously (single dose)	Positive	Miyashita et al. (1987)
Chromosomal aberrations	Male albino mouse bone marrow and skin cells	1000 mg/kg bw intra-peritoneally (single dose)	Positive	Barale et al. (1992)
Chromosomal aberrations	Male albino mouse bone marrow and skin cells	300–8000 mg/kg bw topical (single dose)	Positive	Barale et al. (1992)
Chromosomal aberrations	Female C57BL/6N mouse bone marrow	0, 5000 mg/l in drinking-water for 12 weeks; or 10 000 or 15 000 mg/l in drinking-water for 3 weeks followed by 9 weeks tap water (equivalent to 750, 1500 or 2250 mg/kg bw per day)	Positive (acentric chromosome fragments)	Director et al. (1998)
Mutations	lacZ ⁻ transgenic mouse lung, spleen and liver	900 mg/kg bw intraperitoneally (single dose)	Positive	Williams et al. (1998)
Unscheduled DNA synthesis	Male B6C3F1 mouse early spermatid stages	50–750 mg/kg bw intra-peritoneally (single dose)	Positive (no clear dose–response relationship)	Sotomayor et al. (1994)
DNA binding	Male B6C3F1 mouse	10–1000 mg/kg bw intra-peritoneally (single dose)	Positive in liver, testes and sperm heads	Sotomayor et al. (1994)
Modified dominant lethal assay	ICR/Ha Swiss mouse	1000–1200 mg/kg bw intra-peritoneally (single dose)	Negative	Epstein et al. (1972)
Dominant lethal assay	Male CBA and C3HeB/Fe mouse	1500 mg/kg bw intra-peritoneally (single dose)	Negative	Tutikawa (1968)

Table 4. (contd)

Test system	Test object	Concentration	Results	Reference
Dominant lethal assay	Male mouse (strain not specified)	1500 mg/kg bw per day intraperitoneally for 1 or 3 successive days	Negative	Bateman (1967)
Dominant lethal assay	CD-1 mouse	Males received 1250 or 1750 mg/kg bw intraperitoneally (single dose) and were mated to untreated females 1 week after treatment	Negative	Edwards et al. (1999)
Dominant lethal assay	CD-1 mouse	Males received 1250 or 3750 mg/l in drinking-water for 10 and 9 weeks, respectively (equal to 190 or 370 mg/kg bw per day), and were mated with untreated females (investigation on pregnancy day 17)	Negative	Edwards et al. (1999)
Specific locus test (germ cell mutations)	Male (101 × C3H)F1 mouse (32 800 offspring were observed)	1750 mg/kg bw intraperitoneally (single dose)	Negative	Russell et al. (1987)
Spermatid micronucleus test	Male rat (strain not specified)	500 mg/kg bw intraperitoneally (single dose)	Negative	J. Lähdetie, unpublished, cited in Adler et al. (1996)
Inhibition of testicular DNA synthesis	Male mouse (strain not specified)	500 mg/kg bw intraperitoneally (single dose)	Positive	Seiler (1977)

^a In the presence and absence of metabolic activation.

^b In the presence of metabolic activation only.

^c In the absence of metabolic activation only.

lung (females) and liver (males) were increased in offspring of treated animals, but not significantly (Yu et al., 1999).

Nine-week-old male ICR/JCL mice received a single subcutaneous ethyl carbamate dose of 1500 mg/kg bw and were mated with untreated females at weekly intervals for 10 weeks after treatment. A total of 168 of 177 females became pregnant, and 81 of them were sacrificed on day 19 for examination of corpora lutea, total implants, pre-implantation losses, embryonic deaths, living fetuses and external malformations. A dominant lethal effect was evident only in matings in week 3 after treatment of the males (i.e. treatment corresponding to the spermatid stage), in which pre-implantation losses plus early deaths/corpora lutea were significantly increased (19.7%, 24/122), compared with controls (7.5%, 48/641) or other mating intervals (10.0%, 93/931). Malformations were said to be significantly increased in offspring of treated males (2.0%, 18/897), but the rate for controls was not stated (Nomura, 1975a).

Female ICR/JCL mice received a single subcutaneous ethyl carbamate dose of 1500 mg/kg bw and were mated with untreated males at weekly intervals for 10 weeks after treatment, producing 177 pregnancies from 199 matings. The average number of corpora lutea (12.3) was significantly lower than in controls (13.3). Pre-implantation losses plus early deaths/corpora lutea were significantly increased in the matings at 2- to 3-week intervals after treatment (14.4%, 39/270), compared with controls or other time intervals (10.4%, 77/741). Malformations (open eyelid, kinky tail, cleft palate, dwarfism) in the offspring from treated females were also increased (3.2%, 27/856) compared with controls (0.7%, 4/551) (Nomura, 1975a).

In a separate experiment, male mice were treated with a single subcutaneous ethyl carbamate dose of 1500 mg/kg bw and females were treated with a single subcutaneous ethyl carbamate dose of 1000 or 1500 mg/kg bw before mating to untreated females and males, respectively. The resulting offspring were followed until 32 weeks of age. The offspring from treated males and from treated females had significantly increased incidences of tumours (12% and 16%, respectively) compared with the offspring from untreated controls (7%). The majority of tumours in both treated and control groups were lung tumours (Nomura, 1975a).

A further extensive series of experiments conducted by Nomura between 1967 and 1981 on parental exposure to ethyl carbamate before mating and the incidence of tumours and malformations in their offspring has been summarized in one publication (Nomura, 1982). Mice were given a single subcutaneous injection of ethyl carbamate at a dose of 1500 or 2000 mg/kg bw (males) or 1000 or 1500 mg/kg bw (females) from 1 to 80 days before mating, to cover ovulation and the whole of the spermatogenic cycle. Treated animals were then mated with untreated animals. Untreated males were mated with untreated females as controls. Some of the pregnant females were killed on day 19 of gestation to examine the fetuses, and others were allowed to litter out and the offspring examined at 7 days of age for malformations or at 8 months of age for tumours. Malformation rates in fetuses were significantly increased by pre-mating treatment of either the male or female parent. Malformations were present in 2.1% (10/477) and 2.2% (65/2923) of fetuses from males given 2000 or 1500 mg/kg bw, respectively, and

in 4.1% (52/1262) of fetuses from females given 1500 mg/kg bw, compared with 0.4% (4/1026) of fetuses from untreated controls. In offspring examined at 7 days of age, malformation rates were also significantly increased in offspring of treated females; the incidence was 1.0% (16/1637, non-significant), 2.0% (22/1087) and 1.4% (12/885) in 1500 mg/kg bw males, 1500 mg/kg bw females and 1000 mg/kg bw females, respectively, compared with 0.1% (1/809) in untreated controls. Malformation rates were lower than those observed in the fetuses, presumably because some malformations were lethal. The malformations included cleft palate, kinked and/or short tail, open eyelid, exencephaly, hydrocephaly, gastroschisis, polydactyly, syndactyly, dwarfism and abnormal lobulation of the lung and liver. The incidence of tumours in the offspring at 8 months of age was significantly increased to 10.9% (136/1254), 14.4% (139/963) and 14.9% (115/772) in offspring of 1500 mg/kg bw males, 1500 mg/kg bw females and 1000 mg/kg bw females, respectively, compared with 5.3% (29/548) in untreated controls. The majority of tumours in both treated and control groups were in the lung (Nomura, 1982).

A later collation of studies using the same doses of ethyl carbamate given pre-mating, including many of the data published in 1982 and adding further data, confirmed the finding of significant increases in malformation rates in offspring of treated males mated to untreated females and treated females mated to untreated males. Malformation rates in the offspring of males ranged up to 4%, depending on the timing of treatment in relation to spermatogenesis, with more abnormalities arising from ethyl carbamate exposure of spermatozoa than from exposure of spermatogonia. Malformation rates in the offspring of females ranged up to 10%, depending only on dose and not on the timing in relation to ovulation. Heritability of some of the ethyl carbamate-induced abnormalities was confirmed by mating of surviving F1 offspring with untreated mice to produce further generations, resulting in 9.9% of F3-generation offspring having tail anomalies, open eyelid or dwarfism. Crossing of male and female offspring with open eyelid with normal ICR mice yielded 42–100% expressivity in F2 to F12 generations (Nomura, 1988).

It is unclear from many of the publications of Nomura whether the statistical significance of tumour incidence in the offspring was based on comparisons with concurrent controls or on compilations of historical controls from several studies. In this regard, Cattanaach et al. (1998) commented that their failure to replicate the work of Nomura on tumorigenic effects in offspring following male parental X-ray treatment coupled with postnatal exposure to ethyl carbamate could be due to use of historical rather than concurrent controls in the Nomura studies. Cattanaach et al. (1998) found that there were significant variations in tumour incidence among replicates, showing that concurrent controls are essential for this type of work. It should also be noted that in the studies described above, information on tumour incidence is given in offspring according to treatment group, but no analysis is provided of the distribution of tumours among litters. Other authors have commented that retention of litter information in analysis of multigeneration carcinogenesis experiments is particularly important when the outcome of interest is the incorporation of a mutation into the germ line (Turusov & Cardis, 1989).

In a study to investigate malformations and tumours in offspring of treated males, male CD-1 mice were given a single intraperitoneal injection of ethyl

carbamate at 1250 or 1750 mg/kg bw or were given ethyl carbamate in the drinking-water at 1250 or 3750 mg/l for 9 or 10 weeks. Controls were given tap water intraperitoneally or as drinking-water. Consumption of drinking-water containing ethyl carbamate was reduced by around 20% at the low dose and 50% at the high dose. The mean calculated intakes of ethyl carbamate from drinking-water were 190 and 370 mg/kg bw per day. One week after the end of each of the treatments, the surviving mice were paired for mating with two untreated females for 1 week. Half the pregnant females were killed on day 17 of gestation for examination of the contents of the uterus, and the other half were allowed to deliver and rear litters. About 100 male and female offspring from each of the high-dose and control groups were retained for 18 months (acute intraperitoneal study) or 12 months (subacute drinking-water study) to be examined for tumour development. Just over half of the males given the higher doses intraperitoneally or via drinking-water died before mating; the only other death was one male given 1250 mg/kg bw intraperitoneally. The high-dose treatments also reduced the mean body weight of surviving males. At the higher and lower intraperitoneal doses, fewer females were mated (41/54 and 39/48, respectively, compared with 49/50 in controls, $P < 0.001$), and there were fewer pregnancies (36/41 compared with 49/49, $P < 0.001$). These parameters were unaffected in the drinking-water study, but mean time to coition and gestation period were slightly, but significantly, increased at 3750 mg/l. There was no evidence of a dominant lethal effect, and there was no increase in abnormal karyotypes or malformations in any group. The mean number of post-implantation deaths was slightly, but significantly, increased in the 1750 mg/kg bw intraperitoneal group; consequently, the mean litter size at birth was significantly decreased. There were no effects on litter size at weaning or on litter weight at birth or weaning. The ratio of male to female offspring was unaffected. In the offspring examined when adult, tumours were found in treated and control groups in liver, kidney, limb, lung, pancreas, thymus, urogenital system, ovaries and uterus. Liver was the most common site affected in males, and the onset of liver tumours was significantly earlier and the incidence significantly higher in the 1750 mg/kg bw intraperitoneal group compared with controls (Edwards et al., 1999). Thus, this study failed to confirm the findings of Nomura (1982, 1988) in a different strain of mouse with respect to malformations in offspring of intraperitoneally treated males, but did indicate a similar, although smaller, effect of intraperitoneal treatment on the rate for the tumour with the highest spontaneous occurrence in the mouse strain used (liver).

2.2.6 Reproductive toxicity

(a) Fertility and multigeneration reproductive toxicity

(i) Mice

Oral route

Ethyl carbamate was used as a positive control substance in a multi-generation study with DDT. Groups of 60 male and 60 female 6- to 7-week-old CF-1 mice (P generation) were given ethyl carbamate at a concentration of 0.01% (100 mg/l) in the drinking-water *ad libitum* for their lifetime (equivalent to about 0.5

mg per mouse, or 15 mg/kg bw, assuming a mouse drinks 5 ml water per day and weighs 35 g). Twenty-three of the P females were mated (presumably to treated males, but not stated) at 9–10 weeks of age to obtain the F1 generation, which was also given the same concentration of ethyl carbamate in the drinking-water. All produced litters, and there was no effect of ethyl carbamate on litter size or preweaning mortality. In the P and F1 generations, adult mortality was higher after 90 weeks compared with controls. Survivors of the P and F1 generations were kept until 140 or 130 weeks of age, respectively, and major organs showing abnormalities and the lungs were examined microscopically. The numbers of tumour-bearing animals were higher in animals exposed to ethyl carbamate (P generation, ethyl carbamate, 96% and 93%, compared with controls, 70% and 76%, for males and females, respectively; F1 generation, ethyl carbamate, 97% and 97%, compared with controls, 62% and 85%, for males and females, respectively). This was attributable to an increased incidence and earlier onset of lung tumours (P generation, ethyl carbamate, 83% and 70%, compared with controls, 42% and 23%, for males and females, respectively; F1 generation, ethyl carbamate, 92% and 82%, compared with controls, 31% and 40%, for males and females, respectively). No other types of tumour were affected by ethyl carbamate (Tomatis et al., 1972).

Intraperitoneal route

Ethyl carbamate doses of 1000, 2500 or 5000 mg/kg bw were administered intraperitoneally to male C57BL/6 × C3H/He mice on 5 consecutive days. No sperm abnormalities were seen microscopically (Bruce & Heddle, 1979).

Male (101 × C3H)F1 mice were exposed to the maximum tolerated intraperitoneal ethyl carbamate dose of 1750 mg/kg bw and mated at weekly intervals to two females. In total, 32 828 offspring from successive weekly matings were observed. Litter sizes gave no indication of a dominant lethal effect. There was a slight effect on the very earliest stage of sperm development (reduction of Type A₁ and Type A_S spermatogonia in seminiferous tubule cross-sections, but not of Types A₂–A₄, intermediate, Type B spermatogonia or preleptotene spermatocytes) and a borderline decrease in the number of litters conceived during the 8th and 9th post-treatment weeks (Russell et al., 1987).

(b) Developmental toxicity

It should be noted that the majority of experiments conducted on the developmental toxicity of ethyl carbamate in rodents have used maximum tolerated doses or doses close to the LD₅₀ (around 1500–1750 mg/kg bw in mice) or doses causing anaesthesia/narcosis (1000 mg/kg bw in rats), with little or no description of maternal toxicity. In most of the studies, the malformation rates have been stated on a per fetus basis, rather than a per litter basis. This is widely regarded as an unacceptable method for statistical comparisons, since the dam is considered to be the treatment unit. The types of malformation induced by ethyl carbamate largely reflect the vulnerability of the tissues and organs at the time of development at which the ethyl carbamate is given.

(i) *Mice*

Oral route

In a study described only in an abstract, single oral ethyl carbamate doses of between 300 and 1000 mg/kg bw were administered to pregnant NMRI mice on day 11 of pregnancy, and animals were killed on day 18 of gestation. The predominant abnormalities in fetuses were lateral-ventral fusion of thoracic and lumbar vertebrae. The authors calculated the effective doses on a per fetus basis using probit analysis: ED₁₀, ED₅₀ and ED₉₀ for all anomalies were 420, 860 and 1768 mg/kg bw, respectively; the respective values for ventral-lateral fusion of vertebrae were 621, 941 and 1426 mg/kg bw, respectively (Platzek et al., 1992).

Intraperitoneal route

An early study found reduced fetal weight, short tail, polydactyly, syndactyly, brachydactyly and palatal defects following intraperitoneal injection of ethyl carbamate at 1500 mg/kg bw to mice on days 9–12 of gestation. Injection of the same dose at earlier times between days 3 and 8 of gestation caused high incidences of resorptions but no malformations (Nishimura & Kuginuki, 1958).

Strain differences in malformation rates were shown in a study on colony-bred hybrids and H, dd and C3H mice given a single intraperitoneal injection of ethyl carbamate at 1200 mg/kg bw on day 9 of gestation. At term, 95% of the hybrid strain fetuses, 78% of the dd strain, 76% of the H strain and 69% of the C3H strain were malformed (polydactyly, macrodactyly, brachydactyly, syndactyly, clubfoot, cleft palate, open eye, tail and brain defects) (Kageyama, 1961).

Groups of 3–10 A/He mice were treated orally or by intraperitoneal injection of ethyl carbamate at 0.5, 5 or 50 mg/kg bw every other day starting on day 1 of pregnancy. Treatment was repeated through five pregnancies, each separated by intervals of 7 weeks. Progeny of the first pregnancy were reared and bred to untreated males for three successive generations with 10-week intervals between filial generations; the females were treated during each pregnancy. Groups of 6–10 controls were given saline only. The authors stated that as there was no difference in fertility between oral and intraperitoneal routes of administration, results were combined. At 50 mg/kg bw, only one dam gave birth, and all the offspring died in the 1st week. At 5 mg/kg bw, the number carrying pregnancies to term decreased with successive pregnancies until the fifth pregnancy, when no offspring were born. At 0.5 mg/kg bw, there was no effect on pregnancy, except that it was noted that in the third to fifth litters there were more males than females (7/1, 19/4, 9/2 males/females, respectively). In the study on successive generations, in those given 0.5 mg/kg bw during each pregnancy, 83–93% of fetuses were born alive. The remainder were stillborn. In the F1 generation, 5/7 stillborns were malformed (brain, eye, lip and palate), and 40% of the live births died in the 1st week. In those given 50 mg/kg bw, fewer animals gave birth (F1 7/10, F2 4/6, F3 1/3), 7 of the 10 stillborns were malformed and 31–38% of the live births died in the 1st week in the three generations (DiPaolo & Elis, 1970). It should be noted

that there were small numbers of animals in some treatment groups and no controls in the study on successive generations.

In a study described only in an abstract, a single intraperitoneal injection of ethyl carbamate at 1500 mg/kg bw in CBA and C3HeB mice on day 8.5 of gestation induced a strain-specific frequency of exencephaly in the offspring. In a similar experiment (no details given) with *N*-hydroxyethyl carbamate, a key metabolite of ethyl carbamate, a higher incidence of exencephaly was produced, but with similar strain differences as for ethyl carbamate. F1 offspring of various crosses of inbred strains of mice treated with ethyl carbamate during pregnancy (no details given) showed a similar frequency of white spots in their mid-ventral fur, suggesting that ethyl carbamate kills prospective pigment cells (Tutikawa & Harada, 1972).

In another study described only in an abstract, a single intraperitoneal injection of ethyl carbamate at 1500 mg/kg bw was given on one of days 9–13 of gestation to CBA and C3HeB female mice mated to PW males. Injection on day 12 or 13 resulted in F1 offspring with abnormalities of tooth development and position when examined as adults (Tutikawa & Tutikawa, 1972).

A single intraperitoneal injection of ethyl carbamate in distilled water at 1500 mg/kg bw was given on day 7.5 of gestation to C57BL/lcr mice. Controls were untreated or given intraperitoneal isotonic saline on day 7.5 of gestation. Subgroups of 8–12 animals per group were killed on day 13.5 of gestation, and the remaining 8–9 animals per group were killed on day 20 of gestation. At 13.5 days of gestation, fetal mortality was 32% in the ethyl carbamate group compared with 15% in saline controls and 0% in untreated controls. Of the surviving fetuses in the ethyl carbamate group, 47% had subcutaneous haemorrhages, mainly in the extremities but in some fetuses covering most of the body surface, and 35% had eye defects of the retina and lens, compared with no such defects in saline or untreated controls. In the ethyl carbamate group, 17.5% of fetuses had "miscellaneous anomalies" (not further described), compared with 18.5% and 7.1% in saline and untreated controls, respectively. At 20 days of gestation, there was 35%, 21% and 7% mortality and 40%, 10% and 8% miscellaneous anomalies (mainly haemorrhages) in fetuses in the ethyl carbamate group, saline controls and untreated controls, respectively (Diwan & Batra, 1972). In an earlier study, intraperitoneal injection of a single dose of ethyl carbamate into mice on gestation day 9.5 or 11.5 did not produce teratogenic effects (Diwan et al., 1970).

Ethyl carbamate was given as a single intraperitoneal injection at a dose of 250, 500, 750 or 1000 mg/kg bw on day 9 of gestation or at 1000 mg/kg bw on one of days 7–15 of gestation to groups of 20–47 CL/Fr mice, which have a high spontaneous incidence of cleft lip with cleft palate (around 30% of live fetuses). A group of 120 animals served as untreated controls, and all animals were killed on day 18 of gestation. Treatment with ethyl carbamate at any dose on day 9 of gestation decreased the incidence of cleft lip with cleft palate, but slightly increased the rate of cleft palate alone, increased the rate of tail abnormalities (absent, short or kinked tail) and significantly reduced fetal weight. Treatment with 1000 mg/kg bw on one of days 7–10 produced similar effects. Treatment on one of

days 11–15 had no effects on abnormality rates, but did reduce fetal weight. Intrauterine mortality was increased by treatment with ethyl carbamate at 750 mg/kg bw on day 9 and at 1000 mg/kg bw on day 8 or 9 of gestation. Caffeine given by subcutaneous injection at 50 mg/kg bw within 48 h following the administration of ethyl carbamate significantly reduced the incidence of malformations (Nakane & Kameyama, 1986).

Ten pregnant NMRI mice were treated with a single intraperitoneal dose of ethyl carbamate at 800 mg/kg bw on gestation day 14 and killed on day 18 of gestation. Of 95 (living and dead) fetuses, 19.2% were malformed; 11.7% showed polydactyly, 3.2% microphthalmia, 2.1% cleft palate and 2.1% kidney dysplasia. Only 6% of 85 non-treated fetuses showed malformations (all polydactyly). When pregnant females were additionally treated with irradiation at 5 or 20 cGy, with vitamin C at 3.3 g/kg bw intraperitoneally or with chloroquine at 0.1 g/kg bw intraperitoneally, 6 h after ethyl carbamate injection, total malformations were significantly reduced to between 4.6% and 8.6% (Burkard & Fritz-Niggli, 1987).

Subcutaneous route

The first recorded developmental toxicity study on ethyl carbamate reported neural tube defects in the mouse fetus following subcutaneous injection of 15 mg on day 7 or 8 of gestation. A dose of 30 mg administered subcutaneously was lethal to the embryos and fetuses and, if given 4 or 5 times on alternate days of gestation, was lethal to the dams (Sinclair, 1950).

When ethyl carbamate at 1000 mg/kg bw was injected subcutaneously into 17 female ICR/JCL mice on day 9 of gestation, lung and tail anomalies were induced in more than 70% of offspring (compared with 0.08% lung anomalies and no tail anomalies in 22 control animals). These malformations were not induced by treatment on day 5, 7, 11, 13, 15, 17 or 19 of gestation (Nomura & Okamoto, 1972).

A single subcutaneous ethyl carbamate dose of 500, 1000 or 1500 mg/kg bw was given to ICR/JCL mice on one of days 3–19 of gestation (treatment groups were 3–9 mice, using a total of 212 pregnant mice). A control group of 336 mice received no treatment. Mice were killed on day 19 of gestation. When ethyl carbamate at 1000 or 1500 mg/kg bw was given before implantation on day 3, 66–83% pre-implantation loss was observed; 500 mg/kg bw was without effect. Treatment with ethyl carbamate at 1500 mg/kg bw just after implantation on day 7 or 8 caused complete resorption of the embryos in 6 of 9 and 3 of 9 pregnant animals, respectively. A statistically significant increase in early deaths (deaths before the completion of placenta) was observed when ethyl carbamate at 1500 mg/kg bw was given on day 8 of gestation, and the incidence of late deaths (deaths after day 10) was significantly increased with this dose administered on day 8, 9, 10 or 11. When ethyl carbamate at 1500 mg/kg bw was given on one of days 8–12, a significant increase in malformations was observed (18–95% of fetuses with malformation, 10–50 fetuses per treatment group examined, compared with 0.3% [1/320] in controls). Malformations consisted of tail anomalies, cleft palate, adactyly, syndactyly, polydactyly, lung anomalies, liver anomalies,

asplenia and diaphragmatic hernia. The incidence of malformations following administration of ethyl carbamate as a single dose on gestation day 8, 9, 10, 11 or 12 dropped sharply from 18–95% at 1500 mg/kg bw to 0–26% at 1000 mg/kg bw and 0–16% at 500 mg/kg bw (Nomura, 1974).

Female ICR/JCL mice received subcutaneous injections of ethyl carbamate at 150 mg/kg bw on days 9, 10 and 11 of gestation and were killed on day 19. Out of 41 implantations (numbers of treated mice not stated), there were no significant effects on early or late embryonic or fetal deaths, but 2 fetuses (5.5%) were malformed, with kinky tail, compared with 1 (0.3%) of 351 implantations from untreated controls (Nomura, 1975b). This suggests that 150 mg/kg bw may be a lowest-observed-adverse-effect level (LOAEL).

Groups of 8–16 female ICR/JCL mice received a single subcutaneous injection of ethyl carbamate at 450, 800, 1000, 1350 or 1500 mg/kg bw on day 10 of gestation and were killed on day 19 for examination of the contents of the uterus. The incidence of late fetal deaths was unaffected except at the highest dose of 1500 mg/kg bw, at which about 60% of fetuses died. The incidence of malformations (cleft palate, tail anomalies, polydactyly) was increased in a dose-related manner at 1000 mg/kg bw and above, with 60–90% of live fetuses showing abnormalities (Nomura et al., 1983).

Nicotinamide significantly reduced ethyl carbamate-induced malformations in groups of 17–18 ICR/JCL mice when it was given immediately after a single subcutaneous injection of ethyl carbamate at 1000 mg/kg bw on day 9 of gestation. Ethyl carbamate alone caused malformations (mainly polydactyly, cleft palate and tail abnormalities) in about 70% of fetuses. The three doses of nicotinamide (0.1, 0.3 or 0.5 g/kg bw) were given intraperitoneally 5 times at 6-h intervals. The reduction in incidence of malformations increased with the dose of nicotinamide. The incidences of polydactyly and tail anomalies were markedly reduced, but the incidence of cleft palate was not. The incidence of ethyl carbamate-induced malformations was also reduced when nicotinamide was given at 0.5%, 1% and 3% in the diet. Nicotinic acid was without effect (Gotoh et al., 1988).

The influence of maternal immune stimulation on ethyl carbamate-induced abnormalities has been investigated in a series of experiments. Pyran copolymer given by intraperitoneal injection at 1 or 10 mg/kg bw on day 3 of gestation or *Bacillus Calmette-Guérin* (BCG) given by intraperitoneal injection at 10 µg/g bw before mating significantly reduced the incidence of malformations following a single subcutaneous injection of ethyl carbamate at 1000 mg/kg bw to groups of 10–14 ICR mice on day 9 of gestation. Ethyl carbamate alone caused malformation in about 50% of live fetuses examined on day 18 of gestation, including cleft palate, tail anomalies and polydactyly (Nomura et al., 1990).

A similar experiment was conducted, also using groups of 5 ICR mice and the same subcutaneous dosing regimen for ethyl carbamate of 1000 mg/kg bw on day 9 of gestation. With ethyl carbamate alone, 19% of fetuses had digital defects, compared with none given BCG 2 weeks before mating. Similarly, 70% of fetuses from mice given subcutaneous ethyl carbamate alone at 1500 mg/kg bw on day 10

of gestation had cleft palate; this was reduced to 48% and 26% in mice also given intraperitoneal interferon- γ ($0.5\text{--}1.0 \times 10^3$ IU) on the morning of day 10 of gestation or Freund's complete adjuvant (FCA) at 30 μ l in the footpad at 5 and 3 days before mating (Holladay et al., 2000). Investigation of the expression of five genes controlling the cell cycle or apoptosis showed that ethyl carbamate reduced gene expression and interferon- γ and FCA increased or restored to normal gene expression levels in fetal heads. The authors suggest that stimulation of the maternal immune system may protect against ethyl carbamate-induced malformation via regulation of fetal gene expression (Sharova et al., 2000). Further work by the same group using the same model has suggested that up-regulation of expression of maternal splenic leukocyte genes for cytokines such as transforming growth factor beta-3 (TGF β 3) and granulocyte-macrophage colony stimulating factor (GM-CSF) caused by maternal immune stimulation may also play a role in mitigating the teratogenic effect of ethyl carbamate (Sharova et al., 2002) and that maternal immune stimulation also mitigates the adverse effects of ethyl carbamate on placental structure and on down-regulation of placental growth factor and cell cycle gene expression (Sharova et al., 2003).

Injection of pregnant mice with total ethyl carbamate doses of 500 or 1000 mg/kg bw over days 7–16 of gestation has been shown to cause elevated leukocyte counts, a trend towards increased spleen and thymus size and suppressed humoral immune response (as measured by the immunoglobulin M [IgM] response to sheep erythrocytes) in the offspring postnatally (Luebke et al., 1986).

Inhalation route

Groups of 8–24 ICR/JCL mice were exposed to air containing sublimed ethyl carbamate gas at 1.28 mg/l for 12, 24, 36 or 48 h starting on the morning of day 9 of gestation. Controls were housed in the same inhalation chambers for 48 h but exposed only to air. The animals were killed on gestation day 18, and the contents of the uterus examined. There was a marked effect on intrauterine mortality in those exposed to ethyl carbamate for 48 h, in which 97 of 102 implants were late fetal deaths and the 1 live fetus was multiply malformed. With shorter exposures, there was no effect on intrauterine mortality; however, following 24 or 36 h of exposure, 44% and 59%, respectively, of live fetuses were malformed, compared with none in controls. The malformations included cleft palate, polydactyly, tail anomalies and vertebral anomalies. Groups of four pregnant mice were subjected to the same inhalation treatment for 48 h and killed at intervals of 3–72 h after the end of treatment. Examination of cells from whole embryos in these mice revealed a high proportion of cells with chromosome aberrations (36%) 3 h after the end of treatment, which had returned to control levels by 48 h after the end of treatment (Nomura et al., 1996).

(ii) Rats

Oral route

Wistar rats were given ethyl carbamate at 1000 mg/kg bw by oral gavage daily for 6 or 7 successive days on days 0–5, 6–12 or 13–19 of gestation, on 2

successive days during organogenesis (days 6–13) or on single days (day 8 or 9) of gestation. The dose caused anaesthesia with reduced food intake and decreased maternal body weight gain. Fetal body weight was decreased and resorptions were increased in all treated groups. There were no gross malformations in those treated on days 0–5 or 13–19 of gestation, but absent tail and exencephaly were observed (incidence not stated) in those treated on days 6–12. Increases in skeletal abnormalities were observed in fetuses from dams treated for 6 or 7 successive days from all three treatment periods (malformed vertebrae and ribs and mislocated sternebrae). Similar effects on fetal weight, resorptions and gross and skeletal malformations were observed in fetuses from dams treated on only 1 or 2 successive days of gestation (Takaori et al., 1966).

Intraperitoneal route

The first report of malformations in rats following ethyl carbamate treatment was of eye defects in 12 rat fetuses following intraperitoneal injection of ethyl carbamate at 900–1600 mg/kg bw on days 8–11 of gestation (Hall, 1953).

Ethyl carbamate or its metabolite hydroxyethyl carbamate was given by intraperitoneal injection to groups of 1–29 Wistar rats on one of days 9, 10, 11 or 12 of gestation, at doses of 62–2000 mg/kg bw. Hydroxyethyl carbamate doses of 700 mg/kg bw and higher caused high maternal mortality. Hydroxyethyl carbamate doses of 350 mg/kg bw and higher caused 52–72% embryo-fetal mortality, and doses of 225 mg/kg bw and higher caused a high incidence of malformations, ranging from 30% to 100% of surviving fetuses. The abnormalities included exencephaly, encephalocoele, cleft palate, retarded or clubbed rear leg and short, curled or absent tail. In contrast, when ethyl carbamate was given, no malformations were observed in fetuses that survived, although at 1000 mg/kg bw on day 11 of gestation, 42% of embryos and fetuses were resorbed (Chaube & Murphy, 1966).

Sprague-Dawley CD rats were given either corn oil intraperitoneally (16 rats) or ethyl carbamate intraperitoneally in corn oil at 1000 mg/kg bw (17 rats) on day 11 of gestation and killed on gestation day 20. Ethyl carbamate produced maternal anaesthesia for 18 h, reduced food consumption on days 11–12 to almost zero and caused a loss of about 30 g in maternal body weight for that day. Accordingly, other pregnant rats were food-deprived for 18 h to serve as pair-fed controls. There were no significant effects on numbers of implantations, live fetuses, dead fetuses or resorptions in the ethyl carbamate group, but mean fetal weight (2.98 g) was significantly reduced compared with both corn oil controls (3.48 g) and pair-fed controls (3.29 g). There were no frank malformations, but ossification was significantly delayed in fetuses from the ethyl carbamate-treated group compared with corn oil controls, as judged by the lower number of ossified sternebrae. Maternal hepatic metallothionein levels were increased 14-fold in ethyl carbamate-treated dams, which was about 50% greater than that produced by food deprivation alone. Zinc concentrations were reduced in maternal plasma, increased in maternal liver cytosol and unchanged in the embryo, compared with corn oil and pair-fed controls. Exposure of 11-day-old whole rat embryos in culture to ethyl carbamate at concentrations of 250–2250 µg/ml for 48 h had no effect on

embryonic development. The authors suggested that the disturbed maternal zinc status may contribute to the developmental toxicity of ethyl carbamate (Daston et al., 1991).

(iii) *Hamsters*

In hamsters injected intravenously with 25 mg ethyl carbamate on day 8 of gestation, 33% (10) of fetuses examined on day 9, 10 or 11 had neural tube defects and/or cardiac malformations. In hamsters given 50 mg ethyl carbamate intravenously or intraperitoneally on day 8, fetotoxicity as well as abnormalities were observed in fetuses examined on days 10 and 11. When 100 or 150 mg ethyl carbamate were given on day 8, fetotoxicity and a wider range of neural and cardiac abnormalities were observed. These doses were equivalent to about 200–1200 mg/kg bw (Ferm, 1966). It is noted that some of these findings (failure of closure of the neural tube and convoluted cardiac tubes) may represent retardations rather than true malformations in fetuses observed close to the time of treatment, rather than at term.

Ethyl carbamate was injected intraperitoneally at doses of 500, 1000, 1500 or 3000 mg/kg bw to groups of three pregnant Syrian golden hamsters on day 8 of gestation, and fetuses were examined for malformations on day 13. All pregnant animals in the highest dose group died. In the other three dose groups, there was a dose-related increase in the incidence of dead or resorbed fetuses, from 18% to 44%, compared with 11% in saline controls, and 7–10% of total implantations were living but malformed fetuses. Malformations consisted of exencephaly, encephalocele, microcephaly, anophthalmia/microphthalmia, omphalocele and malformed extremity (DiPaolo & Elis, 1967).

(c) *Transplacental carcinogenicity*

(i) *Mice*

Intraperitoneal or intravenous route

The first ever observation of transplacental carcinogenicity using a chemical was in animals exposed prenatally to ethyl carbamate (Larsen, 1947). Female A strain mice were injected with a single intraperitoneal or intravenous injection of 25 mg ethyl carbamate 1, 2, 3, 4 or 5 days before parturition. This dose caused narcosis. The surviving offspring were killed and examined for lung tumours at 6 months of age. One hundred per cent of those exposed 1 day before birth developed lung tumours, with an average of 9–10 tumours per mouse. The earlier administrations resulted in 61–83% developing 1–2 lung tumours per mouse. Another laboratory confirmed the finding of a high incidence of lung tumours in offspring exposed 1 day before birth using the same A strain of mice and the same intravenous dose (25 mg) of ethyl carbamate (DiPaolo, 1962). Similar results were also reported in A × C mice injected intraperitoneally with 25 mg ethyl carbamate. After injection on day 17, 18 or 19 of gestation, 57% of offspring developed an average of 0.9 tumours per mouse, and 97% of offspring of mice injected 8–20 h before parturition had an average of 7.6 tumours per mouse (Klein, 1952).

Groups of 3–10 A/He mice were treated orally or by intraperitoneal injection with ethyl carbamate at 0.5, 5 or 50 mg/kg bw every other day starting on day 1 of pregnancy, and the results were combined. Treatment was repeated through five pregnancies, each separated by intervals of 7 weeks. Where possible, surviving offspring were examined for malignancies, with particular attention being given to the lung. Offspring of animals given repeated doses of 50 mg/kg bw all had multiple tumours (mean 28 per mouse). Offspring from the 0.5 mg/kg bw group also all had tumours (mean 5 tumours per mouse). Where the same treatments were given through pregnancies of three successive generations, offspring showed a 3-fold increase in lung tumours (mean 16 tumours per mouse) (DiPaolo & Elis, 1970).

(C57BL \times C3H)F1 mice were given ethyl carbamate at 500 mg/kg bw by intraperitoneal injection on days 7–11, 11–15 or 14–18 of gestation. Others were given the same treatment on days 12, 15 and 18 of gestation, and a portion of their offspring were also given the same dose intraperitoneally in the neonatal period, on postnatal days 1 and 4 or 1, 4, 7, 10 and 13. Other groups were left untreated prenatally and neonatally (controls) or were treated only neonatally on days 1, 4 and 7 or 1, 4, 7, 10 and 13. Surviving offspring were killed at 48 or 90 weeks of age. Prenatal exposure for 5 consecutive days on gestation days 11–15 or 14–18 significantly increased the incidence of lung adenoma, hepatoma and ovarian tumours. Treatment on days 12, 15 and 18 had no effect, unless combined with neonatal exposure, when significant increases were observed in leukaemia, lung adenoma, Harderian gland cystadenoma, hepatoma and ovarian tumours. Animals treated neonatally developed a wider range of tumours and a higher incidence of tumours than animals exposed only *in utero* (Vesselinovitch et al., 1971).

BALB/c mice were given a single intraperitoneal injection of ethyl carbamate at 1000 mg/kg bw on day 17 of gestation. Controls were injected intraperitoneally with saline on the same day or left untreated. The offspring were killed at intervals after birth between 10 and 54 weeks of age. A high incidence of lung tumours was observed in the offspring exposed prenatally to ethyl carbamate, appearing as two distinct crops; a smaller but earlier crop appeared at 10–12 weeks of age and a larger crop at 8–12 months of age, coincident with the appearance of a lower incidence of spontaneous lung tumours in controls (Anderson, 1978).

Subcutaneous route

Thirty-three female Swiss mice and 26 C3H mice were given ethyl carbamate at 500 mg/kg bw by subcutaneous injection for 5 consecutive days on days 7–11 or 11–15 of gestation, respectively. Groups of 18 Swiss mice and 16 C3H mice were given saline subcutaneously on the same days as controls. The offspring were kept for 65–105 weeks, depending on sex and strain. The treatment reduced the number of animals giving birth to live offspring from 94% in controls to 73% (C3H) and 60% (Swiss) and increased the incidence of stillborn litters from 6% in controls to 23% (C3H) and 12% (Swiss). Postnatal mortality in the first 10 weeks of life was higher among offspring of treated animals (16%) compared with controls (3%). In C3H mice, the incidence of hepatomas was increased in both

sexes of offspring from ethyl carbamate-treated animals, in males from 42% in controls to 92% in offspring from treated animals and in females from 0% in controls to 22% in offspring of treated females. There were no hepatomas in Swiss mice. In female offspring, ovarian tumours were increased in C3H mice from 2% in controls to 37% in offspring of treated animals and in Swiss mice from 5% in controls to 71% in offspring of treated animals. Lung adenomas were significantly increased in C3H offspring of both sexes from treated animals compared with controls (males 25% vs 6%, females 9% vs 2%). Lung adenomas were observed in 12–16% of Swiss mice, but there was no effect of treatment (Vesselinovitch et al., 1967). It is noted that few statistical analyses were performed.

Groups of 3–17 ICR/JCL mice were given 1000 mg ethyl carbamate injected subcutaneously on one of days 5, 7, 9, 11, 13, 15, 17 or 19 of gestation or 5 days after parturition. Groups of 2–5 controls were injected on the same days with distilled water. Thirty-five per cent of ethyl carbamate-treated mothers died during the experiment. At 32 weeks of age, surviving offspring were killed and examined for tumours. Significantly fewer ethyl carbamate-treated animals gave birth to live offspring (66% compared with 95% in controls), and a higher proportion gave birth to stillborns (19% compared with 0% in controls). Postnatal survival was significantly reduced in those exposed to ethyl carbamate on day 9 or 11 of gestation, but there were no effects on body weight gain in offspring. The incidence of lung tumours was significantly increased in those exposed to ethyl carbamate on one day of gestation at or after day 13 or postpartum (31–71% in treated groups compared with 0–11% in controls). In those exposed on day 19 of gestation, the incidences of lung tumours and nodules per mouse were significantly higher in those injected less than 24 h before birth than in those injected more than 24 h before birth (Nomura & Okamoto, 1972). A subsequent study showed that radio-labelled ethyl carbamate given subcutaneously to pregnant mice on one of days 11–19 of gestation is rapidly distributed to all organs and tissues of the mother and fetus, irrespective of the stage of pregnancy or dose. Ethyl carbamate is retained longer in offspring exposed on day 19 of gestation than in earlier-stage fetuses or adults; the shorter the interval between exposure and birth, the longer the ethyl carbamate is retained, which correlates with the tumour incidence (Nomura et al., 1973; Nomura, 1976).

ICR/JCL mice were given ethyl carbamate at 1000 mg/kg bw subcutaneously on one of days 7, 13, 15 or 17 of gestation or at 500 mg/kg bw subcutaneously on day 9 or 11 of gestation. Other mice were given ethyl carbamate at 1000 mg/kg bw subcutaneously during lactation only on day 2, days 2, 4, 6 and 8, days 2, 7, 12 and 17 or days 14, 16, 18 and 20 postpartum. Other mice were treated during pregnancy and lactation with ethyl carbamate at 1000 mg/kg bw subcutaneously on one of days 7–17 of gestation and additionally on days 2, 7, 12 and 17 postpartum. Controls were injected with distilled water on one day (gestation days not specified). Dams given ethyl carbamate developed a high incidence of uterine tumours (22–29% compared with 8% in controls), lung tumours (97–100% compared with 16% in controls) and ovarian cysts (6–14% compared with 0% in controls) during the experiment. At 32 weeks of age, surviving offspring were killed and examined for tumours. In those exposed during gestation only, the incidences

of lung tumours and of liver tumours (male offspring only) were significantly increased in those treated on or after day 11 of gestation (lung tumours 15–69% compared with 5% in controls; hepatomas 4–15% compared with 0% in controls). The later in gestation the mice were exposed, the higher the incidence of lung tumours. In those treated during lactation only, there was a significant increase in lung tumours (41–100% compared with 2.4% in controls). The highest incidence and the most tumours per mouse were in those exposed on days 2, 4, 6 and 8 of lactation. In those exposed during gestation and lactation, the incidence of tumours was higher than in those exposed during gestation only or during lactation only. Lung tumours were said to be significantly increased in all groups treated on or after gestation day 7 (59–90%), hepatomas were increased only in those treated on gestation day 9 (25%) and ovarian cysts were increased in those treated on gestation days 9–15 (9–45%) (statistical significance levels were given, but there were no control data for these combined groups) (Nomura, 1973).

ICR/JCL mice were given a single subcutaneous injection of ethyl carbamate at 200 or 1000 mg/kg bw on one of days 7, 9, 11, 13, 15, 17 or 19 of gestation or subcutaneous injections at 200 mg/kg bw on 3 consecutive days (days 8–10, 12–14 or 16–18) of gestation. Controls were injected subcutaneously with distilled water during pregnancy. Dams given ethyl carbamate developed a high incidence of uterine tumours (8–12% compared with 2% in controls), lung tumours (70–93% compared with 13% in controls) and ovarian cystadenomas (6–10% compared with 0% in controls) during the experiment. At 36 weeks of age, surviving offspring were killed and examined for tumours. In the groups exposed via a single injection of ethyl carbamate, the incidence of lung tumours in the offspring was significantly increased in both dose groups exposed on or after day 11 of gestation (17–100% compared with 7% in controls). Hepatomas in male offspring were increased in both dose groups exposed on day 11 of gestation and in the 1000 mg/kg bw groups exposed on day 12 or 13 of gestation (3–15% compared with 0% in controls). Ovarian cystadenomas in female offspring were increased in both dose groups exposed on day 13 of gestation. In those exposed on day 19 of gestation, the incidence of lung tumours was significantly higher in those injected less than 12 h before birth than in those injected more than 36 h before birth. In the groups exposed to 200 mg/kg bw on 3 consecutive days of gestation, lung tumours were significantly increased to 95% with exposure on days 16–18 and hepatomas were increased in male offspring exposed on days 8–10 (5%) or 12–14 (10%). Ovarian cystadenomas were increased in female offspring exposed on days 8–10 (19%) or 12–14 (21%) (Nomura, 1974).

In multigeneration studies, female mice were untreated or were given ethyl carbamate at 1000 mg/kg bw subcutaneously on day 17 of gestation, and their offspring were cross-fostered from birth onto the opposite treatment group. Controls were given distilled water subcutaneously on day 17 of gestation and allowed to rear their own offspring. A portion of these offspring were then mated within or across treatment groups to produce second and third generations, none of which was treated. In the first generation, there was a high incidence of (mainly lung) tumours in the offspring of treated females reared by untreated females (42/52, 81%) compared with controls (5/72, 7%). Tumours were also increased in

offspring of untreated females reared by treated females (13/70, 19%). Tumour incidences (again mostly lung) were also significantly increased in the second but not the third generations descended from treated females (16%) or from animals reared by treated females (11%), compared with descendants from controls (6%) (Nomura, 1975a).

In a study on the relative sensitivity in female mice of the fetal, young and adult lung to tumour induction by ethyl carbamate administered subcutaneously at 1500 mg/kg bw, it was shown that day 15 of gestation was a more sensitive stage than 21 days of age, and 21 days of age was in turn more sensitive than 64 days of age. Relative sensitivity was assessed by the average number of lung tumours per unit mass of the lung at the time of treatment, by the average diameter of lung tumours and by the ratio of adenocarcinomas to adenomas (Nomura, 1976).

Pregnant BALB/Mo or BALB/c mice were given 35 mg (approximately 1000 mg/kg bw) of ethyl carbamate by subcutaneous injection either 3 days or 3 days and 1 day before parturition. The offspring were killed at 2, 4, 6 or 8 months of age and examined for tumours. BALB/Mo mice carry the Moloney murine leukaemia virus and have a high spontaneous incidence of lymphomas, mostly derived from T-lymphocytes. The development of lung tumours was accelerated in both strains of mice, and the development of lymphomas was accelerated in BALB/Mo mice by prenatal ethyl carbamate exposure (Chieco-Bianchi et al., 1984).

2.2.7 Special studies

(a) Covalent binding to nucleic acids and/or proteins

It has been recognized for many years that ethyl carbamate undergoes activation to a reactive metabolite that binds covalently to nucleic acids and proteins (Boyland & Williams, 1969; Prodi et al., 1970; Lawson & Pound, 1971; Chavan & Bhide, 1973; Dahl et al., 1980). Recent studies have focused on the chemical characterization of the adducts and their biological significance.

Male B6C3F1 mice aged 2–2.5 months were given a single intraperitoneal injection of radiolabelled ethyl carbamate adjusted with cold ethyl carbamate to give total doses of 10, 100, 500 or 1000 mg/kg bw. They were killed 12 h after treatment, and binding of ethyl carbamate metabolites to liver and testis DNA and to sperm heads from the vas deferens was measured. Binding of radiolabelled ethyl carbamate to testis DNA was at least 2 orders of magnitude lower than binding to liver DNA but was dose related, as was binding to sperm heads (Sotomayor et al., 1994).

Approximately 7–10 pmol of 1,*N*⁶-ethenoadenosine and 2–3 pmol of 3,*N*⁴-ethenocytidine were found per milligram of liver RNA 6–12 h following the administration of ethyl carbamate (500 mg/kg bw) to male B6C3F1 mice (Miller & Miller, 1983).

The binding to DNA, RNA and protein 6 h after administration of 62 mg [ethyl-1-¹⁴C]ethyl carbamate/kg bw (740 kBq/20 g bw) orally to male SENCAR and BALB/c mice varied with tissue (liver > stomach > skin > lung) but did not differ

with strain. Analysis of the labelled DNA from liver indicated little incorporation of the radiolabel into normal deoxyribonucleotide or deoxyribonucleoside bases (Fossa et al., 1985).

The amounts of 2-oxoethyl adducts in haemoglobin and 7-(2-oxoethyl)guanine in liver DNA increased in a dose-related manner when [^{14}C]ethyl carbamate at 1–260 mg/kg bw was given to male CBA mice by intraperitoneal injection (Svensson, 1988).

Injection of a single dose of [ethyl-1,2- ^3H]- or [ethyl-1- ^{14}C]ethyl carbamate into 12-day-old male (C57BL/6 \times C3H/He)F1 mice or of [ethyl-1,2- ^3H]ethyl carbamate into adult male A/Jax mice resulted in the formation of 1, N^6 -ethenoadenosine and 3, N^4 -ethenocytidine adducts in the hepatic RNA. The levels of adducts in the hepatic RNA 12 h after a single injection (500–600 mg/kg bw) of ethyl carbamate were 6–10 and 2–3 pmol of ethenoadenosine and ethenocytidine, respectively, per milligram RNA. Labelled adducts were not detected after injection of [1- ^{14}C]ethanol, indicating that the radiolabel was not simply a consequence of incorporation into normal bases (Ribovich et al., 1982).

Injection of a single dose of ethyl carbamate (500 mg/kg bw) into 12-day-old male B6C3F1 mice resulted in the formation of 11 pmol 1, N^6 -ethenoadenosine per milligram liver RNA. Treatment of the animals with the cytochrome P450 inhibitors sodium diethyldithiocarbamate or 2-(2,4-dichloro-6-phenyl) phenoxyethyl amine before and after the ethyl carbamate injection reduced the amount of 1, N^6 -ethenoadenosine in the hepatic RNA (Leithauser et al., 1990).

Intraperitoneal administration of ethyl carbamate, vinyl carbamate or vinyl carbamate epoxide to different mouse strains (12-day-old and adult CD-1, B6C3F1, C3H/HeJ and C57BL/6J mice) resulted in the formation of 1, N^6 -ethenodeoxyadenosine and 3, N^4 -ethenodeoxycytidine adducts in the DNA of liver and lung. Young mice received a single intraperitoneal dose of 0.025 or 0.25 mmol/kg bw (equivalent to 2.2 or 22.3 mg/kg bw for ethyl carbamate), and adult mice received five intraperitoneal doses of 0.029, 0.25 or 0.28 mmol/kg bw per day (equivalent to 2.6, 22.3 or 24.9 mg/kg bw for ethyl carbamate); the mice were sacrificed 24 h after the last treatment. Using ^{32}P -postlabelling, 1- N^6 -ethenodeoxyadenosine and 3, N^4 -ethenodeoxycytidine adducts were found in untreated control DNA samples from liver and lung in the range of 2–15 adducts/ 10^9 parent nucleotides. Treatment of adult C3H/HeJ, C57BL/6J and CD-1 mice with 5×0.25 or 5×0.28 mmol/kg bw per day increased these levels to 10–33 adducts/ 10^9 nucleotides for ethyl carbamate and to 24–80 adducts/ 10^9 nucleotides for vinyl carbamate. In general, 2–4 times more adducts were formed with vinyl carbamate than with ethyl carbamate. Very low levels of adducts were found with vinyl carbamate epoxide, which the authors ascribed to reaction with other tissue nucleophiles before interaction with DNA. High levels of adducts were found in treated 12-day-old mice given a single dose of ethyl carbamate or vinyl carbamate (Fernando et al., 1996).

Administration of 1% 2(3)-*tert*-butyl-4-hydroxyanisole in the diet of male A/J mice for 12 days increased the concentrations of glutathione in liver cytosol almost 2-fold and glutathione transferase activity about 5-fold. These changes were associated with a 23% reduction in formation of 2-oxoethylvaline adducts in

haemoglobin during a 50-h measurement period following a single intraperitoneal ethyl carbamate dose of 1000 mg/kg bw on day 13 (Kemper et al., 1995). A protective role of glutathione was supported by associated *in vitro* studies.

After intraperitoneal dosing of radiolabelled ethyl carbamate to mice and rats, the main DNA adduct observed in liver DNA was 7-(2-oxoethyl)guanine. Vinyl carbamate led to about 100 times as much 7-(2-oxoethyl)guanine (on a molar basis) as did ethyl carbamate (Scherer et al., 1986).

Vinyl carbamate epoxide reacts *in vitro* with adenosine to form 1,*N*⁶-etheno-adenosine (Park et al., 1990) and forms 7-(2-oxoethyl)deoxyguanosine, *N*²,3-ethenodeoxyguanosine and 1,*N*⁶-ethenodeoxyadenosine *in vitro* on incubation with DNA and *in vivo* in liver of rats and mice dosed with 3 mg/kg bw intraperitoneally for 8–10 days (Park et al., 1993).

Less than 0.3–8 nmol of 1,*N*²-ethenoguanine and *N*²,3-ethenoguanine per litre were detected in the urine of 13 healthy persons not occupationally exposed to industrial chemicals. Since occupational exposure to chemicals that may form etheno-adducts could be ruled out, the authors concluded that endogenously produced intermediates, such as 2,3-epoxy-4-hydroxynonanal, may be responsible for the formation of etheno-adducts in human DNA and that the background level of the general population has to be taken into account in the investigation of persons occupationally exposed to etheno-adduct-forming chemicals (Gonzalez-Reche et al., 2002).

The *in vitro* formation of 1,*N*⁶-etheno-adenosine adducts from ethyl carbamate in liver samples from 11 humans showed close correlations with chloroxazone 6-hydroxylation ($r = 0.98$; a reaction catalysed by CYP2E1) and with the formation of adducts from vinyl carbamate ($r = 0.99$). The rate of formation of the adduct was 500 times faster for vinyl carbamate than for ethyl carbamate, and the authors noted that this could be the reason why vinyl carbamate has not been detected as a metabolite of ethyl carbamate. Diethyldithiocarbamate, a specific inhibitor of CYP2E1, reduced the formation of 1,*N*⁶-etheno-adenosine adducts from both ethyl and vinyl carbamates in a dose-related manner to less than 10% of control values (Guengerich et al., 1991). This conclusion was supported by the finding that the K_m for the conversion of ethyl carbamate to 1,*N*⁶-etheno-adenosine by human liver microsomes *in vitro* in the presence of adenosine was greater than 2 mmol/l, compared with a K_m of 54 μ mol/l for the same reaction with vinyl carbamate. The rate of formation was considerably slower in the case of ethyl carbamate (Guengerich & Kim, 1991).

N-Hydroxyethyl carbamate caused oxidative DNA damage in the presence of copper(II) by the formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine, which causes the misreplication of DNA. Treatment with esterase increased the extent of DNA damage by a factor of 10. Electron spin resonance spectrometry revealed that esterase-treated *N*-hydroxyethyl carbamate generated nitric oxide in the presence of copper(II). The authors concluded that *N*-hydroxyethyl carbamate was metabolized by esterase to generate hydroxylamine and that it exerts its carcinogenic effect by generating O⁻ and NO to cause oxidation and depurination of DNA (Sakano et al., 2002). Although this study has implicated a role for oxidative

damage in the carcinogenic activity of ethyl carbamate, the *in vivo* significance of the findings is questioned by the low formation of the putative active metabolite *N*-hydroxyethyl carbamate and the artificial nature of the incubation conditions.

The spontaneous formation of 1,*N*⁶-ethenoadenosine on incubation of vinyl carbamate epoxide with adenosine was inhibited by the addition of glutathione, and the extent of inhibition was increased when mouse liver cytosol was added as a source of glutathione transferase. Addition of *S*-octylglutathione, an inhibitor of glutathione transferase, reduced the glutathione-induced inhibition of adduct formation (Kemper et al., 1995).

The significance of the 1,*N*⁶-ethenoadenine as the key adduct in the generation of cancer by ethyl carbamate has been questioned by the recent observation that the incidences of hepatocellular carcinoma were not higher in alkylpurine-DNA-*N*-glycosylase knockout mice given a single dose of vinyl carbamate (30 or 150 µmol/kg bw given intraperitoneally when 14–15 days old) compared with wild-type mice. Alkylpurine-DNA-*N*-glycosylase is the enzyme that releases 1,*N*⁶-ethenoadenine from DNA, and therefore lack of the repair enzyme would have been expected to cause a greater tumorigenic response (Barbin et al., 2003).

The data from several authors (Ribovich et al., 1982; Fossa et al., 1985; Leithauser et al., 1990; Park et al., 1993; Fernando et al., 1996; NTP, 2004) support the metabolic pathway shown in Figure 2, leading to the formation of DNA adducts (7-(2'-oxoethyl)deoxyguanosine, *N*²,3-ethenodeoxyguanosine, 1,*N*⁶-ethenodeoxyadenosine and 3,*N*⁴-ethenodeoxycytidine). As discussed above, the metabolic activation reactions seem to depend on CYP2E1 (Guengerich et al., 1991).

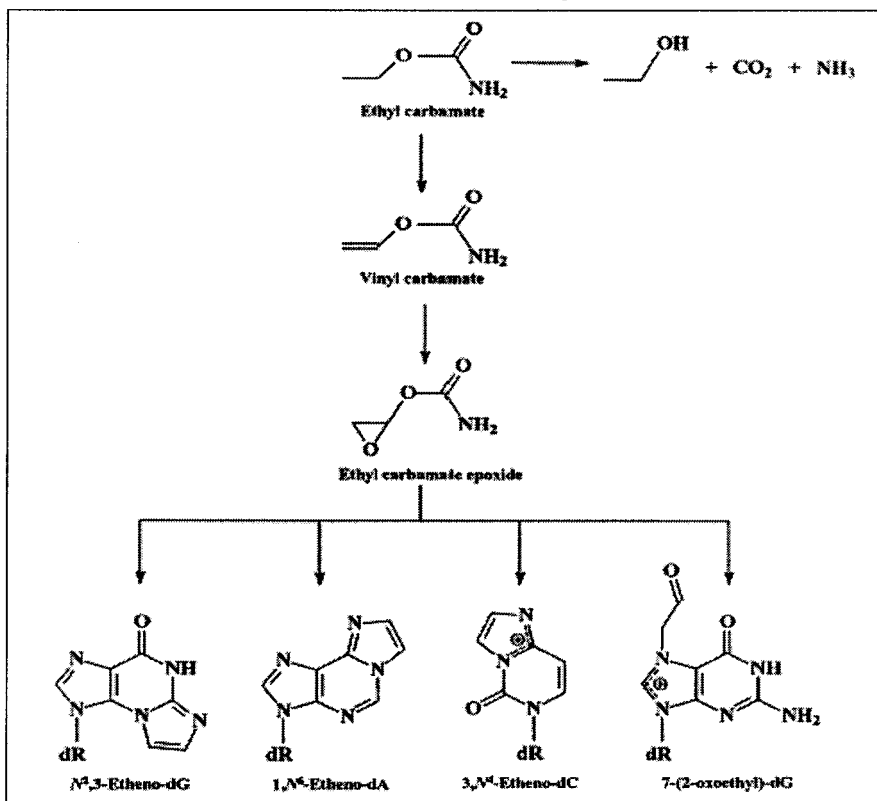
(i) Interaction with ethanol

The concentrations of 1,*N*⁶-ethenoadenine and 7-(2'-oxoethyl)guanine adducts in liver DNA of male F-344 rats given ethyl carbamate at 10 000 mg/l in drinking-water for 2 or 90 days or in 5% ethanol for 2 or 14 days indicated that co-administration with ethanol for 2 days reduced the extent of adduct formation. Unfortunately, the study design did not allow a definitive answer on whether induction of CYP2E1 *in vivo* would increase or decrease the extent of DNA binding, but the available data indicated that if there were an interaction, it would be of a limited extent (Sotomayor & Washington, 1996).

Groups of four male and four female B6C3F1 mice received ethyl carbamate at 0, 10, 30 or 90 mg/l in drinking-water *ad libitum* in the presence of 0%, 2.5% or 5% ethanol for 4 weeks (equal to 0, 0.04, 0.11 and 0.32 mg ethyl carbamate per animal per day in males and 0, 0.03, 0.08 and 0.25 mg ethyl carbamate per animal per day in females, respectively; ethanol consumption was equivalent to 0, 85 and 170 mg per animal per day in males and 0, 70 and 130 mg per animal per day in females). Liver and lung samples were collected for cell proliferation and apoptosis analyses. Ethenoadenosine adduct concentrations in hepatic DNA were significantly increased by exposure to ethyl carbamate (90 mg/l) and decreased by exposure to ethanol (5%). There was not a significant interaction between ethanol

and ethyl carbamate with respect to the extent of covalent binding in lung tissue (NTP, 2004).

Figure 2. Metabolism and DNA adduct formation of ethyl carbamate



From NTP (2004)

(b) Immunotoxicity

(i) Mice

A variety of immunological end-points were assessed in groups of 7–10 female B6C3F1 mice, 5–7 weeks of age, that received daily intraperitoneal injections of ethyl carbamate over a 14-day period to achieve a total dose of 0, 1000, 2000 or 4000 mg/kg bw (i.e. likely 0, 70, 140 and 290 mg/kg bw per day). None of the treated mice died or demonstrated overt toxicity during the exposure period. Weights of body, liver and kidney were reported to be unchanged, whereas spleen weights were 53% of control values at the highest dose (total dose 4000 mg/kg bw). These spleens revealed small follicles with less prominent mantle zones. Increased splenic myelopoiesis was evident histologically in all treated

groups, but most marked in the highest dose group. In addition, thymic atrophy, characterized by cortical lymphoid depletion and mean weights of 60% of control values, was observed at this dose. Haematological parameters were unchanged. The highest dose depressed the splenic lymphoproliferative response to the T lymphocyte mitogen concanavalin A and reduced the number of primary antibody plaque-forming cells following sheep erythrocyte (40% of control values) or *Escherichia coli* lipopolysaccharide (a T-independent antigen) challenge (54% of control values). The number of metastatic lung foci and [125 I]5-iodo-2'-deoxyuridine incorporation in lungs were significantly increased following injection of B16 melanoma cells in the highest dose group. Natural killer cell activity and proliferation of pluripotent stem cells were markedly reduced, while bone marrow cellularity was unaltered. At the lower total ethyl carbamate doses of 1000 and 2000 mg/kg bw, natural killer cell activity and proliferation of pluripotent stem cells were reduced, but the other parameters were not significantly affected. Exposure to methyl carbamate (4000 mg/kg bw total dose) did not cause any alterations in the parameters examined. Noting that bone marrow cellularity was unaffected, the authors suggested that ethyl carbamate selectively affects rapidly proliferating cells (Luster et al., 1982).

The splenic antibody response to sheep erythrocytes was assessed in groups of five female BALB/c mice treated with ethyl carbamate intraperitoneally for 14 consecutive days at 0, 25, 50, 100, 200 or 400 mg/kg bw per day. Thymus weight was decreased by ethyl carbamate treatment in a dose-dependent manner, with statistical significance at doses of 100 mg/kg bw per day and higher; the weight of the spleen was decreased only at the highest dose, 400 mg/kg bw per day. Body and liver weights were unchanged, and there was no effect of treatment on liver function, as assessed by measurements of the activity of serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase. The authors also noted that mice treated with ethyl carbamate became more hyperactive with repeated treatment. The splenic antibody response to sheep erythrocytes was significantly suppressed at doses of 200 mg/kg bw or higher, but there was no effect on the number of cells per spleen. Acute exposure to a single dose of ethyl carbamate relevant to conditions of clinical anaesthetic use (1 g/kg bw) also caused a significant suppression of the sheep erythrocyte antibody response and of thymus weight (interval from treatment to assessment not indicated). The results of a haemagglutination study performed with sera from these mice indicated that the antibody response to sheep red blood cells was most likely to be the IgM antibody response. When mice were pretreated with phenobarbital (80 mg/kg bw) for 3 days to induce P450 enzymes, followed by administration of ethyl carbamate at 100–400 mg/kg bw intraperitoneally for 7 consecutive days, the antibody response was more suppressed than in saline-pretreated mice receiving the same dose of ethyl carbamate. Treatment with aminoacetonitrile, a cytochrome P450 inhibitor, resulted in eradication of the suppression of the antibody response caused by ethyl carbamate. The authors suggested that metabolism of ethyl carbamate by cytochrome P450 may be the critical pathway in the production of metabolites responsible for suppression of antibody response to sheep erythrocytes (Jeong et al., 1995).

A possible role of cytochrome P450 in ethyl carbamate-induced immunosuppression was investigated using the polyclonal antibody response induced by bacterial lipopolysaccharide in splenocyte cultures isolated from female BALB/c mice. Ethyl carbamate added to cultured splenocytes from BALB/c mice at concentrations of 0.05–5.0 mmol/l in the presence of bacterial lipopolysaccharide at 100 µg/ml resulted in a suppression of response at the highest concentration (5.0 mmol/l) only; methyl carbamate had no effect over the same range of concentrations. P450 metabolites of ethyl carbamate, ethyl *N*-hydroxycarbamate and vinyl carbamate, both suppressed the antibody response at concentrations of 0.5 and 0.1 mmol/l and higher, respectively, in association with concentration-dependent cytotoxicity. The polyclonal antibody response was more suppressed by vinyl carbamate and *N*-hydroxyethyl carbamate than by ethyl carbamate. Pre-incubation of splenocytes with phenobarbital-induced mouse liver microsomes and ethyl carbamate enhanced the immunosuppression of antibody response observed with ethyl carbamate alone. Pre-incubation with a cytochrome P450 inhibitor, aminoacetonitrile, prevented the immunosuppressive response observed with ethyl carbamate (Jeong et al., 1996).

The effect of inhibitors of esterase and cytochrome P450 on ethyl carbamate-induced suppression of the antibody response to sheep erythrocytes was assessed in 6- to 8-week-old female BALB/c mice. Pretreatment with the esterase inhibitor diazinon at 20 mg/kg bw intraperitoneally 30 min prior to the administration of ethyl carbamate at 100 or 400 mg/kg bw intraperitoneally for 7 consecutive days completely blocked serum esterase activity. Spleen and thymus weights and spleen cellularity were significantly lower than controls at the 400 mg/kg bw dose, and this effect was exacerbated by pretreatment with diazinon. Diazinon pretreatment also resulted in mortality and increased liver weights in combination with ethyl carbamate treatment at the highest dose. Histopathologically, splenic and thymic atrophy accompanied by cellular depletion were observed at both doses when mice were treated with ethyl carbamate, and this effect was potentiated by the pretreatment with diazinon. Significant suppression of the antibody response to sheep red blood cells was noted at the high dose of ethyl carbamate, and this suppression was enhanced by diazinon pretreatment, becoming significant in the lower ethyl carbamate dose group as well. The authors suggested that the metabolism of ethyl carbamate by esterase may be an inactivation pathway in ethyl carbamate-induced immunosuppression. In addition, *N*-hydroxyethyl carbamate, a cytochrome P450 metabolite, suppressed the lymphoproliferative response induced by lipopolysaccharide and concanavalin A in splenocyte cultures at concentrations of 0.5 mmol/l and of 0.1 and 0.5 mmol/l, respectively. Neither methyl nor ethyl carbamate had an effect on the lymphoproliferative response at concentrations up to 10 mmol/l. The authors suggested that the metabolism of ethyl carbamate by cytochrome P450 may be an activation pathway for immunosuppression by ethyl carbamate (Cha et al., 2000).

The possible role of corticosterone produced by the adrenal gland in the ethyl carbamate-induced suppression of the antibody response to the T-cell-dependent antigen sheep red blood cells and the subpopulation changes of splenocytes and thymocytes were investigated in adrenalectomized female BALB/c mice. The

weights of spleen and thymus were reduced by ethyl carbamate treatment in a dose-dependent manner compared with vehicle controls at doses of 100, 200 or 400 mg/kg bw for 7 consecutive days, which achieved statistical significance at the two top doses; body weights were unchanged from controls. Total cells per spleen and all subpopulations, including macrophages, B cells, total T cells, CD4⁺ T-helper cells and CD8⁺ T-cytotoxic cells, were decreased significantly at 200 and 400 mg/kg bw. Serum corticosterone concentrations were significantly increased in the same manner. When naive, sham-operated and adrenalectomized mice were treated using the same protocol at a dose of 400 mg/kg bw, there were no effects on spleen or on spleen cell subpopulations in the adrenalectomized mice compared with vehicle or non-adrenalectomized controls. Serum corticosterone levels were reduced to very low concentrations in both treated and control adrenalectomized mice. While the effect of adrenalectomy on ethyl carbamate-induced reduction in thymic cell subpopulations was attenuated, it was not completely eradicated, as with splenic cell subpopulations (Cha et al., 2001).

(c) *Metabolites*

The toxicity of vinyl carbamate was compared with that of ethyl carbamate in acute toxicity studies, an Ames *Salmonella* point mutation assay and short-term studies of carcinogenicity. None of the female CD-1 mice given intraperitoneal injections of vinyl carbamate at 62 mg/kg bw died within 10 days, but three of four mice given 125 mg/kg bw did. All mice injected with vinyl carbamate at 250 or 500 mg/kg bw died within 24 h. Administration of ethyl carbamate at doses of 50–1000 mg/kg bw had no apparent gross or histological effect; survival was not reported. Vinyl carbamate was more active than ethyl carbamate in the induction of lung adenomas in female CD-1 mice 20–28 weeks after they received ethyl carbamate at 32–500 mg/kg bw or vinyl carbamate at 16–65 mg/kg bw as a single intraperitoneal dose or up to 10 multiple doses at 1-week intervals. While ethyl carbamate was not mutagenic in *S. typhimurium* strains TA1535, TA100 or TA98, either alone or in the presence of rat or mouse metabolic activation, vinyl carbamate was mutagenic in TA1535 and TA100, which detect missense mutations, in the presence of a rat or mouse liver metabolic activation system that included an NADPH- and NADH-generating system. No mutagenicity of vinyl carbamate for strain TA98 was detected either in the absence or in the presence of fortified liver S13 (Dahl et al., 1978).

Multiple weekly intraperitoneal injections of ethyl carbamate or vinyl carbamate into Fischer rat (five injections) or (C57BL/6J × C3H/HeJ)F1 mouse (eight injections) pups, starting within 24 h of birth, resulted in the induction of similar spectra of tumours at 22–23 months (rats) or 15–16 months (mice) by each of the compounds. In rats, hepatic carcinomas, carcinomas of the ear duct (Zymbal's gland), neurofibrosarcomas of the ear lobe and testicular tumours were the most prominent neoplasms, while hepatomas, lung adenomas, Harderian gland tumours and thymomas were the most prominent neoplasms in mice. Vinyl carbamate was more active in the induction of these tumour types in mice and of hepatic carcinomas and neurofibrosarcomas of the ear lobe in rats (Dahl et al., 1980).

The ability of ethyl carbamate and vinyl carbamate to induce sister chromatid exchanges in cultured human peripheral lymphocytes was assessed in the presence and absence of Aroclor-induced rat liver microsomal activation. At a 10^{-2} mol/l concentration, vinyl carbamate in the presence of microsomal activation was much more potent in inducing sister chromatid exchanges than was vinyl carbamate in the absence of microsomal activation or ethyl carbamate in the absence or presence of microsomal activation (Csukas et al., 1981).

Ethyl carbamate and vinyl carbamate were evaluated for their ability to induce adenomas and sister chromatid exchanges in the lung cells of A/J, C3HeB/FeJ and C57BL/6J strain mice following a single intraperitoneal injection of 30–1000 mg/kg bw and 1–60 mg/kg bw, respectively. The percentage of mice with adenomas and the number of adenomas per mouse were assessed after 24 weeks. Although the relative order of strain sensitivity was the same for both chemicals, vinyl carbamate was more potent than ethyl carbamate in terms of both incidence and multiplicity of adenomas. Ethyl carbamate and vinyl carbamate showed similar potency differences in the induction of sister chromatid exchanges in primary lung cells cultured from treated animals (Allen et al., 1986).

The pre-administration of the CYP2E1 inhibitor, diethyldithiocarbamate, by intraperitoneal injection markedly inhibited the induction of tumours by single intraperitoneal doses of ethyl carbamate or vinyl carbamate as assessed by incidence and multiplicity in the livers, lungs and Harderian glands of infant female B6C3F1 mice. Diethyldithiocarbamate also resulted in reduced multiplicity of liver tumours induced by ethyl or vinyl carbamate in infant male B6C3F1 mice and by vinyl carbamate but not ethyl carbamate in the lungs of adult female A/Jax mice. The mutagenicity of vinyl carbamate for *S. typhimurium* strain TA1535 in the presence of a mouse liver metabolic activation system plus cofactors was markedly reduced by increasing concentrations of diethyldithiocarbamate added to the medium. Vinyl carbamate formed more of an ethenoadenosine adduct from adenosine than did ethyl carbamate in an in vitro system in the presence of cofactor-fortified mouse liver microsomes. The authors concluded from the data from these studies and by analogy with vinyl chloride in the formation of DNA and RNA adducts that vinyl carbamate epoxide is the ultimate electrophilic and carcinogenic metabolite of ethyl carbamate and vinyl carbamate in the mouse (Leithauser et al., 1990).

Studies with vinyl carbamate epoxide demonstrated that in addition to forming the same DNA adducts both in vitro and in vivo that have been identified with ethyl carbamate and vinyl carbamate (7-(2'-oxoethyl)guanine, N^2 ,3-ethenoguanine and 1, N^6 -ethenoadenine), it was a strong direct mutagen in *S. typhimurium* TA1535 and TA100, although it was only weakly active in TA98. Equimolar doses of vinyl carbamate epoxide of 34 or 68 nmol given by intraperitoneal injection to adult female A/Jax mice resulted in greatly reduced survival compared with vinyl carbamate, but did not increase the incidence or multiplicity of lung adenomas. A single intraperitoneal dose of 25 μ g vinyl carbamate epoxide (24 nmol) or vinyl carbamate (29 nmol) per kilogram body weight given to 12-day-old male B6C3F1 mice induced nearly 100% incidence of hepatomas at 9 months; vinyl carbamate epoxide drastically reduced survival compared with vinyl carbamate (15% vs

100%) and induced a 5-fold increase in the number of hepatomas per mouse. A dose of 125 µg ethyl carbamate (1400 nmol) per kilogram body weight was required to produce the same incidence and multiplicity of tumours as vinyl carbamate (Park et al., 1993).

2.3 Observations in humans

2.3.1 Biomarkers of exposure or effects

Analytical methodology has been developed for the quantitative detection of biomarkers in haemoglobin (*N*-(2-oxoethyl)valine) (Cai et al., 1995) and urine (ethenoguanines) (Gonzalez-Reche et al., 2002) that reflect exposure to genotoxic metabolites of ethyl carbamate. In terms of their specificity for estimating exposure to ethyl carbamate, it was noted that oxoethyl adducts of haemoglobin and etheno-adducts of nucleic acids may be formed from exposure to a number of structurally related exogenous chemicals. In addition, significant background levels of these adducts suggested that endogenous substrates are also capable of forming adducts. No reports were found in which biomarkers were used as an estimate of exposure or tissue damage from ethyl carbamate.

2.3.2 Clinical observations

The review of Sokal & Lessmann (1960) states three case-studies with ethyl carbamate given to pregnant women, at doses of 65 mg/kg bw through the entire pregnancy, 50 mg/kg bw starting at month 3.5 to term and an unknown dose through the entire pregnancy. In all cases, mothers suffered from chronic myelogenous leukaemia; children were normal at birth and during follow-up observations up to 3.5 years of age.

Schardein (2000) summarized five case reports of women exposed to ethyl carbamate during early pregnancy. None of the offspring was malformed. It is unclear if these five cases include those reviewed by Sokal & Lessmann (1960).

2.3.3 Epidemiological studies

Ethyl carbamate was in medical use in Japan from 1950 to 1970. It was used as a co-solvent of water-insoluble analgesic and sedative drugs for post-operational pain. An estimate of 100 million 2-ml ampules of 7–15% solutions of ethyl carbamate were injected into patients in this time period. The author noted that the ethyl carbamate dose commonly used for single injection in humans corresponds to 5–10 mg/kg bw, with a total daily dose of 10–50 mg/kg bw per patient. These doses correspond to carcinogenic doses in mice (10 mg/kg bw) (Nomura, 1975b). The need for epidemiological studies of the cancer incidences in Japanese patients who received ethyl carbamate therapeutically has been noted (Miller, 1991).

3. ANALYTICAL METHODS

3.1 Chemistry

Ethyl carbamate ($\text{NH}_2\text{COOCH}_2\text{CH}_3$; also known as urethane) is the ethyl ester of carbamic acid. It has a molecular weight of 89 daltons. It takes the form of colourless crystals or a white granular powder and has a melting point of 48–50 °C and a boiling point of 182–184 °C (Zimmerli & Schlatter, 1991). The limited conjugation involving π -electrons means that ethyl carbamate lacks a strong chromophore for ultraviolet detection and does not fluoresce. As a small organic ester, ethyl carbamate has a relatively high vapour pressure, about 133 Pa at 30–40 °C. The low molecular weight and the volatility of ethyl carbamate affect the choice of methods used for its analysis in food and beverages.

Ethyl carbamate is quite stable at ambient conditions, and the pure chemical has a long storage life. The main reactions of ethyl carbamate come from the ester moiety. It undergoes hydrolysis in aqueous alkaline solutions to form cyanate (NCO^-) and ultimately carbonate (CO_3^{2-}). Acidic conditions also promote hydrolysis.

3.2 Description of analytical methods

3.2.1 Beverages

Ethyl carbamate can be determined in distilled spirit drinks by gas chromatography (GC) with direct injection onto a polar wax column and detection by gas chromatography coupled to mass spectrometry (GC-MS).

This direct technique cannot be applied to beverages with lower alcohol content, for which the favoured techniques use extraction into dichloromethane and concentration prior to analysis by GC-MS (Kobayashi et al., 1987; Clegg & Frank, 1988; Hasegawa et al., 1990; Sen et al., 1992; Canas et al., 1994; Ma et al., 1995; Jagerdeo et al., 2002). Analysis of the extract has also used the chemiluminescent thermal energy analyser operated in nitrogen mode (Canas et al., 1988; Sen et al., 1992; Dyer, 1994). The extraction is generally carried out by using an inert solid as a support to pre-absorb the beverage before extracting with the organic solvent. These methods typically have limits of detection of about 1 µg/l. Other methods reported include continuous extraction with diethyl ether (Fauhl & Wittkowski, 1992) and, more recently, solid-phase microextraction (Whiton & Zoecklein, 2002). Cleanup procedures are not always required but have included the use of magnesium silicate adsorbent columns to remove potential interferences from the extract (Walker et al., 1974; Ough, 1976; Dennis et al., 1989).

Two methods have been tested by international trials, one for application to beers and whiskey (Dennis et al., 1990) and another for application to wine, fortified wine spirits and soya sauce (Canas et al., 1994). The reported ranges of ethyl carbamate covered by the latter method (Canas et al., 1994) are described as 50–350 µg/l in distilled spirits, 40–160 µg/l in fortified wines, 10–50 µg/l in table wines and 15–70 µg/kg in soya sauce.

3.2.2 Solid and semi-solid foods

Ethyl carbamate has generally been extracted from solid or semi-solid food samples by using an inert medium mixed with the food prior to extraction of the sample with organic solvent (Ough, 1976; Canas et al., 1989; Dennis et al., 1989; Hasegawa et al., 1990). For certain fermented soya bean products that are solid, extraction with ethyl acetate followed by C_{18} column chromatography has been used as a preliminary cleanup step (Kim et al., 2000). Other cleanup procedures applied to food extracts have included liquid-liquid partition (Sen et al., 1993) and/or the use of magnesium silicate adsorbent columns (Dennis et al., 1989; Kim et al., 2000) and/or an ion-exchange resin (Dennis et al., 1989). For cheese samples, vacuum distillation has been used as a sample cleanup approach to isolate the volatile fraction containing ethyl carbamate (Canas et al., 1989).

3.2.3 Derivatization approaches

Most published methods conduct GC analysis on ethyl carbamate itself, but derivatization methods have been reported too. Derivatization has been applied as the trifluoroacetyl- (Bailey et al., 1986), the *N,N*-dimethyl- (Bailey et al., 1986) and the *N,N*-dimethylaminomethylene derivatives (Kobayashi et al., 1987). Derivatization is rarely used because of the instability of the derivatives and/or the fact that they do not really offer improved chromatographic properties or mass spectral information compared with the underivatized compound. Ethyl carbamate has been determined as the xanthylamide derivative prepared from the reaction with 9-xanthidrol with detection by GC (Giachetti et al., 1991) or by liquid chromatography (Herbert et al., 2002).

3.2.4 Identification aspects

Many older published methods used flame ionization detection, thermionic detection in nitrogen-specific mode or thermal energy analysis in nitrogen-specific mode. In these methods, the identification of ethyl carbamate rests on the chromatographic retention time and the (albeit limited) specificity of the detector when run in nitrogen-specific mode. This can be sufficient, for example, when monitoring trends in production samples for which it has been established *a priori* that no significant interferences occur. For analytical surveys and for the analysis of less well known samples, however, using these detectors does not provide unambiguous identification and quantification of ethyl carbamate, and the use of MS is obligatory.

Given the ready availability of inexpensive bench-top GC-MS instruments, most laboratories find that this is now the preferred analytical approach. The identity of any ethyl carbamate in the sample is confirmed from the chromatographic retention time and from the responses for ions at mass-to-charge ratios 89, 74 and 62. These ions are diagnostic for the molecular ion $[M]^+$ of ethyl carbamate and for its main fragment ions $[M-CH_3]^+$ and $[M-C_2H_3]^+$, respectively. The molecular ion at m/z 89 is usually weaker than the two fragment ions, and so the sample extract may need to be concentrated further to obtain sufficient

response. Typical confirmation criteria would be that the three ions ascribed to putative ethyl carbamate should all maximize at the correct GC retention time and that their relative intensities should be within $\pm 20\%$ of the ratios for a standard.

3.2.5 Quantification aspects

For quantification, most published procedures include an internal standard of propyl carbamate or butyl carbamate. Quantification is made directly against matrix-matched standards containing known concentrations of added ethyl carbamate or against solution standards, with correction then made for the analytical recovery. In GC-MS analysis, as such or used as a confirmatory technique following a screening analysis, quantification uses the stronger m/z 62 fragment ion. Isotope-labelled ethyl carbamate is available commercially, labelled with deuterium and/or ^{15}N , and its use as an internal standard makes the quantitative aspects more straightforward and more robust.

3.2.6 General conclusions

Analytical methods for ethyl carbamate in foods and especially beverages have been developed to a high state of refinement over the past 30 years. Extraction and cleanup methods have been devised to suit all the types of foods and beverages that may be affected by ethyl carbamate formation. The use of MS ensures that the identification of putative ethyl carbamate meets modern standards of evidence. The methods are capable of detecting ethyl carbamate to typically $1\text{ }\mu\text{g/kg}$ and have limits of quantification in the range $3\text{--}5\text{ }\mu\text{g/kg}$ in foods and beverages.

4. SAMPLING PROTOCOLS

The sampling procedures for ethyl carbamate analysis are not especially demanding. The normal requirement to obtain a representative sample pertains. The substance itself is relatively stable in most matrices. However, it should be taken into account that ethyl carbamate formation can continue after the processing and packaging of the food or beverage. For example, new wine will tend to have lower levels of ethyl carbamate than aged wine. Therefore, the time of sampling and analysis should correspond to the time of consumption. Ethyl carbamate formation can also be promoted by heat and light. Samples should not be unduly exposed to heat or light if this would not also occur for the foodstuff as consumed.

5. EFFECTS OF PROCESSING

Ethyl carbamate is formed in foods, primarily alcoholic beverages, but also in other foods as a consequence of the fermentation process from food components, food additives or food packaging materials. The generation of ethyl carbamate in foods during food processing and storage is discussed in section 8 in the context of prevention and control measures.

6. LEVELS AND PATTERNS OF CONTAMINATION OF FOOD COMMODITIES

Ethyl carbamate is formed naturally during the fermentation of foods. By the mid-1990s, researchers and government regulatory agencies had quantified levels of ethyl carbamate in a number of foods commonly consumed as part of the diet. The highest (albeit still low) levels of ethyl carbamate are typically found in yeast-fermented foods, with low to non-quantifiable levels found in foods fermented by lactic acid bacteria, acetic acid bacteria or moulds (Canas et al., 1989; Battaglia et al., 1990). Additionally, the level of ethyl carbamate in a fermented food or drink can increase when ethanol and appropriate precursors remain in contact for extended periods, such as in the ageing of distilled scotch whiskeys and fruit brandies. Distilled alcoholic beverages, wine and beer contained the highest residual ethyl carbamate levels, with low levels (often at or below the limit of quantification) consistently found in such staples as bread, cheese, vinegar, soya sauces and yoghurt. Many fermented ethnic foods, such as Korean kimchi, African kenkey and Asian tempeh, have also been found to contain trace levels of ethyl carbamate (Nout et al., 1993, 1994; Kim et al., 2000). It is clear from published analyses of ethyl carbamate exposure that a large majority of the population is consuming one or more foods likely to contain some ethyl carbamate (Zimmerli & Schlatter, 1991; Diachenko et al., 1992; Vahl, 1993).

It has long been known that alcoholic beverages contain the highest levels of residual ethyl carbamate. The initial realization of the possible negative health impact of the presence of ethyl carbamate in wines, distilled spirits and beer led to numerous endeavours to discover production methods that resulted in lower ethyl carbamate levels in these beverages. The effectiveness of developing mitigation procedures (see section 8) was monitored through regular surveys of the alcoholic beverage industry. As a result of these efforts, the highly elevated levels seen in early examinations of distilled spirits and fruit brandies have been decreasing over the last decade.

The Committee received a submission concerning residual ethyl carbamate levels in and ethyl carbamate intake from the consumption of wines, fortified wines, beer and distilled spirits from The Wine Institute (USA), which contained the summarized results of ethyl carbamate monitoring analyses completed by the Liquor Control Board of Ontario (Canada) (Wine Institute, 2004). These data included samples of beverages produced throughout the world taken from 1993 through 2001. A report on ethyl carbamate residues in various distilled spirits, wines and beers analysed from 1989 to 1998 was submitted by the Alcohol and Tobacco Tax and Trade Bureau (USA) (Alcohol and Tobacco Tax and Trade Bureau, 2004). Additional data on ethyl carbamate residues in alcoholic beverages were received from the United Kingdom (United Kingdom, 2004) and Japan (sake) (Japan, 2004). The Committee also reviewed a report completed in 2000 from the Food Standards Agency (United Kingdom) on a survey of ethyl carbamate levels in whiskey.

The Committee concludes that the mitigation measures that the alcoholic beverage industry has been implementing (see section 8) have been effective in

reducing residual ethyl carbamate levels in alcoholic beverages. For example, the level of residual ethyl carbamate in vintage-dated wines from throughout the world was reduced from an average of 8 µg/l in 1993 to 4 µg/l in 2001 (Wine Institute, 2004). Similarly, the mean ethyl carbamate level in all wines was reduced from 7.4 µg/l to 4 µg/l over the same period.

Data on concentrations of ethyl carbamate in foods and beverages were submitted by the Food and Drug Administration of the United States, the Food Standards Agency of the United Kingdom and the Wine Institute of the United States. The alcoholic beverages considered in these reports originate from many countries throughout the world.

Means and ranges of concentrations of ethyl carbamate in foods and beverages as reported in publications or data submitted to the Committee are presented in Table 5. For alcoholic beverages, only recent data were included because concentrations have been reduced considerably over time as a result of the application of mitigation measures.

7. DIETARY INTAKE ASSESSMENT

7.1 Methods

The national estimates of exposure to ethyl carbamate from consumption of all foods in the diet published in the 1990s generally combined mean ethyl carbamate residue levels with mean food intakes to generate a mean ethyl carbamate intake estimate (Zimmerli & Schlatter, 1991; Diachenko et al., 1992; Vahl, 1993; Kim et al., 2000). These analyses do not contain the raw ethyl carbamate residue data; as a consequence, it was not possible for the Committee to prepare its own estimates of exposure to ethyl carbamate from all foods using probabilistic (Monte Carlo) techniques. However, the Committee received sufficient raw ethyl carbamate residue data from wines and distilled spirits analysed during the last 10 years to prepare contaminant distribution curves and examine the effect of any potential regulatory maximum levels on exposure to ethyl carbamate. The Committee concluded that the use of older ethyl carbamate residue data from published exposure analyses would result in conservatism, as it is to be expected that mean residual ethyl carbamate levels in foods as well as distilled spirits are declining.

7.2 Estimates of dietary intake

7.2.1 National estimates

The Committee evaluated four published national estimates of intake — from Denmark, the Republic of Korea, Switzerland and the United States (Zimmerli & Schlatter, 1991; Diachenko et al., 1992; Vahl, 1993; Kim et al., 2000) — and two estimates submitted to the Committee by Australia and New Zealand (Australia, 2004; New Zealand, 2004). Mean contamination levels were used in each, as it was assumed that the chronic nature of the hazard posed by ethyl carbamate would allow a consumer to be exposed to an average amount of ethyl carbamate

Table 5. Ethyl carbamate levels in food

Food	Country of data origin	No. of samples	Mean level (µg/kg)	Range found (µg/kg)	Reference
<i>Alcoholic beverages</i>					
Wine	Various	228	8	ND–36	FDA (2004)
	Various	13	10	ND–24	FSA (2005)
	Various	5189	4	ND–61	Wine Institute (2004)
Fortified wines	Various	125	41	ND–262	Wine Institute (2004)
	Various	15	32	14–60	FSA (2005)
Whiskeys	Various	205	29	ND–239	FSA (2000)
	Various	30	32	ND–102	FDA (2004)
Cordials, liqueurs, brandies	Various	14	37 ^a	ND–170, 6131	FSA (2005)
	Various	31	64	ND–243	FDA (2004)
Sake	Japan	90	73	ND–202	Japan (2004)
	Japan	2	122	81, 164 ^b	FSA (2005)
Beer	Various	10	–	ND	FSA (2005)
	Various	52	1	ND–5	FDA (2004)
<i>Other foods</i>					
Bread ^c	United Kingdom	157	–	ND	United Kingdom (2003)
	Denmark	33	4	0.8–12	Vahl (1993)
	United States	3	2		Canas et al. (1997)
	United Kingdom	65	2	0.4–4.5	Dennis et al. (1997a, 1997b)
	Canada	12	3	1.6–4.8	Sen et al. (1993)
Kimchi	Republic of Korea	20	4	ND–16	Kim et al. (2000)
Yoghurt	United Kingdom	4	–	ND	FSA (2005)
	Various	9	1	ND–1.3	Dennis et al. (1989)
	Denmark	19	0.2	ND–0.3	Vahl (1993)

Table 5. (contd)

Food	Country of data origin	No. of samples	Mean level ($\mu\text{g/kg}$)	Range found ($\mu\text{g/kg}$)	Reference
Cheese	Various	17	—	ND	Diachenko et al. (1992)
		8	—	ND	FSA (2005)
Olives	Various	6	—	ND	Diachenko et al. (1992)
		3	—	ND	FSA (2005)
Soya sauce	Japan	10	—	ND	FSA (2005)
		20	16	ND–84	Diachenko et al. (1992)
		18	16	ND–78	Fauhl et al. (1993)
Soya bean paste	Republic of Korea	7	2	ND–8	Kim et al. (2000)
Vinegar	United States	6	9	4–26	Diachenko et al. (1992)
	Republic of Korea	5	1	0.3–2.5	Kim et al. (2000)
Cider	United States	8	0.4	ND–3	Diachenko et al. (1992)
Tempeh ^d	Netherlands	—	—	ND	Nout et al. (1993)
Kenkey ^d	Netherlands	—	—	ND	Nout et al. (1994)

ND, not detected

^a This mean of ethyl carbamate levels excludes the single highest value of 6131 reported (see next column).

^b Only two values are reported.

^c A review of reported ethyl carbamate levels in breads has been prepared for publication (B.J. Canas, personal communication, 2005).

^d Tempeh is a fermented soy product, whereas kenkey is a fermented maize product.

in any given foodstuff over a lifetime. Each of the four published estimates was completed using deterministic methods (point estimates for food consumption and ethyl carbamate concentration); the intakes of fermented foods expected to contain ethyl carbamate were combined with ethyl carbamate levels taken from national surveys of the food supply and summed. The analyses from Australia and New Zealand combined mean ethyl carbamate residual levels taken from the literature (none was derived from foods in either Australia or New Zealand) with national food intakes from surveyed individuals to produce a distribution of ethyl carbamate exposures.

(a) *Literature estimates*

(i) *Denmark*

The Danish analysis of ethyl carbamate intake was compiled using residue data obtained during the period 1988–1990. Ethyl carbamate levels were determined for breads, yoghurts and other acidified milk products, beer, table wine and fortified wines, fruit brandy and other distilled spirits. The highest levels by far were found in fruit brandies and distilled spirits, ranging as high as 5100 µg/l. The levels in most foods ranged from the limit of quantification (0.1–3 µg/l, depending on the food matrix) to 35 µg/l. Food intakes were taken from a survey published in 1986 (Haraldsdottir et al., 1986). The average daily intake was estimated to be approximately 2 µg/person per day. The authors conclude that the 5% of males with the highest level of alcohol intake probably consume more than 7 µg/person per day.

(ii) *Republic of Korea*

The analysis of ethyl carbamate intake in the Republic of Korea was prepared by combining ethyl carbamate levels determined for five food groups: kimchi, soya sauce, soya bean paste, alcoholic beverages and vinegar. The authors determined that these traditional fermented foods are staples of the diet of the Republic of Korea. Ethyl carbamate levels overall were fairly low, ranging from below the limit of detection (not specified) to approximately 75 µg/l. Food intakes were taken from a 1995 Korean Ministry of Health and Welfare Survey (Korean Ministry of Health and Welfare, 1997). Overall intake of ethyl carbamate was estimated to be 2.8 µg/person per day, with the majority coming from the consumption of kimchi. The authors additionally considered the consumption of foods of foreign origin, such as yoghurt and breads. Based on food intake data and published ethyl carbamate levels for the yoghurt and bread products in the Republic of Korea, they concluded that the additional exposure to ethyl carbamate would be less than 0.1 µg/person per day, resulting in a total exposure of approximately 2.9 µg/person per day.

(iii) *Switzerland*

The Swiss analysis utilized ethyl carbamate residue data published in the literature from the late 1980s through 1990 and recognized that the majority of the available data concerned ethyl carbamate in various types of alcoholic beverages. The summarized residue data do not indicate how many samples were included in the mean levels reported. Additionally, there is no indication of the source of food intake data used in preparing the intake estimates. The ethyl carbamate contributed from consumption of soya sauce, bread, yoghurt, cheese, tea, cider and beer was included with that from consumption of a number of different distilled alcoholic spirits and wines (including fortified wines). The authors concluded that the “unavoidable” ethyl carbamate intake from food, primarily bread, is 10–20 ng/kg bw per day (approximately 1 µg/person per day, assuming a 60-kg individual). They noted that the smoking of cigarettes could double this intake. It was

additionally noted that the consumption of wine can lead to a 3-fold increase in the unavoidable baseline ethyl carbamate intake, assuming a daily consumption of 200–300 ml of table wine contaminated at an ethyl carbamate level of 10 µg/l, whereas consumption of 30 ml of stone-fruit brandy contaminated at 2000 µg/l could lead to a 60-fold increase in ethyl carbamate intake.

(iv) United States

The USA analysis utilized ethyl carbamate residue data collected and reported in the literature through the late 1980s. As with the Swiss analysis, the large majority of the reported ethyl carbamate levels were taken from alcoholic beverages. Over 1000 samples of alcoholic beverages were included. Additionally, approximately 150 samples from fermented foods were taken into account. These foods included bread and bakery products, soya sauce, soya bean paste (miso), cider, vinegar, yoghurt, cheese and olives. The ethyl carbamate levels in fermented foods were all lower than 20 µg/kg, with soya sauce being the only food with an ethyl carbamate level greater than 9 µg/kg. The ethyl carbamate levels in fruit brandies were the highest reported, averaging nearly 1200 µg/kg. Food consumption data from a 14-day survey of the frequency of consumption of foods were used to estimate ethyl carbamate intake. The estimate of overall consumption of ethyl carbamate was 4 µg/person per day, with greater than 90% of intake due to the consumption of alcoholic beverages.

(b) National estimates submitted to the Committee

(i) Australia

The Australian analysis utilized ethyl carbamate residue levels in foods reported in the literature from the late 1980s through the year 2000. Mean (or median where necessary) ethyl carbamate residue levels taken from literature reports were used (the number of samples included in each mean was not reported). None of the analyses was completed using foods from Australia. The foods and beverages included in the analysis were similar to those used in the Swiss and USA analyses: milk products, including yoghurt and cheese, soya sauce, breads, beer, wine and distilled alcoholic beverages. Food consumptions from a 1995 1-day intake survey were combined with the ethyl carbamate residue data to estimate overall ethyl carbamate intake. The mean ethyl carbamate intake was reported to be 1.4 µg/person per day, with greater than 99% of the population exposed to ethyl carbamate from the diet. Beer and wine consumption accounted for greater than 50% of the estimated total ethyl carbamate intake. The highest non-beverage contribution to ethyl carbamate intake was from the consumption of bread products (15%).

(ii) New Zealand

The New Zealand analysis was prepared in a manner analogous to that from Australia. Ethyl carbamate residue levels were identical to those used in the Australian analysis, taken from the literature. The same foods were considered:

milk products, including yoghurt and cheese, soya sauce, breads, beer, wine and distilled alcoholic beverages. Food consumptions from a 1997 1-day intake survey were combined with the ethyl carbamate residue data to estimate overall ethyl carbamate intake. The mean ethyl carbamate intake was reported to be 1.4 µg/person per day, with greater than 99% of the population exposed to ethyl carbamate from the diet. Beer and wine consumption accounted for greater than 50% of the estimated total ethyl carbamate intake. The highest non-beverage contribution to ethyl carbamate intake was from the consumption of bread products (18%).

7.2.2 International estimates

The Committee prepared international estimates of exposure to ethyl carbamate from foods using the five regional diets of the Global Environment Monitoring System Food Contamination Monitoring and Assessment Programme (GEMS/Food) database (i.e. African, European, Far Eastern, Latin American, Middle Eastern; see Table 6). The foods included in the analyses were bread, fermented milk products (including yoghurt and cheese) and soya sauce. Alcoholic beverages are not included in the GEMS/Food database and were excluded from the analyses. The ethyl carbamate levels used were taken from published summaries of ethyl carbamate residues in foodstuffs. All of the regional diets show ethyl carbamate intake to be less than 1 µg/person per day. This result is not surprising, as it has been shown that consumption of alcoholic beverages would be expected to contribute the greatest part of an individual's exposure to ethyl carbamate.

7.2.3 Special considerations — alcohol consumption

The Committee has concluded that the mitigation measures that the alcoholic beverage industry has been implementing (see section 8) have been effective in reducing residual ethyl carbamate levels in alcoholic beverages and, hence, that the older data used in making the initial estimates of exposure to ethyl carbamate published in the early 1990s (Zimmerli & Schlatter, 1991; Diachenko et al., 1992; Vahl, 1993) are no longer appropriate. The exposure analyses described above for fermented foods in general all included consideration of alcoholic beverage consumption, albeit with very limited information (typically only a mean and/or a range of ethyl carbamate levels in the beverages being considered) on the data used for preparing the residual ethyl carbamate level used.

Zimmerli & Schlatter (1991) concluded that consumption of 500 g of table wine per day would increase the risk from exposure to ethyl carbamate 5-fold, while consumption of 20–40 g of stone-fruit distillates per day increased the burden 60-fold. The increase in ethyl carbamate exposure from consumption of wine assumed an average ethyl carbamate level of 10 µg/l. The data submitted to the Committee suggest that this level has been lowered to 5 µg/l or less. The assumed level of ethyl carbamate in stone-fruit distillates of 2000 µg/l, leading to the 60-fold increase in ethyl carbamate exposure from consumption of 30 ml/day, has been reduced to a mean level of less than 100 µg/l. The overall effect of the reduction of mean levels of residual ethyl carbamate in alcoholic beverages is that

Table 6. International estimates of exposure to ethyl carbamate from foods using the five regional diets of the GEMS/Food database

GEMS/ Food code	Commodity	Reported ethyl carbamate level (µg/kg)	Middle Eastern		Far Eastern		African		Latin American		European	
			Comm. intake ^a	EC intake ^b	Comm. intake ^a	EC intake ^b	Comm. intake ^a	EC intake ^b	Comm. intake ^a	EC intake ^b	Comm. intake ^a	EC intake ^b
CP 1211	White bread	2	0.2153	0.4306	0.076	0.152	0.0189	0.0378	0.0373	0.0746	0.1172	0.2344
CP 1211br	Wholemeal bread	2	0.1077	0.2154	0.038	0.076	0.0094	0.0188	0.0747	0.1494	0.0586	0.1172
	Soya sauce	15	0.0015	0.0225	0.011	0.165	0	0	0	0	0	0
AO 31	Total milk products	1	0.1324	0.1324	0.0328	0.0328	0.0422	0.0422	0.1679	0.1679	0.3361	0.3361
	Total ethyl carbamate intake			0.8009		0.4258		0.0988		0.3919		0.6877

^a Comm. intake = Total dietary intake of commodity, in kg/person per day.

^b EC intake = Ethyl carbamate intake, in µg/person per day.

the background intake would be increased by less than a factor of 10 for the majority of alcoholic beverage consumers. The Committee considered the specific case of consumption of wine in France and the resulting ethyl carbamate exposure. The mean and 95th-percentile consumption levels of wine in France were reported to be 1170 and 5342 ml/week (167 and 763 ml/day, respectively) (D'Hauteville et al., 2001). At a mean ethyl carbamate level of 4 µg/l, the ethyl carbamate exposure from wine consumption would be less than 1 µg/day at the mean and 3 µg/day for the 95th-percentile consumer, approximately doubling the expected background exposure to ethyl carbamate.

8. PREVENTION AND CONTROL

The keys to successful prevention and control have been the identification of the main precursors responsible for the formation of ethyl carbamate in foods and an understanding of the influence of the main external factors of heat, light and time, leading to a mechanistic understanding from which control measures have been devised. Two review articles are available that provide an excellent summary of the main factors involved (Battaglia et al., 1990; Zimmerli & Schlatter, 1991).

8.1 Formation from cyanide



8.1.1 Stone-fruit brandies

By far the highest concentrations reported in surveys were found in alcoholic beverages — up to 20 mg/l in stone-fruit brandies (Baumann & Zimmerli, 1986) — and this area has received the most focus in the literature. Manufacturers of a number of these products have used the research into ethyl carbamate formation to change the production processes and so reduce this formation. The main source of ethyl carbamate is hydrogen cyanide, a natural constituent of stone fruits and other fruits. Ethyl carbamate formation occurs in the mash — especially when heated — but forms mainly in the brandy after distillation. Oxidation of cyanide to cyanate is necessary, and this can be accelerated by light, especially with photosensitizing dicarbonyl compounds such as biacetyl present. Cyanate then reacts with ethanol to form ethyl carbamate. The procedures for reducing ethyl carbamate in stone-fruit brandies therefore centre on reducing the cyanide available for oxidation to cyanate (by complexing with copper, silver or ion-exchange resins) and by protection of the distillate from light.

8.1.2 Whiskey

Again, the main source of ethyl carbamate formation post-distillation is hydrogen cyanide. This is produced by thermal decomposition of the cyanohydrin of isobutyraldehyde present in the fermented mash. The key to control is to limit the concentrations of hydrogen cyanide and of copper concentrations to the minimum level possible. The Scottish whiskey industry has taken steps to reduce levels in

malt whiskeys by carefully controlling the distillation and also by selecting grain varieties that do not promote ethyl carbamate formation. The success of this approach has been demonstrated in a survey of whiskey, where it was found that mean levels of ethyl carbamate were considerably lower than were found in previous surveys (FSA, 2000).

8.2 Formation from urea



8.2.1 Wine (including rice wine, sake)

Urea is considered to be the most important precursor of ethyl carbamate in wine, although other sources and pathways may occur too. Urea is formed by the metabolism of arginine by arginases in the yeasts. The formation of urea depends on the yeast strain. The urea in turn dissociates to ammonium cyanate ($\text{NH}_4^+ : \text{NCO}^-$), and it is the cyanate reacting with ethanol that is the ultimate precursor of ethyl carbamate. So, for example, young wines have a tendency to have lower concentrations of ethyl carbamate than old wines, because when the urea is formed during alcoholic fermentation, there is less chance of ethanolysis occurring in the short time between bottling and consumption (Uthurry et al., 2004). Citrulline has also been identified as an important precursor. Citrulline can be formed during arginine biosynthesis.

Removal of the major precursor, urea, by an acid urease from wine (Ough & Trioli, 1988; Trioli & Ough, 1989) or sake (Yoshizawa & Takahashi, 1988) has been examined. Control measures, including the use of urease, have allowed the wine industry to reduce the levels of ethyl carbamate in table and dessert wines (Muller & Fugelsang, 1996). Clearly, using urea as a yeast food additive is also not advisable if ethyl carbamate formation may occur.

An alternative approach is to remove or suppress the metabolism of arginine to urea. Thus, in order to minimize the production of urea, a sake yeast was transformed into arginase-deficient mutants (Kitamoto et al., 1991). The stability of the culture was checked after about 100 generations, and the fermentation profiles and the sensory properties of the resulting sake were checked too. All parameters were said to be satisfactory. No urea or ethyl carbamate was detected in the sake made with transformed yeast.

A second approach is to engineer yeast strains that have enhanced urease activity, as an alternative to adding external urease activity. These urea-degrading yeasts are reported to substantially reduce the formation of ethyl carbamate in wine (First Venture, 2004).

8.3 Formation from citrulline and other *N*-carbamyl compounds

Most *N*-carbamyl compounds will react with ethanol, especially at acid pH, to form ethyl carbamate. These compounds include urea, citrulline, *N*-carbamyl α -amino acids, *N*-carbamyl β -amino acids, allantoin and carbonyl phosphate (Ough

et al., 1988). In the case of soya sauce, an accumulation of citrulline in raw soya sauce was concluded to be responsible for the occurrence of ethyl carbamate in soya sauce (Matsudo et al., 1993). It was proposed that an accumulation of citrulline during the mash stage of production was due to an abnormal lactic acid fermentation.

8.4 Formation from exogenous substances

8.4.1 Diethylpyrocarbonate

Diethylpyrocarbonate (Et-O-CO-O-CO-Et, also known as diethyl dicarbonate) has been used as a fermentation inhibitor in alcoholic and non-alcoholic beverages. It can react with ammonia to form ethyl carbamate. The formation of ethyl carbamate can be kept to low $\mu\text{g/kg}$ levels if the conditions are controlled strictly (Ough, 1976). However, because of the potential for the formation of ethyl carbamate, the previous acceptance of diethylpyrocarbonate was revoked by JECFA at its seventeenth meeting (Annex 1, reference 32).

8.4.2 Azodicarbonamide

Baxter (1992) found that bottled beer contained more ethyl carbamate than canned beer, which in turn contained more ethyl carbamate than draught beer. Baxter (1992) demonstrated that this was due to the use of azodicarbonamide ($\text{H}_2\text{N-CO-N=N-CO-NH}_2$) as a blowing agent to make the soft, foamed, polyvinyl chloride sealing gasket in the beer bottle caps. Dennis et al. (1997b) confirmed these findings and found that the content of ethyl carbamate increased with the alcohol content of the beer. They also showed that ethyl carbamate was formed when sealing gaskets from caps were refluxed with a 5% ethanol solution but not when the gaskets were refluxed with dichloromethane. Ethyl carbamate was also formed when azodicarbonamide was itself refluxed with 5% ethanol. This led the authors to conclude that azodicarbonamide was the precursor for ethyl carbamate formation. The avoidance measure is clear — either to use alternative blowing agent(s) or to prevent the use of such gaskets, made using azodicarbonamide, in contact with alcoholic beverages.

As well as use as a blowing agent, azodicarbonamide has also been used for many years as an additive to flour. The use of azodicarbonamide as a flour improver is not permitted within the European Union, but it is permitted in the United States and in other countries. Usage levels up to 45 mg/kg in flour are permitted in these countries. At the maximum usage levels, azodicarbonamide can give rise to a slight increase in the formation of ethyl carbamate in bread, especially in toasted bread (Canas et al., 1997; Dennis et al., 1997a, 1997b).

When used as a blowing agent or as a flour additive, azodicarbonamide decomposes mainly to biurea. It has been assumed that azodicarbonamide itself is the immediate precursor of ethyl carbamate formation in these two cases, reacting directly with ethanol. However, as urea is a well known precursor for ethyl carbamate formation (Zimmerli & Schlatter, 1991), it may be that biurea formed

from azodicarbonamide is the precursor to ethyl carbamate in these cases (T. Hallas-Moller, personal communication, 2002).

9. DOSE-RESPONSE ANALYSIS AND ESTIMATION OF CARCINOGENIC/TOXIC RISK

9.1 Contribution of above data to assessment of risk

9.1.1 Pivotal data from biochemical and toxicological studies

Ethyl carbamate undergoes activation to a reactive metabolite that binds covalently to nucleic acids and proteins, including to etheno-adducts that result in base-pair substitutions in DNA in tumour tissue. CYP2E1 activity is required for activation to the toxic metabolite(s). It has been proposed that activation of ethyl carbamate proceeds via oxidation of the carbon side chain of ethyl carbamate to vinyl carbamate, followed by the rapid oxidation to vinyl carbamate epoxide. Evidence for the formation of vinyl carbamate from ethyl carbamate in human liver microsomes has been reported.

In studies conducted mostly in rodents, ethyl carbamate has been shown to be genotoxic, a multisite carcinogen, a reproductive and developmental toxicant and an immunosuppressant. The most sensitive end-point of ethyl carbamate activity by the oral route was carcinogenicity. A new study of carcinogenicity in the mouse conducted by the United States National Toxicology Program was the only assay in which ethyl carbamate was administered by the oral route that satisfied the criteria for adequate group size, continuous exposure throughout the lifetime of the animals, the concurrent use of more than one dose level and adequate controls, use of a range of doses that enables the characterization of a dose-response relationship and adequate conduct of the study, survival of the animals and reporting of results. The interaction of concomitant administration of ethanol at concentrations of 2.5% and 5.0% in the drinking-water, the latter reflecting the concentration of ethanol in commonly consumed alcoholic beverages, was also investigated in this study. No data in other species meeting the above criteria were available.

In this assay, ethyl carbamate was administered at concentrations of 0, 10, 30 or 90 mg/l in drinking-water containing 0%, 2.5% or 5% ethanol *ad libitum* for 2 years, equal to 0, 1.2/0.9, 3.3/2.8 and 10.1/8.2 mg/kg bw per day in males/females for the 0% ethanol groups. Treatment with ethyl carbamate resulted in increased incidences of alveolar/bronchiolar adenoma/carcinoma, hepatocellular adenoma/carcinoma, hepatic haemangiosarcoma and Harderian gland adenoma/carcinoma, compared with their respective drinking-water control groups in both males and females, and of mammary gland adenocarcinoma or adenocarcinoma in female mice. Smaller increases in the incidence of haemangiosarcoma of the heart and squamous cell papilloma/carcinoma of the forestomach and skin in males and of haemangiosarcoma of the spleen, benign/malignant granulosa cell tumour of the ovary in females and non-neoplastic lesions in both sexes affecting the blood vessels of the liver, heart and uterus as well as eosinophilic foci in the liver were also noted. The most prominent dose-response effect was seen with

increased incidences of alveolar/bronchiolar adenoma/carcinoma and Harderian gland adenoma/carcinoma. The co-administration of ethyl carbamate and ethanol resulted in marginal changes in the incidence of some of the neoplasms attributed to ethyl carbamate alone; overall, however, there did not appear to be a definitive effect of ethanol on the carcinogenicity of ethyl carbamate.

9.1.2 Pivotal data from human clinical/epidemiological studies

No information was available from human clinical or epidemiological studies that could assist in the risk assessment of ethyl carbamate consumed in food.

9.1.3 Biomarker studies

Analytical methodology has been developed for the quantitative detection of biomarkers in haemoglobin (*N*-(2-oxoethyl)valine) (Cai et al., 1995) and urine (ethenoguanines) (Gonzalez-Reche et al., 2002) that reflect exposure to genotoxic metabolites of ethyl carbamate. In terms of their specificity for estimating exposure to ethyl carbamate, it was noted that oxoethyl adducts of haemoglobin and etheno-adducts of nucleic acids may be formed from exposure to a number of structurally related exogenous chemicals. In addition, significant background levels of these adducts suggested that endogenous substrates are also capable of forming adducts. No reports were found in which biomarkers were used as an estimate of exposure or tissue damage from ethyl carbamate.

9.2 General modelling considerations

From the available data, the most critical effect for the risk assessment of ethyl carbamate was carcinogenicity. In line with the Committee's general considerations for formulation of advice on compounds that are both genotoxic and carcinogenic (Annex 1, reference 174), dose–response modelling of the toxicological data was used to determine a basis for hazard characterization. In general, dose–response modelling of toxicological data is used to determine a point of departure for further risk assessment. Dose–response data were used to derive a lower confidence limit of the benchmark dose (BMDL) for a 10% incidence of tumours, which served as a point of departure, as described in section 2.1 of the report of the sixth-fourth meeting (Annex 1, reference 174), reproduced here as Annex 5.

9.2.1 Selection of data

The 2004 National Toxicology Program 2-year bioassay in the mouse was considered to be the pivotal study for risk assessment. This was the only study that met the criteria for modelling of the dose–response data for lifetime exposure to ethyl carbamate in foods. In this study, the effects of co-administration of ethanol were included. The most prominent dose–response effect of ethyl carbamate in this study was seen with increased incidences of alveolar and bronchiolar adenoma and carcinoma and of Harderian gland adenoma and carcinoma.

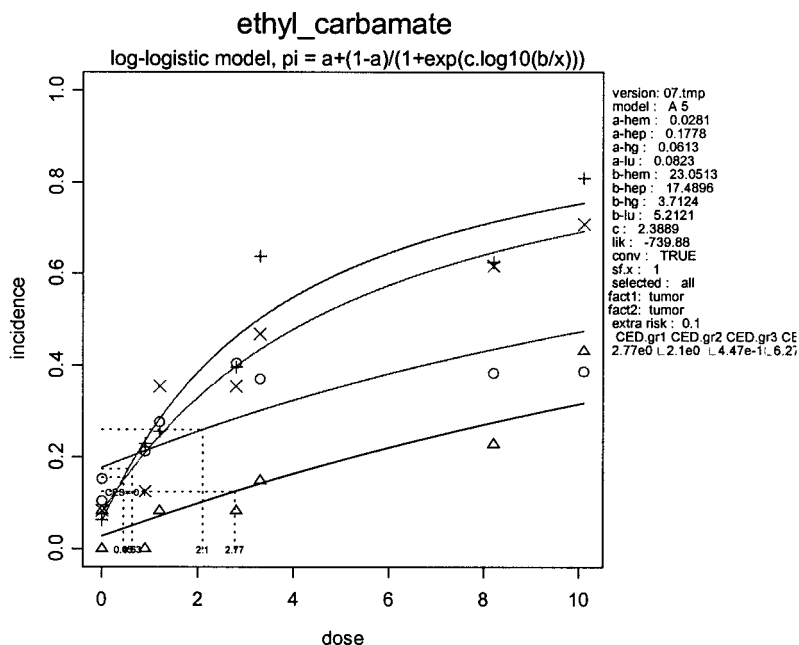
9.2.2 Measure of exposure

Ethyl carbamate was administered in the drinking-water of mice in the National Toxicology Program bioassay. The dose of ethyl carbamate in mg/kg bw per day received over the 2-year study period was calculated for each dose group from ethyl carbamate concentration in water, water consumption and body weight values over the lifetime of the animals.

9.2.3 Measure of response

Tumour incidence data for the four most prominent tumour types — alveolar and bronchiolar adenoma and carcinoma, Harderian gland adenoma and carcinoma, hepatocellular adenoma and carcinoma and hepatic haemangiosarcoma — are shown in Figure 3. The lung and Harderian gland tumours were more sensitive end-points than either of the tumour types occurring in the liver. The analysis of the data indicated that for tumours of the lung and liver, co-exposure to ethanol resulted in less steep dose-response curves than with ethyl carbamate alone.

Figure 3. Incidences of four tumour types. Plusses: Harderian glands (hg), crosses: lung (lu), circles: hepatocellular (hep), triangles: haemangiosarcoma (hem). Dose is in mg/kg bw per day.



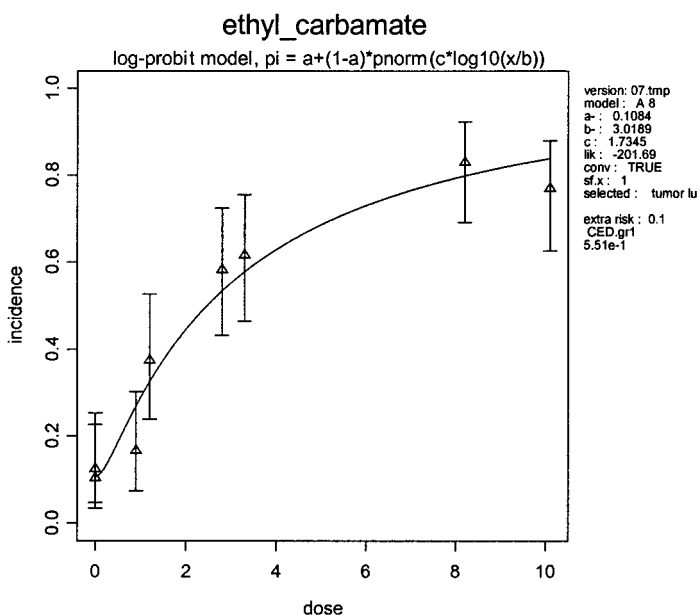
9.2.4 Selection of mathematical model

(a) Lung tumours

Figure 4 shows the observed incidences of lung tumours, with the log-probit model fitted to them. Here, both sexes are plotted, and a single curve is fitted to these data, since the analysis revealed no significant differences in dose-response between sexes. The observed incidences provide good information on the dose-response relationship and are quite suitable for dose-response modelling.

The best fit of the Weibull model resulted in a curve with infinite slope at dose zero. Therefore, this model was refit imposing the constraint on the shape parameter precluding infinite slope at dose zero. Table 7 summarizes the results of the fits of the models used.

Figure 4. Observed incidences of lung tumours, with the log-probit model fitted to them. Dose is in mg/kg bw per day.



(b) Harderian gland tumours

Figure 5 shows the observed incidences of Harderian gland tumours, with the log-probit model fitted to them. Again, both sexes are plotted, as no significant differences in dose-response between sexes were found. The observed incidences provide good information on the dose-response relationship and are quite suitable for dose-response modelling.

Table 7. Summary of modelling results for (all) lung tumours^a

Model	Log-likelihood	No. of parameters	mg/kg bw per day	
			BMD ₁₀	BMDL ₁₀ ^b
One-stage	-204.03	2	0.55	0.46
Two-stage	-204.03	3		
Log-logistic	-201.69	3	0.50	0.26
Log-probit	-201.69	3	0.55	0.30
Weibull ^c	-202.95	3		
Weibull ^d	-204.03	3	0.55	0.47
Proast M2	-212.78	2		
Proast M4	-200.55	3	0.63	0.51
Maximal model	-198.67	8		

BMD₁₀, benchmark dose for a 10% incidence of tumours; BMDL₁₀, lower confidence limit of the benchmark dose for a 10% incidence of tumours

- ^a No significant differences between sexes.
- ^b Based on 500 bootstrap runs.
- ^c Fitted without constraint on the shape parameter, resulting in fitted model having infinite slope at dose 0.
- ^d Fitted with constraint on shape parameter to avoid infinite slope at dose zero.

Figure 5. Observed incidences of Harderian gland tumours, with the log-probit model fitted to them. Dose is in mg/kg bw per day.

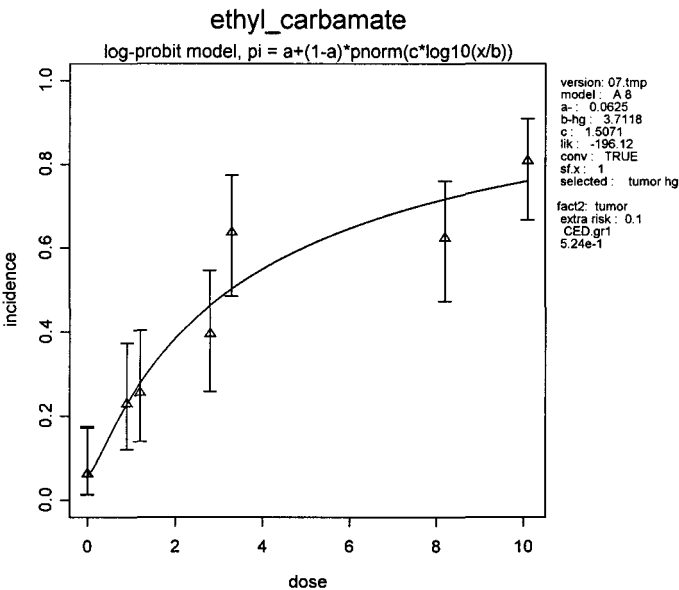


Table 8. Summary of modelling results for Harderian gland tumours^a

Model	Log-likelihood	No. of parameters	mg/kg bw per day	
			BMD ₁₀	BMDL ₁₀ ^b
One-stage	-197.96	2	0.66	0.57
Two-stage	-197.96	3		
Log-logistic	-196.16	3	0.47	0.36
Log-probit	-196.12	3	0.52	0.28
Weibull ^c	-197.96	3	0.66	0.61
Proast M2	-206.00	2		
Proast M4	-196.55	3	0.76	0.59
Maximal model	-192.60	8		

BMD₁₀, benchmark dose for a 10% incidence of tumours; BMDL₁₀, lower confidence limit of the benchmark dose for a 10% incidence of tumours

^a No significant differences between sexes.

^b Based on 100 bootstrap runs.

^c Model fitted by constraining the shape parameter to avoid infinite slope at dose zero.

As for the lung tumours, the best fit of the Weibull model resulted in a curve with infinite slope at dose zero, and therefore the constraint to avoid this was imposed in fitting the model. Table 8 summarizes the results of the fits of the models used.

10. COMMENTS

10.1 Absorption, distribution, metabolism and excretion

Ethyl carbamate is well absorbed from the gastrointestinal tract (and skin) and is rapidly and evenly distributed throughout the body. Elimination is also rapid, with >90% being eliminated as carbon dioxide within 6 h in mice. Metabolic pathways of potential importance include hydrolysis to ethanol and ammonia and side-chain oxidation to vinyl carbamate. In rats and mice, CYP2E1 activity is responsible for about 95% of the metabolism of ethyl carbamate to carbon dioxide. Ethyl carbamate undergoes CYP2E1-mediated metabolic activation to vinyl carbamate epoxide, which binds covalently to nucleic acids and proteins, resulting in the formation of adducts, including those that have been shown to induce base-pair substitutions in DNA from tumour tissue. It has been hypothesized that co-administration of ethyl carbamate with ethanol reduces CYP2E1-mediated activation and increases elimination by esterase-mediated hydrolysis. High doses of ethanol (4 ml/kg bw in one study and 5 g/kg bw in another) given to mice 1 h before, or at the same time as, ethyl carbamate delayed elimination as carbon dioxide; in contrast, pretreatment with 10% ethanol in drinking-water for 3 weeks had no effect.

10.2 Toxicological data

The acute oral toxicity of ethyl carbamate is low, the oral LD₅₀ in rodents being approximately 2000 mg/kg bw. In rodents, single doses of 1000 mg/kg bw cause anaesthesia.

In mice and rats given drinking-water containing ethyl carbamate for 13 weeks, there was an increase in mortality at doses of about 500–600 mg/kg bw per day. In the same study, mice given ethyl carbamate at doses of ≥ 150 mg/kg bw per day showed reduced body weight gain and effects on the lungs, liver, kidney, heart, spleen, lymph nodes, thymus, bone marrow and ovaries. No such effects were seen at 50 mg/kg bw per day. The same organs, with the exception of the lungs, were affected in rats given drinking-water containing ethyl carbamate at the same concentrations as those at which these effects were observed in mice. A treatment-related decrease in serum lymphocyte and leukocyte counts was observed in rats; no effects were seen in male rats at a dose of about 10 mg/kg bw per day, the lowest dose tested, while a decrease was observed in the females at this dose. Serum haematological parameters were not assessed in mice, but cellular depletion of the spleen and thymus were noted in assays of immunotoxicity in mice treated intraperitoneally with ethyl carbamate at a higher range of doses (100–400 mg/kg bw per day) for 1–2 weeks. Co-administration of 5% ethanol in drinking-water with ethyl carbamate at concentrations spanning the range used in the 13-week study in mice and rats (110–10 000 mg/l) attenuated many of the adverse effects of ethyl carbamate.

Ethyl carbamate has been tested in a large number of studies of genotoxicity *in vitro* and *in vivo*. The results of assays for point mutations were uniformly negative for mouse lymphoma cells, while assays in bacterial, yeast and other types of mammalian cells produced variable results. Results of assays in somatic cells *in vivo* (including tests for induction of chromosomal aberrations, micronucleus formation and sister chromatid exchange) were almost uniformly positive. The assay for micronucleus formation in mice showed the strongest positive response, and co-administration of ethanol delayed rather than prevented the genotoxicity of ethyl carbamate in this assay.

There was no evidence for genotoxicity in mammalian germ cells *in vivo*, according to the results of assays for dominant lethal mutations or in specific locus tests in mice given ethyl carbamate by intraperitoneal injection or in drinking-water. Treatment of mice with high doses of ethyl carbamate administered by the subcutaneous or intraperitoneal route before mating resulted in increased incidences of tumours in adult offspring.

In most studies of developmental toxicity in which mice, rats or hamsters were given ethyl carbamate at high doses administered by various routes, very high rates of embryonic/fetal mortality and malformations were revealed. In the only two available studies in which ethyl carbamate was administered orally, dose-related increases in skeletal anomalies were observed in mice given ethyl carbamate as a single dose at ≥ 300 mg/kg bw on day 11 of gestation, and increases in external malformations and skeletal abnormalities were observed in rats given ethyl carbamate at a dose of 1000 mg/kg bw for 1, 2 or 7 days during the period of

organogenesis. Oral doses of ethyl carbamate that show no effect have not been established. No multigeneration studies that met currently accepted standard protocols were available.

Ethyl carbamate is a multisite carcinogen with a short latency period. Single doses or short-term oral dosing at 100–2000 mg/kg bw have been shown to induce tumours in mice, rats and hamsters. The upper range of these doses overlaps with the standard anaesthetic dose (1000 mg/kg bw) and the values for LD₅₀s in rodents. In addition, in non-human primates given ethyl carbamate at a dose of 250 mg/kg bw per day by oral administration for 5 years, a variety of tumour types that were analogous to those observed in rodents (including adenocarcinoma of the lung, hepatocellular adenoma and carcinoma and hepatic haemangiosarcoma) were induced over an observation period of up to 22 years. Treatment of female mice with single or multiple doses of ethyl carbamate during gestation or lactation was found to increase the incidence or multiplicity of tumours in the adult offspring compared with untreated controls.

In a newly available lifetime study of carcinogenicity, male and female B6C3F1 mice were given drinking-water containing ethyl carbamate at a concentration of 0, 10, 30 or 90 mg/l together with ethanol at a concentration of 0%, 2.5% or 5%. Results from the animals that did not receive ethanol were used as the basis of the present evaluation. In these animals, intakes of ethyl carbamate were equal to approximately 0, 1, 3 or 9 mg/kg bw per day, respectively. Treatment with ethyl carbamate resulted in dose-dependent increased incidences of alveolar and bronchiolar, hepatocellular and Harderian gland adenoma or carcinoma, hepatic haemangiosarcoma and mammary gland adenoacanthoma or adenocarcinoma (females only). Smaller, but still statistically significant, increases were observed in the incidence of haemangiosarcoma of the heart (males only) and spleen (females only), squamous cell papilloma or carcinoma of the forestomach and skin (males only) and benign or malignant ovarian granulosa cell tumours. Dose-related increases in non-neoplastic lesions affecting the blood vessels of the liver, heart and uterus as well as eosinophilic foci of the liver were also observed. The most sensitive sites for tumour induction (i.e. those at which a significant increase in tumours was observed at the lowest dose tested) were the lung and Harderian gland. The incidences of combined alveolar and bronchiolar adenoma or carcinoma were 5/48, 18/48, 29/47, 37/48 (males); and 6/48, 8/48, 28/48, 39/47 (females). The incidences of combined Harderian gland adenomas or carcinomas were 3/47, 12/47, 30/47, 38/47 (males); and 3/48, 11/48, 19/48, 30/48 (females).

There was also a treatment-related increase in the combined incidence of any tumour type at any site (males: 33/48, 39/48, 46/47, 47/48; females: 37/48, 35/48, 45/48, 47/48). The co-administration of ethyl carbamate and ethanol resulted in marginal changes in the incidence of some of the neoplasms attributed to ethyl carbamate alone, but overall, co-administration of ethanol had no consistent effect on the carcinogenicity of ethyl carbamate. The absence of a clear interaction between ethyl carbamate and ethanol with regard to tumour incidence is consistent with data on CYP2E1, glutathione and apoptosis in the liver, PCNA labelling in the lung and etheno-adducts in liver and lung reported in a related 4-week study in mice given the same treatment regimens.

10.3 Observations in humans

Very few data were available, and these were not of a quality that could be used for hazard characterization.

10.4 Analytical methods

Over the past 30 years, methods have been developed for the extraction and analysis of ethyl carbamate in all the food and beverage types in which the substance is known to be formed. These methods have been tested in two international collaborative trials, for application to beers and whiskeys and to wine, fortified wine, spirits and soya sauce.

As a small organic ester, ethyl carbamate is suitable for analysis using GC. Ethyl carbamate labelled with heavy isotopes (deuterium and/or ^{15}N) is available for use as an internal standard during analysis. The use of GC coupled with MS gives confidence in the analytical aspects of correct identification and quantification in food and beverages. The methods are capable of routinely detecting ethyl carbamate at concentrations of 1 $\mu\text{g/kg}$ and have limits of quantification in the range of 3–5 $\mu\text{g/kg}$ in food and beverages. Sensitivity is further improved if extra sample cleanup and concentration steps are performed.

10.5 Levels and pattern of food contamination

Data on concentrations of ethyl carbamate in foods and beverages were submitted by the Food and Drug Administration of the United States, the Food Standards Agency of the United Kingdom and the Wine Institute of the United States. The alcoholic beverages considered in these reports originate from many countries throughout the world. Means and ranges of concentrations of ethyl carbamate in foods and beverages as reported in publications or data submitted to the Committee are presented in Table 5 above. For alcoholic beverages, only recent data were included because concentrations have been reduced considerably over time as a result of the application of mitigation measures.

10.6 Prevention and control

The key to successful prevention and control for ethyl carbamate has been the identification of the main precursor substances responsible for the formation of ethyl carbamate in food and beverages, together with an understanding of the influence of the main external factors of light, time and temperature. This information has led to a mechanistic understanding from which control measures have been devised. Ethyl carbamate can be formed from various substances derived from food and beverages, including hydrogen cyanide, urea, citrulline and other *N*-carbamyl compounds. Cyanate is probably the ultimate precursor in most cases, reacting with ethanol to form the carbamate ester. Over the past years, major reductions in concentrations of ethyl carbamate have been achieved using two approaches: first, by reducing the concentration of the main precursor substances in the food or beverage; second, by reducing the tendency for these substances to

react to form cyanate, e.g. by the exclusion of light from bottled stone-fruit brandies.

Diethylpyrocarbonate, which is an inhibitor of fermentation, can form ethyl carbamate, and for this reason the previous acceptance of diethylpyrocarbonate was revoked by the Committee at its seventeenth meeting (Annex 1, reference 32). A second exogenous precursor for ethyl carbamate, azodicarbonamide, which has been used as a blowing agent to make certain sealing gaskets, is not recommended for bottling alcoholic beverages. The use of azodicarbonamide as a dough maturing agent is permitted in some countries; at the maximum usage levels, it can give rise to a slight increase in the formation of ethyl carbamate in bread.

10.7 Dietary intake assessment

The Committee evaluated four published national estimates of intake (Denmark, Republic of Korea, Switzerland and the United States) and two estimates submitted to the Committee by Australia and New Zealand. The national estimates of mean intake of ethyl carbamate from both food and alcoholic beverages for the population as a whole ranged from approximately 1 to 4 µg/person per day, equivalent to approximately 15–65 ng/kg bw per day. The more recent national estimates of mean intake from Australia (1.4 µg/person per day), Republic of Korea (0.6 µg/person per day) and New Zealand (1.4 µg/person per day) used concentrations of ethyl carbamate in alcoholic beverages that were much lower than those considered at the time of the assessments in Denmark, Switzerland and the United States, which were conducted in the early 1990s. The Committee noted that mitigation measures have been effective in reducing residual concentrations of ethyl carbamate in alcoholic beverages and that, consequently, the older data published in the early 1990s and used to make the initial estimates of intake of ethyl carbamate no longer accurately reflect current intake from alcoholic beverages.

The Committee prepared international estimates of intake of ethyl carbamate from foods using the five regional diets of the GEMS/Food database. The relevant foods included in the analyses were bread, fermented milk products (including yoghurt and cheese) and soya sauce; alcoholic beverages (with the exception of wine) are not included in the GEMS/Food database and consequently were not considered in the analyses. The concentrations of ethyl carbamate used were mean values taken from published summaries. The mean intake of ethyl carbamate from food was estimated to be approximately 1 µg/person per day, equivalent to about 15 ng/kg bw per day, for the five regional diets. This value was consistent with the contribution of ethyl carbamate to intake from food in the national estimates, when alcoholic beverages were excluded.

The intake of ethyl carbamate for a high-percentile consumer of alcoholic beverages was modelled using an average concentration of ethyl carbamate of 4 µg/kg in wines (data from 2001) and a 95th-percentile intake of approximately 750 ml of wine (data from France). It was calculated that an additional 3 µg/person per day could be consumed, which when added to the international and national

estimates of intake from food of approximately 1 µg/person per day resulted in a total intake of ethyl carbamate of up to 5 µg/person per day after rounding to one significant figure (equivalent to 80 ng/kg bw per day). The Committee was aware that high concentrations of ethyl carbamate can be found in stone-fruit brandies and, therefore, that high consumption of such brandies could lead to higher intakes of ethyl carbamate than those considered here.

10.8 Dose-response analysis

The Committee concluded that ethyl carbamate is genotoxic and is a multisite carcinogen in all species tested. The pivotal study for risk assessment was a recent long-term study of carcinogenicity in mice. The increased incidence of alveolar and bronchiolar adenoma or carcinoma in mice was considered to be a critical response, and the associated dose-response data were analysed. The dose-response data for animals with Harderian gland tumours were also analysed. The dose-response data for the total number of tumour-bearing animals were not considered suitable for modelling, since the background incidence was already about 75%. In the dose-response analysis, eight different statistical models were fitted to the experimental data considered relevant for further consideration. Those resulting in acceptable fits based on biological and statistical considerations were selected to derive the BMD and BMDL for a 10% extra risk of tumours. This procedure results in a range of BMD and BMDL values for each end-point considered (Table 9). The dose-response relationships appeared not to differ statistically significantly between males and females, and the models were fitted to the combined data for both sexes. For both site-specific tumour types, the dose-response data left relatively little uncertainty about the shape of the dose-response curve. As a result, the ranges of the BMD and BMDL values were quite narrow, while the BMDLs were not much lower than their associated BMDs.

Choosing lung tumours as the critical end-point, the values for BMDLs ranged from 0.3 to 0.5 mg/kg bw per day. The Committee decided to use the more conservative lower end of this range of values for the evaluation. The Committee used these BMDLs to estimate margins of exposure (MOEs).

11. EVALUATION

When the estimated intake of ethyl carbamate in foods (15 ng/kg bw per day) is compared with the lower end of the range of BMDL values obtained for the incidence of alveolar and bronchiolar neoplasms in male and female mice (0.3 mg/kg bw per day), the resulting MOE is 20 000. With the inclusion of alcoholic beverages in the estimated intakes (80 ng/kg bw per day), the resulting MOE is 3800. On the basis of these considerations, the Committee concluded that exposure to ethyl carbamate in food would be of low concern. The MOE for all intakes, food and beverages, is of concern, and therefore mitigation measures to reduce concentrations of ethyl carbamate in some alcoholic beverages should be continued.

Table 9. Ranges of BMD and BMDL values for tumours associated with administration of ethyl carbamate

Tumour type	Range of BMD values (mg/kg bw per day)	Range of BMDL values (mg/kg bw per day)
Lung adenoma or carcinoma	0.50–0.63	0.26–0.51
Harderian gland adenoma or carcinoma	0.47–0.76	0.28–0.61

BMD, benchmark dose for 10% extra risk of tumours; BMDL, 95% lower confidence limit for the benchmark dose. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls.

12. REFERENCES

- Abe, S. & Sasaki, M. (1977) Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. *J. Natl. Cancer Inst.*, **58**, 1635–1641.
- Abe, S. & Sasaki, M. (1982) Induction of sister-chromatid exchanges by indirect mutagens/carcinogens in cultured rat hepatoma and esophageal tumor cells and in Chinese hamster Don cells co-cultivated with rat cells. *Mutat. Res.*, **93**, 409–418.
- Abraham, S.K. & Graf, U. (1996) Protection by coffee against somatic genotoxicity in *Drosophila*: role of bioactivation capacity. *Food Chem. Toxicol.*, **34**, 1–14.
- Adler, I.D., Anderson, D., Benigni, R., Ehling, U.H., Laehdetie, J., Pacchierotti, F., Russo, A. & Tate, A.D. (1996) Synthesis report of the step project detection of germ cell mutagens. *Mutat. Res.*, **353**, 65–84.
- Agrelo, C.E. & Severin, B.J. (1981) A simplified method for measuring scheduled and unscheduled DNA synthesis in human fibroblasts. *Toxicology*, **21**, 151–158.
- Alcohol and Tobacco Tax and Trade Bureau (2004) Submission to the 64th JECFA meeting: 1998 surveillance data. Washington, D.C.: United States Department of the Treasury.
- Aldovini, A. & Ronchese, F. (1983) Different susceptibility of BALB/Mo and BALB/c mice to cytogenetic damage induced by urethan. *Tumori*, **69**, 387–390.
- Allen, J.W., Langenbach, R. & Leavitt, S. (1982a) SCE and gene mutation studies with ethyl carbamate, ethyl *N*-hydroxycarbamate, and vinyl carbamate: Potencies and species, strain, tissue specificities. *Banbury Rep.*, **13**, 293–305.
- Allen, J.W., Langenbach, R., Nesnow, S., Sasseville, K., Leavitt, S., Campbell, J., Brock, K. & Sharief, Y. (1982b) Comparative genotoxicity studies of ethyl carbamate and related chemicals: further support for vinyl carbamate as a proximate carcinogenic metabolite. *Carcinogenesis*, **3**, 1437–1441.
- Allen, J.W., Stoner, G.D., Pereira, M.A., Backer, L.C., Sharief, Y., Hatch, G.G., Campbell, J.A., Stead, A.G. & Nesnow, S. (1986) Tumorigenesis and genotoxicity of ethyl carbamate and vinyl carbamate in rodent cells. *Cancer Res.*, **46**, 4911–4915.
- Altmann, H.J., Dusemund, B., Goll, M. & Grunow, W. (1991) Effect of ethanol on the induction of lung tumours by ethyl carbamate in mice. *Toxicology*, **68**, 195–201.
- Amacher, D.E. & Turner, G.N. (1982) Mutagenic evaluation of carcinogens and non-carcinogens in the L5178Y/TK assay utilizing postmitochondrial fractions (S9) from normal rat liver. *Mutat. Res.*, **97**, 49–65.

- Anderson, L.M. (1978) Two crops of primary lung tumours in BALB/c mice after a single transplacental exposure to urethane. *Cancer Lett.*, **5**, 55–59.
- Ashby, J., Tinwell, H. & Callander, R.D. (1990) Activity of urethane and *N,N*-dimethyl-urethane in the mouse bone-marrow micronucleus assay: Equivalence of oral and intraperitoneal routes of exposure. *Mutat. Res.*, **245**, 227–230.
- Australia (2004) Submission to the 64th JECFA meeting. Canberra: Food Standards Australia New Zealand.
- Bailey, R., North, D., Myatt, D. & Lawrence, J.F. (1986) Determination of ethyl carbamate in alcoholic beverages by methylation and gas-chromatography with nitrogen-phosphorus thermionic detection. *J. Chromatogr.*, **369** (1), 199–202.
- Balansky, R., Blagoeva, P. & Mircheva, Z. (1992) Clastogenic activity of urethane in mice. *Mutat. Res.*, **281**, 99–103.
- Barale, R., Scapoli, C., Falezza, A., Ventura, L., Bernacchi, F., Loprieno, N. & Barrai, I. (1992) Skin cytogenetic assay for the detection of clastogens–carcinogens topically administered to mice. *Mutat. Res.*, **271**, 223–230.
- Barbin, A., Wang, R., O'Connor, P.J. & Elder, R.H. (2003) Increased formation and persistence of 1,*N*⁶-ethenoadenine in DNA is not associated with higher susceptibility to carcinogenesis in alkylpurine-DNA-*N*-glycosylase knockout mice treated with vinyl carbamate. *Cancer Res.*, **63**, 7699–7703.
- Bateman, A.J. (1967) A failure to detect any mutagenic action of urethane in the mouse. *Mutat. Res.*, **4**, 710–712.
- Battaglia, R., Conacher, H.B.S. & Page, B.D. (1990) Ethyl carbamate (urethane) in alcoholic beverages and foods: a review. *Food Addit. Contam.*, **7** (4), 477–496.
- Baumann, U. & Zimmerli, B. (1986) Gaschromatographische bestimmung von urethan (ethylcarbamate) in alkoholischen getränken. *Mitt. Geb. Lebensmittelunters. Hyg.*, **77**, 327–322.
- Baxter, E.D. (1992) Food safety issues in relation to small packaged beers. *Fermentation*, **5** (1), 79–81.
- Beland, F.A., Benson, R.W., Mellick, T.W., Kovatch, R.M., Roberts, D.W., Fang, J.L. & Doerge, D.R. (2005) Effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in B6C3F1 mice. *Food Chem. Toxicol.*, **41** (1), 1–19.
- Boiato, L., Mirvish, S.S. & Berenblum, I. (1966) The carcinogenic action and metabolism of *N*-hydroxyurethane in newborn mice. *Int. J. Cancer*, **1**, 265–269.
- Boyland, E. (1968) The biochemistry of urethane. *N. Z. Med. J.*, **67**, 4–7.
- Boyland, E. & Nery, R. (1965) The metabolism of urethane and related compounds. *Biochem. J.*, **94**, 198–208.
- Boyland, E. & Rhoden, E. (1949) The distribution of urethane in animal tissues, as determined by a microdiffusion method, and the effect of urethane treatment on enzymes. *Biochem. J.*, **44**, 528–531.
- Boyland, E. & Williams, K. (1969) Reaction of urethane with nucleic acids in vivo. *Biochem. J.*, **111**, 121–127.
- Bruce, W.R. & Heddle, J.A. (1979) The mutagenic activity of 61 agents as determined by the micronucleus, *Salmonella*, and sperm abnormality assays. *Can. J. Genet. Cytol.*, **21**, 319–334.
- Bryan, C.E., Skipper, H.E. & White, L. (1949) Carbamates in the chemotherapy of leukemia. IV. The distribution of radioactivity in tissues of mice following injection of carbonyl-labeled urethane. *J. Biol. Chem.*, **177**, 941–950.

- Burkard, W. & Fritz-Niggli, H. (1987) Antiteratogenic and anticarcinogenic effects of X-rays in urethane-treated NMRI mice. *Int. J. Radiat. Biol.*, **51**, 1031–1039.
- CAC (2003) *Report of the Thirty-fifth Session of the Codex Committee on Food Additives and Contaminants, Arusha, Tanzania, 17–21 March 2003*. Rome: Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ALINORM 03/12A; <http://www.codexalimentarius.net/web/archives.jsp?year=03>).
- Cai, J., Myers, S.R. & Hurst, H.E. (1995) Measurement of the hemoglobin N-(2-oxoethyl)-valine adduct in ethyl carbamate-treated mice. *Toxicol. Appl. Pharmacol.*, **131**, 73–79.
- Canas, B.J., Havery, D.C. & Joe, F.L. (1988) Rapid gas-chromatographic method for determining ethyl carbamate in alcoholic beverages with thermal-energy analyzer detection. *J. Assoc. Off. Anal. Chem.*, **71** (3), 509–511.
- Canas, B.J., Havery, D.C., Robinn, L.R., Sullivan, M.P., Joe, F.L. & Diachenko, G.W. (1989) Ethyl carbamate levels in selected fermented foods and beverages. *J. Assoc. Off. Anal. Chem.*, **72** (6), 873–876.
- Canas, B.J., Joe, F.L., Diachenko, G.W. & Burns, G. (1994) Determination of ethyl carbamate in alcoholic beverages and soy-sauce by gas-chromatography with mass-selective detection — collaborative study. *J. AOAC Int.*, **77** (6), 1530–1536.
- Canas, B.J., Diachenko, G.W. & Nyman, P.J. (1997) Ethyl carbamate levels resulting from azodicarbonamide use in bread. *Food Addit. Contam.*, **14** (1), 89–94.
- Carlson, G.P. (1994) The effect of inducers and inhibitors of urethane metabolism on its in vitro and in vivo metabolism in rats. *Cancer Lett.*, **87**, 145–150.
- Carmichael, N.G., Debruyne, E.L.M. and Bigot-Lasserre, D. (2000) The p53 heterozygous knockout mouse as a model for chemical carcinogenesis in vascular tissue. *Environ. Health Perspect.*, **108**, 61–65.
- Cattanach, B.M., Papworth, D., Patrick, G., Goodhead, D.T., Hacker, T., Cobb, L. & Whitehill, E. (1998) Investigations of lung tumour induction in C3H/HeH mice, with and without tumour promotion with urethane, following paternal X-irradiation. *Mutat. Res.*, **403**, 1–12.
- Cha, S.W., Gu, H.K., Lee, K.P., Lee, M.H., Han, S.S. & Jeong, T.C. (2000) Immunotoxicity of ethyl carbamate in female BALB/c mice: role of esterase and cytochrome P450. *Toxicol. Lett.*, **115**, 173–181.
- Cha, S.W., Lee, H.J., Cho, M.H., Lee, M.H., Koh, W.S., Han, S., Kim, J., Nam, D. & Jeong, T.C. (2001) Role of corticosterone in ethyl carbamate-induced immunosuppression in female BALB/c mice. *Toxicol. Lett.*, **119**, 173–181.
- Chaube, S. & Murphy, M.L. (1966) The effects of hydroxyurea and related compounds on the rat fetus. *Cancer Res. Part 1*, **26**, 1448–1457.
- Chavan, B.G. & Bhide, S.V. (1973) Binding of urethane with macromolecules from cell organelles. *J. Natl. Cancer Inst.*, **50**, 1459–1461.
- Cheng, M., Conner, M.K. & Alarie, Y. (1981a) Multicellular in vivo sister-chromatid exchanges induced by urethane. *Mutat. Res.*, **88**, 223–231.
- Cheng, M., Conner, M.K. & Alarie, Y. (1981b) Potency of some carbamates as multiple tissue sister chromatid exchange inducers and comparison with known carcinogenic activities. *Cancer Res.*, **41**, 4489–4492.
- Chieco-Bianchi, L., Aldovini, A., Ronchese, F., De Rossi, A., Majone, F., Montaldi, A. & Levis, A.G. (1984) Short- and long-term studies on chemical carcinogenesis in BALB/Mo mice. *Toxicol. Pathol.*, **12**, 361–368.

- Choy, W.N., Black, W., Mandakas, G., Mirro, E.J. & Black, H.E. (1995) A pharmacokinetic study of ethanol inhibition of micronuclei induction by urethane in mouse bone marrow erythrocytes. *Mutat. Res.*, **341**, 255–263.
- Choy, W.N., Mandakas, G. & Paradisin, W. (1996) Co-administration of ethanol transiently inhibits urethane genotoxicity as detected by a kinetic study of micronuclei induction on mice. *Mutat. Res.*, **367**, 237–244.
- Cividalli, G., Mirvish, S.S. & Berenblum, I. (1965) The catabolism of urethan in young mice of varying age and strain, and in x-irradiated mice, in relation to urethan carcinogenesis. *Cancer Res.*, **25**, 855–858.
- Clegg, B.S. and Frank, R. (1988) Detection and quantitation of trace levels of ethyl carbamate in alcoholic beverages by selected ion monitoring. *J. Agric. Food Chem.*, **36** (3), 502–505.
- Conner, M.K. (1986) Induction of sister chromatid exchange by ethyl carbamate and vinyl carbamate. *IARC Sci. Publ.*, **70**, 313–320.
- Conner, M.K. & Cheng, M. (1983) Persistence of ethyl carbamate-induced DNA damage in vivo as indicated by sister chromatid exchange analysis. *Cancer Res.*, **43**, 965–971.
- Csukas, I., Gungl, E., Fedorcsak, I., Vida, G., Antoni, F., Turtoczky, I. & Solymosy, F. (1979) Urethane and hydroxyurethane induce sister-chromatid exchanges in cultured human lymphocytes. *Mutat. Res.*, **67**, 315–319.
- Csukas, I., Gungl, E., Antoni, F., Vida, G. & Solymosy, F. (1981) Role of metabolic activation in the sister chromatid exchange-inducing activity of ethyl carbamate (urethane) and vinyl carbamate. *Mutat. Res.*, **89**, 75–82.
- Dahl, G.A., Miller, J.A. & Miller, E.C. (1978) Vinyl carbamate as a promutagen and a more carcinogenic analog of ethyl carbamate. *Cancer Res.*, **38**, 3793–3804.
- Dahl, G.A., Miller, E.C. & Miller, J.A. (1980) Comparative carcinogenicities and mutagenicities of vinyl carbamate, ethyl carbamate, and ethyl *N*-hydroxycarbamate. *Cancer Res.*, **40**, 1194–1203.
- Daston, G.P., Overmann, G.J., Taubemeck, M.W., Lehman-McKeenan L.D., Rogers, J.M. & Keen, C.L. (1991) The role of metallothionein induction and altered zinc status in maternally mediated developmental toxicity: comparison of the effects of urethane and styrene in rats. *Toxicol. Appl. Pharmacol.*, **110**, 450–463.
- Dean, B.J. (1969) Chemical-induced chromosome damage. *Lab. Anim.*, **3**, 157–174.
- De Flora, S., Astengo, M., Serra, D. & Bennicelli, C. (1986) Inhibition of urethane-induced lung tumors in mice by dietary *N*-acetylcysteine. *Cancer Lett.*, **32**, 235–241.
- Dennis, M.J., Howarth, N., Key, P.E., Pointer, M. & Massey, R.C. (1989) Investigation of ethyl carbamate levels in some fermented foods and alcoholic beverages. *Food Addit. Contam.*, **6** (3), 383–389.
- Dennis, M.J., Massey, R.C., Pointer, M. & Willetts, P. (1990) Co-operative trial studies on the analysis of ethyl carbamate using capillary gas-chromatography. *J. High Resolut. Chromatogr.*, **13** (4), 247–251.
- Dennis, M.J., Massey, R.C., Ginn, R., Parker, I., Crews, C., Zimmerli, B., Zoller, O., Rhyn, P. & Osborne, B. (1997a) The effect of azodicarbonamide concentrations on ethyl carbamate concentrations in bread and toast. *Food Addit. Contam.*, **14** (1), 95–100.
- Dennis, M.J., Massey, R.C., Ginn, R., Willetts, P., Crews, C. & Parker, I. (1997b) The contribution of azodicarbonamide to ethyl carbamate formation in bread and beer. *Food Addit. Contam.*, **14** (1), 101–108.
- D'Hauteville, F., Laporte, J.P., Morrot, G. & Sirieix, L. (2001) *La consommation de vin en France: comportements, attitudes et représentations. Resultats d'enquête ONIVINS-*

- INRA 2000. Office National Interprofessionnel des Vins (ONIVINS) and National Institute for Agricultural Research (INRA).
- Diachenko, G.M., Canas, B.J., Joe, F.L. & DiNovi, M. (1992) Ethyl carbamate in alcoholic beverages and fermented foods. In: Finley, J.W., Robinson, S.F. & Armstrong, D.J., eds., *Food Safety Assessment*. Washington, D.C.: American Chemical Society, pp. 419–428 (ACS Symposium Series 48).
- DiPaolo, J.A. (1962) Effect of oxygen concentration on carcinogenesis induced by transplacental exposure to urethane. *Cancer Res.*, **22**, 299–304.
- DiPaolo, J.A. & Elis, J. (1967) The comparison of teratogenic and carcinogenic effects of some carbamate compounds. *Cancer Res.*, **27**, 1696–1701.
- DiPaolo, J.A. & Elis, J. (1970) Effect of actinomycin D and urethan on successive litters and generations of mice. *Teratology*, **3**, 53–58.
- Director, A.E., Tucker, J.D., Ramsey, M.J. & Nath, J. (1998) Chronic ingestion of clastogens by mice and the frequency of chromosome aberrations. *Environ. Mol. Mutagen.*, **32**, 139–147.
- Diwan, B.A. & Batra, B.K. (1972) Effects of urethane on embryonic development in the mouse. *Indian J. Exp. Biol.*, **10**, 81–83.
- Diwan, B.A., Batra, B.K. & D'Souza, A.V. (1970) Effects of urethane on embryonic development in the mouse. I. The period of organogenesis. *Indian J. Exp. Biol.*, **8**, 167–170.
- Dragani, T.A., Sozzi, G. & Della, P.G. (1983) Comparison of urethane-induced sister-chromatid exchanges in various murine strains, and the effect of enzyme inducers. *Mutat. Res.*, **121**, 233–239.
- Dyer, R.H. (1994) Determination of ethyl carbamate (urethane) in alcoholic beverages using capillary gas-chromatography with thermal-energy analyzer detection — collaborative study. *J. AOAC Int.*, **77** (1), 64–66.
- Edwards, A.J., Anderson, D., Brinkworth, M.H., Myres, B. & Parry, J.M. (1999) An investigation of male-mediated F1 effects in mice treated acutely and sub-chronically with urethane. *Teratogen. Carcinogen. Mutagen.*, **19**, 87–103.
- Endo, A. & Watanabe, T. (1988) Individual and strain differences in patterns of long-term persistence of urethane-induced sister chromatid exchanges (SCEs) in mouse lymphocytes and their relation to carcinogen susceptibility. *Environ. Mol. Mutagen.*, **12**, 375–383.
- Epstein, S.S., Arnold, E., Andrea, J., Bass, W. & Bishop, Y. (1972) Detection of chemical mutagens by the dominant lethal assay in the mouse. *Toxicol. Appl. Pharmacol.*, **23**, 288–325.
- Evans, E.L. & Mitchell, A.D. (1981) Effects of 20 coded chemicals on sister chromatid exchange frequencies in cultured Chinese hamster cells. In: de Serres, F.J. & Ashby, J., eds., *Evaluation of Short-Term Tests for Carcinogens*. New York: Elsevier/North-Holland, pp. 538–550 (Progress in Mutation Research, Vol. 1).
- Fauhl, C. & Wittkowski, R. (1992) Determination of ethyl carbamate in wine by GC-SIM-MS after continuous extraction with diethyl-ether. *J. High Resolut. Chromatogr.*, **15** (3), 203–205.
- Fauhl, C., Catsburg, R. & Wittkowski, R. (1993) Determination of ethyl carbamate in soy sauces. *Food Chem.*, **48** (3), 313–316.
- FDA (2004) Submission to the 64th JECFA meeting: 1998 ethyl carbamate surveillance data. College Park, Maryland: United States Food and Drug Administration.

- Ferm, V.H. (1966) Severe developmental malformations. *Arch. Pathol.*, **81**, 174–177. As cited by Shepard, T.H. (1989) Urethan. In: *Catalog of Teratogenic Agents*, 6th ed. Baltimore, Maryland: The Johns Hopkins University Press, p. 654; and as cited by Collins, T.F.X. & Sotomayor, R.E. (1989) Developmental toxicity of urethane. *Toxicol. Ind. Health*, **5**, 1045–1060.
- Fernando, R.C., Nair, J., Barbin, A., Miller, J.A. & Bartsch, H. (1996) Detection of 1,*N*⁶-ethenodeoxyadenosine and 3,*N*⁴-ethenodeoxycytidine by immunoaffinity/³²P-postlabelling in liver and lung DNA of mice treated with ethyl carbamate (urethane) or its metabolites. *Carcinogenesis*, **17**, 1711–1718.
- First Venture (2004) News Release (29 November 2004): First Venture Reports Substantial Reduction in Ethyl Carbamate Formation in Wine Fermentation Tests. Vancouver, B.C.: First Venture Technologies (<http://www.firstventuretech.com/s/NewsReleases.asp?ReportID=94143>).
- Forkert, P.-G. & Lee, R.P. (1997) Metabolism of ethyl carbamate by pulmonary cytochrome P450 and carboxylesterase isozymes: involvement of CYP2E1 and hydrolase A. *Toxicol. Appl. Pharmacol.*, **146**, 245–254.
- Forkert, P.-G., Lee, R.P. & Reid, K. (2001) Involvement of CYP2E1 and carboxylesterase enzymes in vinyl carbamate metabolism in human lung microsomes. *Drug Metab. Dispos.*, **29**, 258–263.
- Fossa, A.A., Baird, W.M. & Carlson, G.P. (1985) Distribution of urethane and its binding to DNA, RNA, and protein in SENCAR and BALB/c mice following oral and dermal administration. *J. Toxicol. Environ. Health*, **15**, 635–654.
- Foureman, P., Mason, J.M., Valencia, R. & Zimmering, S. (1994) Chemical mutagenesis testing in *Drosophila*. IX. Results of 50 coded compounds tested for the National Toxicology Program. *Environ. Mol. Mutagen.*, **23**, 51–63.
- Froelich, A. & Wuergler, F.E. (1990) Genotoxicity of ethyl carbamate in the *Drosophila* wing spot test: dependence on genotype-controlled metabolic capacity. *Mutat. Res.*, **244**, 201–208.
- FSA (2000) *Survey of Ethyl Carbamate in Whisky*. London: United Kingdom Food Standards Agency (Food Surveillance Information Sheet No. 02/00) (<http://www.food.gov.uk/science/surveillance/fsis-2000/2whisky>).
- FSA (2005) Submission to the 64th JECFA meeting: Results of the analysis of 100 liquid products and food samples for ethyl carbamate. London: United Kingdom Food Standards Agency.
- Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimp, J., Margolin, B.H., Resnik, M.A., Anderson, B. & Zeiger, E. (1987) Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. *Environ. Mol. Mutagen.*, **10**, 1–175.
- Giachetti, C., Assandri, A. & Zanol, G. (1991) Gas-chromatographic mass-spectrometric determination of ethyl carbamate as the xanthylamide derivative in Italian aqua vitae (grappa) samples. *J. Chromatogr.*, **585** (1), 111–115.
- Gonzalez-Reche, L.M., Koch, H.M., Weiss, T., Muller, J., Drexler, H. & Angerer, J. (2002) Analysis of ethenoguanine adducts in human urine using high performance liquid chromatography–tandem mass spectrometry. *Toxicol. Lett.*, **134**, 71–77.
- Goon, D. & Conner, M.K. (1984) Simultaneous assessment of ethyl carbamate-induced SCEs in murine lymphocytes, bone marrow and alveolar macrophage cells. *Carcinogenesis*, **5**, 399–492.

- Gotoh, H., Nomura, T., Nakajima, H., Hasegawa, C. & Sakamoto, Y. (1988) Inhibiting effects of nicotinamide on urethane-induced malformations and tumors in mice. *Mutat. Res.*, **199**, 55–63.
- Graf, U. & van Schaik, N. (1992) Improved high bioactivation cross for the wing somatic mutation and recombination test in *Drosophila melanogaster*. *Mutat. Res.*, **271**, 59–67.
- Graf, U., Abraham, S.K., Guzman-Rincon, J. & Wurgler, F.E. (1998) Antigenotoxicity studies in *Drosophila melanogaster*. *Mutat. Res.*, **402**, 203–209.
- Guengerich, F.P. & Kim, D.-H. (1991) Enzymatic oxidation of ethyl carbamate to vinyl carbamate and its role as an intermediate in the formation of 1,N⁶-ethenoadenosine. *Chem. Res. Toxicol.*, **4**, 413–421.
- Guengerich, F.P., Kim, D.-H. & Iwasaki, M. (1991) Role of human cytochrome P-450 1IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem. Res. Toxicol.*, **4**, 168–179.
- Gupta, R. & Dani, H.M. (1989) In vitro formation of organ-specific ultimate carcinogens of 4-dimethylaminoazobenzene and urethane by microsomes. *Toxicol. Lett.*, **45**, 49–54.
- Hall, E.K. (1953) Developmental anomalies in the eye of the rat after various experimental procedures. *Anat. Rec.*, **116**, 383–393. As cited by Shepard, T.H. (1989) Urethane. In: *Catalog of Teratogenic Agents*, 6th ed. Baltimore, Maryland: The Johns Hopkins University Press, p. 654; and as cited by Collins, T.F.X. & Sotomayor, R.E. (1989) Developmental toxicity of urethane. *Toxicol. Ind. Health*, **5**, 1045–1060.
- Haraldsdottir, J., Holm, L., Jensen, J.H. & Møller, A. (1986) [Dietary Habits in Denmark, 1985.] Copenhagen: National Food Agency of Denmark, p. 27 (Publication No. 136) (in Danish with English summary).
- Hasegawa, Y., Nakamura, Y., Tonogai, Y., Terasawa, S., Ito, Y. & Uchiyama, M. (1990) Determination of ethyl carbamate in various fermented foods by selected ion monitoring. *J. Food Prot.* **53** (12), 1058–1061.
- He, S. & Baker, R. (1991) Micronuclei in mouse skin cells following in vivo exposure to benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene, chrysene, pyrene and urethane. *Environ. Mol. Mutagen.*, **17**, 163–168.
- Herbert, P., Santos, L., Bastos, M., Barros, P. & Alves, A. (2002) New HPLC method to determine ethyl carbamate in alcoholic beverages using fluorescence detection. *J. Food Sci.*, **67** (5), 1616–1620.
- Hoffler, U., El-Masri, H.A. & Ghanayem, B.I. (2003) Cytochrome P450 2E1 (CYP2E1) is the principal enzyme responsible for urethane metabolism: comparative studies using CYP2E1-null and wild-type mice. *J. Pharmacol. Exp. Ther.*, **305**, 557–564.
- Holladay, S.D., Sharova, L., Smith, B.J., Gogal, R.M., Ward, D.L. & Blaylock, B.L. (2000) Nonspecific stimulation of the maternal immune system. I. Effects on teratogen-induced fetal malformations. *Teratology*, **62**, 413–419.
- Holmstrom, M. (1990) Induction of micronuclei in bone marrow of mice exposed to 1, 2 or 3 daily doses of urethane. *Mutat. Res.*, **234**, 147–154.
- Hübner, P., Groux, P.M., Weibel, B., Sengstag, C., Horlbeck, J., Leong-Morgenthaler, P.M. & Lüthy, J. (1997) Genotoxicity of ethyl carbamate (urethane) in *Salmonella*, yeast and human lymphoblastoid cells. *Mutat. Res.*, **390**, 11–19.
- Hynes, G.M., Torous, D.K., Tometsko, C.R., Burlinson, B. & Gatehouse, D.G. (2002) The single laser flow cytometric micronucleus test: a time course study using colchicine and urethane in rat and mouse peripheral blood and acetaldehyde in rat peripheral blood. *Mutagenesis*, **17**, 15–23.

- IARC (1974) Urethane. In: *Some Antithyroid and Related Substances, Nitrofurans and Industrial Chemicals*. Lyon: International Agency for Research on Cancer, pp. 111–140 (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Vol. 7).
- Inai, K., Arihiro, K., Takeshima, Y., Yonehara, S., Tachiyama, Y., Khatun, N. & Nishisaka, T. (1991) Quantitative risk assessment of carcinogenicity of urethane (ethyl carbamate) on the basis of long-term oral administration to B6C3F1 mice. *Jpn. J. Cancer Res.*, **82**, 380–385.
- Innes, J.R., Ulland, B.M., Valerio, M.G., Petrucelli, L., Fishbein, L., Hart, E.R., Pallotta, A.J., Bates, R.R., Falk, H.L., Gart, J.J., Klein, M., Mitchell, I. & Peters, J. (1969) Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. *J. Natl. Cancer Inst.*, **42**, 1101–1114.
- Itoh, A. & Matsumoto, N. (1984) Organ-specific susceptibility to clastogenic effect of urethane, a trial of application of whole embryo culture to testing system for clastogen. *J. Toxicol. Sci.*, **9**, 175–192.
- Jagannath, D.R., Vultaggio, D.M. & Brusick, D.J. (1981) Genetic activity of 42 coded compounds in the mitotic gene conversion assay using *Saccharomyces cerevisiae* strain D4. In: de Serres, F.J. & Ashby, J., eds., *Evaluation of Short-Term Tests for Carcinogens*. New York: Elsevier/North-Holland, pp. 456–467 (Progress in Mutation Research, Vol. 1).
- Jagerdeo, E., Dugar, S., Foster, G.D. & Schenck, H. (2002) Analysis of ethyl carbamate in wines using solid-phase extraction and multidimensional gas chromatography/mass spectrometry. *Agric. Food Chem.*, **50** (21), 5797–5802.
- Japan (2004) Submission to the 64th JECFA meeting: Survey of Japanese sake contamination with ethyl carbamate.
- Jeong, T.C., Cha, S.W., Park, J.I., Ha, C.S., Han, S.S. & Roh, J.K. (1995) Role of metabolism in ethyl carbamate-induced suppression of antibody response to sheep erythrocytes in female Balb/C mice. *Int. J. Immunopharmacol.*, **17**, 1035–1044.
- Jeong, T.C., Kim, H.J., Cha, S.W., Park, J.I., Shin, H.C., Kim, D.H., Han, S.S. & Roh, J.K. (1996) Effects of ethyl carbamate and its metabolites on the antibody response in splenocyte cultures from female Balb/C mice. *Immunopharmacol. Immunotoxicol.*, **18**, 91–103.
- Jotz, M.M. & Mitchell, A.D. (1981) Effects of 20 coded chemicals on the forward mutation frequency at the thymidine kinase locus in L5178Y mouse lymphoma cells. In: de Serres, F.J. & Ashby, J., eds., *Evaluation of Short-Term Tests for Carcinogens*. New York: Elsevier/North-Holland, pp. 580–593 (Progress in Mutation Research, Vol. 1).
- Kageyama, M. (1961) Differences in susceptibility to induction of congenital malformations by ethylurethan among various strains of mice. *Acta Anat. Nippon*, **36**, 1–9.
- Kauffman, S.L. (1971) Alteration in cell proliferation in mouse lung following urethane exposure. II. Effects of chronic exposure on terminal bronchiolar epithelium. *Am. J. Pathol.*, **64**, 531–538.
- Kauffman, S.L. (1972) Alterations in cell proliferation in mouse lung following urethane exposure. 3. Effects of chronic exposure on type 2 alveolar epithelial cell. *Am. J. Pathol.*, **68**, 317–326.
- Kaye, A.M. (1960) A study of the relationship between the rate of ethyl carbamate (urethan) catabolism and urethan carcinogenesis. *Cancer Res.*, **20**, 237–241.

- Kaye, A.M. (1968) Urethan carcinogenesis and nucleic acid metabolism: in vivo incorporation of pyrimidines-¹⁴C and urethane-¹⁴C into mouse organs. *Cancer Res.*, **28**, 1047–1054.
- Kemper, R.A., Myers, S.R. & Hurst, H.E. (1995) Detoxification of vinyl carbamate epoxide by glutathione: evidence for participation of glutathione-S-transferases in metabolism of ethyl carbamate. *Toxicol. Appl. Pharmacol.*, **135**, 110–118.
- Kim, S.G., Surh, Y.J. & Miller, J.A. (1999) Inhibitory effects of chlorophyllin on micronucleus formation induced by ethyl carbamate and its proximate and ultimate carcinogenic forms in mouse peripheral reticulocytes. *Environ. Mol. Mutagen.*, **34**, 57–60.
- Kim, Y.K.L., Koh, E., Chung, H. & Kwon, H. (2000) Determination of ethyl carbamate in some fermented Korean foods and beverages. *Food Addit. Contam.*, **17** (6), 469–475.
- Kishi, M., Horiguchi, Y., Watanabe, S. & Hayashi, M. (1992) Validation of the mouse peripheral blood micronucleus assay using acridine orange supravital staining with urethane. *Mutat. Res.*, **278**, 205–208.
- Kitamoto, K., Oda, K., Gomi, K. & Takahashi, K. (1991) Genetic engineering of a sake yeast producing no urea by successive disruption of arginase gene. *Appl. Environ. Microbiol.*, **57** (1), 301–306.
- Klein, M. (1952) The transplacental effect of urethane on lung tumorigenesis in mice. *J. Natl. Cancer Inst.*, **12**, 1003. As cited in IARC (1974), pp. 124–127.
- Kobayashi, K., Toyoda, M. & Saito, Y. (1987) Determination of ethyl carbamate in sake by alkylation and gas-chromatography with a flame ionization detector. *J. Food Hyg. Soc. Jpn.*, **28** (5), 330–335.
- Korean Ministry of Health and Welfare (1997) '95 National Nutrition Survey Report. Seoul, Republic of Korea.
- Kristiansen, E., Clemmensen, S. & Meyer, O. (1990) Chronic ethanol intake and reduction of lung tumours from urethane in strain A mice. *Am. J. Epidemiol.*, **28**, 35–38.
- Kristiansen, E., Vahl, M., Ladefoged, O., Meyer, O., Otergaard, G. & Lam, H.R. (1994) The area under the plasma concentration curve (AUC) of urethane in mice and the influence of concomitant administration of ethanol. *Pharmacol. Toxicol.*, **75**, 324–326.
- Kurata, N., Kemper, R.A., Hurst, H.E. & Waddell, W.J. (1990) Inhibition of the metabolism of ethyl carbamate by acetaldehyde. *Drug Metab. Dispos.*, **18**, 504–507.
- Kurata, N., Hurst, H.E., Benz, F.W., Kemper, R.A. & Waddell, W.J. (1991a) Studies on inhibition and induction of metabolism of ethyl carbamate by acetone and related compounds. *Drug Metab. Dispos.*, **19**, 388–393.
- Kurata, N., Hurst, H.E., Kemper, R.A. & Waddell, W.J. (1991b) Studies on induction of metabolism of ethyl carbamate in mice by ethanol. *Drug Metab. Dispos.*, **19**, 239–240.
- Larsen, C.D. (1947) Pulmonary tumour induction by transplacental exposure to urethane. *J. Natl. Cancer Inst.*, **8**, 63–70. As cited in IARC (1974), pp. 124–127.
- Lawson, T.A. & Pound, A.W. (1971) The reaction of urethane with mouse liver nucleic acids in vivo. *Pathology*, **3**, 223–225.
- Lee, R.P. & Forkert P.-G. (1999) Inactivation of cytochrome P-450 (CYP2E1) and carboxylesterase (Hydrolase A) enzymes by vinyl carbamate in murine pulmonary microsomes. *Drug Metab. Dispos.*, **27**, 233–239.
- Leithauser, M.T., Liem, A., Stewart, B.C., Miller, E.C. & Miller, J.A. (1990) 1,N⁶-etheno-adenosine formation, mutagenicity and murine tumor induction as indicators of the generation of an electrophilic epoxide metabolite of the closely related carcinogens ethyl carbamate (urethane) and vinyl carbamate. *Carcinogenesis*, **11**, 463–473.

- Luebke, R.W., Riddle, M.M., Rogers, R.R., Rowe, D.G., Garner, R.J. & Smialov, R.J. (1986) Immune function in adult C57BL/6J mice following exposure to urethan pre- or postnatally. *J. Immunopharmacol.*, **8**, 243–257.
- Luster, M.I., Dean, J.H., Boorman, G.A., Dieter, M.P. & Hayes, H.T. (1982) Immune functions in methyl and ethyl carbamate treated mice. *Clin. Exp. Immunol.*, **50**, 223–230.
- Ma, Y.P., Deng, F.Q., Chen, D.Z. & Sun, S.W. (1995) Determination of ethyl carbamate in alcoholic beverages by capillary multidimensional gas-chromatography with thermionic specific detection. *J. Chromatogr. A*, **695** (2), 259–265.
- MacGregor, J.T., Wehr, C.M., Henika, P.R. & Shelby, M.D. (1990) The in vivo erythrocyte micronucleus test: measurement at steady state increases assay efficiency and permits integration with toxicity studies. *Fundam. Appl. Toxicol.*, **14**, 513–522.
- Majone, F., Montaldi, A., Ronchese, F., De Rossi, A., Chieco-Bianchi, L. & Levis, A.G. (1983) Sister chromatid exchanges induced in vivo and in vitro by chemical carcinogens in mouse lymphocytes carrying endogenized Moloney leukemia virus. *Carcinogenesis*, **4**, 33–37.
- Matsudo, T., Aoki, T., Abe, K., Fukuta, N., Higuchi, T., Sasaki, M. and Uchida, K. (1993) Determination of ethyl carbamate in soy-sauce and its possible precursor. *J. Agric. Food Chem.*, **41** (3), 352–356.
- Miller, J.A. (1991) The need for epidemiological studies of the medical exposures of Japanese patients to the carcinogen ethyl carbamate (urethane) from 1950 to 1975. *Jpn. J. Cancer Res.*, **82**, 1323–1324.
- Miller, J.A. & Miller, E.C. (1983) The metabolic activation and nucleic acid adducts of naturally-occurring carcinogens: recent results with ethyl carbamate and the spice flavors safrole and estragole. *Br. J. Cancer*, **48**, 1–15.
- Mirvish, S.S. (1966) The metabolism of *N*-hydroxyurethane in relation to its carcinogenic action: conversion into urethane and an *N*-hydroxyurethane glucuronide. *Biochim. Biophys. Acta*, **117**, 1–12.
- Mirvish, S.S., Smyrk, T., Payne, S., Tuatoo, H. & Chen, S.C. (1994) Weak carcinogenicity of 2-hydroxyethyl carbamate in strain A mice: indication that this is not a proximal metabolite of ethyl carbamate. *Cancer Lett.*, **77**, 1–5.
- Miyashita, N., Migita, S. & Moriwaki, K. (1987) Effects of H-2 complex and non-H-2 background on urethane-induced chromosomal aberrations in mice. *Mutat. Res.*, **176**, 59–67.
- Muller, C.J. & Fugelsang, K.C. (1996) *3a,6a-Dimethylglycoluril, the Product of the Interaction of Urea and Diacetyl, as a Source of Post-bottling Ethyl Carbamate in Wines*. Fresno, California: California State University, California Agricultural Technology Institute, Viticulture & Enology Research Center (CATI Publication No. 960502) (<http://cati.csufresno.edu/verc/rese/>).
- Nakane, K. & Kameyama, Y. (1986) Effect of maternal urethane administration on the manifestation of cleft lip and palate in CL/Fr mice. *J. Craniofacial Genet. Dev. Biol. Suppl.*, **2**, 109–112.
- Neeper-Bradley, T.L. & Conner, M.K. (1989) Intralitter variation in murine fetal sister chromatid exchange responses to the transplacental carcinogen ethyl carbamate. *Environ. Mol. Mutagen.*, **14**, 90–97.
- Neeper-Bradley, T.L. & Conner, M.K. (1990) Comparative in vivo sister chromatid exchange induction by ethyl carbamate in maternal and fetal tissues of tumor-susceptible and -resistant murine strains. *Teratogen. Carcinogen. Mutagen.*, **10**, 1–10.

- Neeper-Bradley, T.L. & Conner, M.K. (1992) Tumor formation and sister chromatid exchange induction by ethyl carbamate: Relationships among non-pregnant murine females, gravid dams, and transplacentally exposed offspring. *Teratogen. Carcinogen. Mutagen.*, **12**, 167–177.
- Neft, R.E., Conner, M.K. & Takeshita, T. (1985) Long-term persistence of ethyl carbamate-induced sister chromatid exchanges in murine lymphocytes. *Cancer Res.*, **45**, 4115–4121.
- Nery, R. (1968) Some aspects of the metabolism of urethane and *N*-hydroxyurethane in rodents. *Biochem. J.*, **106**, 1–13.
- New Zealand (2004) Submission to the 64th JECFA meeting. Canberra: Food Standards Australia New Zealand.
- Nishimura, H. & Kuginuki, M. (1958) Congenital malformations induced by ethyl-urethan in mouse embryos. *Okajima Folia Anat. Jpn.*, **31**, 1–10. As cited by Shepard, T.H. (1989) Urethan. In: *Catalog of Teratogenic Agents*, 6th ed. Baltimore, Maryland: The Johns Hopkins University Press, p. 654; and as cited by Collins, T.F.X. & Sotomayor, R.E. (1989) Developmental toxicity of urethane. *Toxicol. Ind. Health*, **5**, 1045–1060.
- Nomeir, A.A., Ioannou, Y.M., Sanders, J.M. & Matthews, H.B. (1989) Comparative metabolism and disposition of ethyl carbamate (urethane) in male Fischer 344 rats and male B6C3F1 mice. *Toxicol. Appl. Pharmacol.*, **97**, 203–215.
- Nomura, T. (1973) Carcinogenesis by urethane via mother's milk and its enhancement of transplacental carcinogenesis in mice. *Cancer Res.*, **33**, 1677–1683.
- Nomura, T. (1974) An analysis of the changing urethane response of the developing mouse embryo in relation to mortality, malformation, and neoplasm. *Cancer Res.*, **34**, 2217–2231.
- Nomura, T. (1975a) Transmission of tumors and malformations to the next generation of mice subsequent to urethane treatment. *Cancer Res.*, **35**, 264–266.
- Nomura, T. (1975b) Urethan (ethyl carbamate) as a cosolvent of drugs commonly used parenterally in humans. *Cancer Res.*, **35**, 2895–2899.
- Nomura, T. (1976) Comparison of tumour susceptibility among various organs of foetal, young and adult ICR/Jcl mice. *Br. J. Cancer*, **33**, 521–533.
- Nomura, T. (1979) Potent mutagenicity of urethan (ethyl carbamate) gas in *Drosophila melanogaster*. *Cancer Res.*, **39**, 4224–4227.
- Nomura, T. (1982) Parental exposure to x rays and chemicals induces heritable tumours and anomalies in mice. *Nature*, **296**, 575–577.
- Nomura, T. (1988) X-ray- and chemically induced germ-line mutation causing phenotypical anomalies in mice. *Mutat. Res.*, **198**, 309–320.
- Nomura, T. & Okamoto, W. (1972) Transplacental carcinogenesis by urethane in mice: teratogenesis and carcinogenesis in relation to organogenesis. *Gann*, **63**, 731–742.
- Nomura, T., Takebe, H. & Okamoto, E. (1973) Long retention of urethan transferred into newborn mice transplacentally, as a possible cause of high carcinogenesis. *Gann*, **64**, 29–40.
- Nomura, T., Enomoto, T., Shibata, K., Kanzaki, T., Tanaka, H., Hata, S., Kimura, S., Kusafuka, T., Sobue, K., Miyamoto, S., Nakano, H. & Gotoh, H. (1983) Antiteratogenic effects of tumor inhibitors, caffeine, antipain, and retinoic acid in mice. *Cancer Res.*, **43**, 5156–5162.
- Nomura, T., Hata, S. & Kusafuka, T. (1990) Suppression of developmental anomalies by maternal macrophages in mice. *J. Exp. Med.*, **192**, 1325–1330.

- Nomura, T., Tanaka, S., Kurokawa, N., Shibata, K., Nakajima, H., Kurishita, A., Hongyo, T. & Ishii, Y. (1996) Cytogenotoxicities of sublimed urethane gas to the mouse embryo. *Mutat. Res.*, **368**, 59–64.
- Nout, M.J.R., Ruikes, M.M.W., Bouwmeester, H.M. and Beljaars, P.R. (1993) Effect of processing conditions on the formation of biogenic amines and ethyl carbamate in soybean tempe. *J. Food Saf.*, **13**, 293–303.
- Nout, M.J.R., Nche, P.F. and Hollman, P.C.H. (1994) Investigation of the presence of biogenic amines and ethyl carbamate in kenkey made with maize and maize-cowpea mixtures as influenced by process conditions. *Food Addit. Contam.*, **11** (3), 397–402.
- NTP (1996) *NTP Technical Report on Toxicity Studies of Urethane in Drinking Water and Urethane in 5% Ethanol Administered to F344/N Rats and B6C3F1 Mice*. Research Triangle Park, North Carolina: United States Department of Health and Human Services, Public Health Service, National Institutes of Health, National Toxicology Program (Toxicity Report Series No. 52; NIH Publication No. 96-3937).
- NTP (2004) *NTP Technical Report on the Toxicology and Carcinogenesis Studies of Urethane, Ethanol, and Urethane/Ethanol (Urethane, CAS No. 51-79-6; Ethanol, CAS No. 64-17-5) in B6C3F1 Mice (Drinking Water Studies)*. Research Triangle Park, North Carolina: United States Department of Health and Human Services, Public Health Service, National Institutes of Health, National Toxicology Program (NIH Publication No. 04-4444).
- NTP (2005) Urethane. In: *Report on Carcinogens*, 11 ed. Research Triangle Park, North Carolina: United States Department of Health and Human Services, Public Health Service, National Institutes of Health, National Toxicology Program (<http://ntp.niehs.nih.gov/ntp/roc/elevanth/profiles/s184uret.pdf>).
- O'Flaherty, E.J. & Sichak, S.P. (1983) The kinetics of urethane elimination in the mouse. *Toxicol. Appl. Pharmacol.*, **68**, 354–358.
- Osswald, H. (1959) [On the question of mitosis-inhibition by polyvalent carbamic acid esters in Ehrlich's carcinoma.] *Arzneimittelforschung*, **9**, 595–598.
- Ough, C.S. (1976) Ethylcarbamate in fermented beverages and foods. II. Possible formation of ethylcarbamate from diethyl decarbonate addition to wine. *J. Agric. Food Chem.*, **24**, 328–331.
- Ough, C.S. & Trioli, G. (1988) Urea removal from wine by an acid urease. *Am. J. Enol. Viticult.*, **39**, 303–307.
- Ough, C.S., Crowell, E.A. & Gutlove, B.R. (1988) Carbamyl compound reactions with ethanol. *Am. J. Enol. Viticult.*, **39**, 239–242.
- Page, D.A. & Carlson, G.P. (1994) The effect of pyridine on the in vitro and in vivo metabolism of ethyl carbamate (urethane) by rat and mouse. *Carcinogenesis*, **15**, 2177–2181.
- Park, K.K., Surh, Y.J., Stewart, B.C. & Miller, J.A. (1990) Synthesis and properties of vinyl carbamate epoxide, a possible ultimate electrophilic and carcinogenic metabolite of vinyl carbamate and ethyl carbamate. *Biochem. Biophys. Res.*, **169**, 1094–1098.
- Park, K.K., Liem, A., Stewart, B.C. & Miller, J.A. (1993) Vinyl carbamate epoxide, a major strong electrophilic, mutagenic and carcinogenic metabolite of vinyl carbamate and ethyl carbamate (urethane). *Carcinogenesis*, **14**, 441–450.
- Pereira, M.A., Khoury, M.M., Glauert, H.P. & Davis, R.A. (1991) Screen of five alkyl carbamates for initiating and promoting activity in rat liver. *Cancer Lett.*, **57**, 37–44.

- Perry, P.E. & Thomson, E.J. (1981) Evaluation of the sister chromatid exchange method in mammalian cells as a screening system for carcinogens. *Prog. Mutat. Res.*, **1**, 560–569.
- Pietra, G. & Shubik, P. (1960) Induction of melanotic tumors in the Syrian golden hamster after administration of ethyl carbamate. *J. Natl. Cancer Inst.*, **25**, 627–630.
- Platzek, T., Bochert, G. & Pauli, B. (1992) Dose–response relationship of teratogenicity of ethyl carbamate in mice. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **345**, R34 (abstract).
- Prodi, G., Rocchi, P. & Grilli, S. (1970) In vivo interaction of urethane with nucleic acids and proteins. *Cancer Res.*, **30**, 2887–2892.
- Rencüzogullari, E. & Topaktas, M. (1998) Sister chromatid exchange in cultured human lymphocytes treated with carbosulfan, ethyl carbamate and ethyl methanesulfonate separately and in mixtures. *Turk. J. Biol.*, **22**, 369–387.
- Rencüzogullari, E. & Topaktas, M. (2000) Chromosomal aberrations in cultured human lymphocytes treated with the mixtures of carbosulfan, ethyl carbamate and ethyl methanesulfonate. *Cytologia*, **65**, 83–92.
- Ribovich, M.L., Miller, J.A., Miller, E.C. & Timmins, L.G. (1982) Labeled 1,*N*⁶-etheno-adenosine and 3,*N*⁴-ethenocytidine in hepatic RNA of mice given (ethyl-1,2-³H or ethyl-1-¹⁴C) ethyl carbamate (urethan). *Carcinogenesis*, **3**, 539–546.
- Richold, M. & Jones, E. (1981) Mutagenic activity of 42 coded compounds in the *Salmonella*/microsome assay. In: de Serres, F.J. & Ashby, J., eds., *Evaluation of Short-Term Tests for Carcinogens*. New York: Elsevier/North-Holland, pp. 314–322 (Progress in Mutation Research, Vol. 1).
- Roberts, G.T. & Allen, J.W. (1980) Tissue-specific induction of sister chromatid exchanges by ethyl carbamate in mice. *Environ. Mutagen.*, **2**, 17–26.
- Russell, L.B., Hunsicker, P.R., Oakberg, E.F., Cummings, C.C. & Schmoyer, R.L. (1987) Tests for urethane induction of germ-cell mutations and germ-cell killing in the mouse. *Mutat. Res.*, **188**, 335–342.
- Sakano, K., Oikawa, S., Hiraku, Y. & Kawanishi, S. (2002) Metabolism of carcinogenic urethane to nitric oxide is involved in oxidative DNA damage. *Free Radic. Biol. Med.*, **33**, 703–714.
- Salamone, M.F., Heddle, J.A. & Katz, M. (1981) Mutagenic activity of 41 compounds in the in vivo micronucleus assay. In: de Serres, F.J. & Ashby, J., eds., *Evaluation of Short-Term Tests for Carcinogens*. New York: Elsevier/North-Holland, pp. 686–697 (Progress in Mutation Research, Vol. 1).
- Salmon, A.G., Painter, P., Dunn, A.J., Wu-Williams, A., Monserrat, L. & Zeise, L. (1991) Carcinogenic effects. In: Salmon, A.G. & Zeise, L., eds., *Risks of Carcinogenesis from Urethane Exposure*. Boca Raton, Florida: CRC Press, pp. 48–77.
- Sanderson, B.J. & Clark, A.M. (1993) Micronuclei in adult and foetal mice exposed in vivo to heliotrine, urethane, monocrotaline and benzidine. *Mutat. Res.*, **285**, 27–33.
- Schardein, J. (2000) Cancer chemotherapeutic agents. In: Schardein, J., ed., *Chemically Induced Birth Defects*, 3rd ed. New York: Marcel Dekker Inc., pp. 577–578.
- Scherer, E., Winterwerp, H. & Emmelot, P. (1986) Modification of DNA and metabolism of ethyl carbamate in vivo: formation of 7-(2-oxoethyl)guanine and its sensitive determination by reductive tritiation using ³H-sodium borohydride. *IARC Sci. Publ.*, **70**, 109–125.
- Schlatter, J. & Lutz, W.K. (1990) The carcinogenic potential of ethyl carbamate (urethane): risk assessment at human dietary exposure levels. *Food Chem. Toxicol.*, **28**, 205–211.

- Schlegel, R. & MacGregor, J.T. (1984) The persistence of micronucleated erythrocytes in the peripheral circulation of normal and splenectomized Fischer 344 rats: implications for cytogenetic screening. *Mutat. Res.*, **127**, 169–174.
- Schmähl, D., Port, R. & Wahrendorf, J. (1977) A dose–response study on urethane carcinogenesis in rats and mice. *Int. J. Cancer*, **19**, 77–80.
- Schönenberger, H., Schmidt, F. & Bindl, L. (1974) Tumorerhemmende *N*-[bis-(2-chloräthyl)-aminomehtyl]-urethane. *Z. Krebsforsch. Klin. Onkol.*, **84**, 227–240.
- Seiler, J.P. (1977) Inhibition of testicular DNA synthesis by chemical mutagens and carcinogens. Preliminary results in the validation of a novel short term test. *Mutat. Res.*, **46**, 305–310.
- Sen, N.P., Seaman, S.W. & Weber, D. (1992) A method for the determination of methyl carbamate and ethyl carbamate in wines. *Food Addit. Contam.*, **9** (2), 149–160.
- Sen, N.P., Seaman, S.W., Boyle, M. & Weber, D. (1993) Methyl carbamate and ethyl carbamate in alcoholic beverages and other fermented foods. *Food Chem.*, **48** (4), 359–366.
- Sharief, Y., Campbell, J., Leavitt, S., Langenbach, R. & Allen, J.W. (1984) Rodent species and strain specificities for sister-chromatid exchange induction and gene mutagenesis effects from ethyl carbamate, ethyl *N*-hydroxycarbamate, and vinyl carbamate. *Mutat. Res.*, **126**, 159–167.
- Sharova, L., Sura, P., Smith, B.J., Gogal, R.M., Sharov, A.A., Ward, D.L. & Holladay, S.D. (2000) Nonspecific stimulation of the maternal immune system. II. Effects on gene expression in the fetus. *Teratology*, **62**, 420–428.
- Sharova, L., Gogal, R.M., Sharov, A.A., Chrisman, M.V. & Holladay, S.D. (2002) Immune stimulation in urethane-exposed pregnant mice increases expression level of spleen leukocyte genes for TGF β 3 GM-CSF and other cytokines that may play a role in reduced chemical-induced birth defects. *Int. Immunopharmacol.*, **2**, 1477–1489.
- Sharova, L., Sharov, A.A., Sura, P., Gogal, R.M., Smith, B.J. & Holladay, S.D. (2003) Maternal immune stimulation reduces both placental morphologic damage and down-regulated placental growth-factor and cell cycle gene expression caused by urethane: are these events related to reduced teratogenesis? *Int. Immunopharmacol.*, **3**, 945–955.
- Sharp, D.C. & Parry, J.M. (1981) Induction of mitotic gene conversion by 41 coded compounds using the yeast culture JD1. In: de Serres, F.J. & Ashby, J., eds., *Evaluation of Short-Term Tests for Carcinogens*. New York: Elsevier/North-Holland, pp. 491–501 (Progress in Mutation Research, Vol. 1).
- Shiraishi, Y. (1986) Hypersensitive character of Bloom syndrome B-lymphoblastoid cell lines usable for sensitive carcinogen detection. *Mutat. Res.*, **175**, 179–187.
- Sinclair, J.G.A. (1950) Specific transplacental effect of urethane in mice. *Texas Rep. Biol. Med.*, **8**, 623–632. As cited by Shepard, T.H. (1989) Urethan. In: *Catalog of Teratogenic Agents*, 6th ed. Baltimore, Maryland: The Johns Hopkins University Press, p. 654; and as cited by Collins, T.F.X. & Sotomayor, R.E. (1989) Developmental toxicity of urethane. *Toxicol. Ind. Health*, **5**, 1045–1060.
- Skipper, H.E., Bennett, L.L., Bryan, C.E., White, L., Newton, M.A. & Simpson, L. (1951) Carbamates in the chemotherapy of leukemia. VIII. Over-all tracer studies on carbonyl-labeled urethan, methylene-labeled urethan, and methylene-labeled alcohol. *Cancer Res.*, **11**, 49–51.
- Skopek, T.R., Andon, B.M., Kaden, D.A. & Thilly, W.G. (1981) Mutagenic activity of 42 coded compounds using 8-azaguanine resistance as a genetic marker in *Salmonella*

- typhimurium*. In: de Serres, F.J. & Ashby, J., eds., *Evaluation of Short-Term Tests for Carcinogens*. New York: Elsevier/North-Holland, pp. 371–375 (Progress in Mutation Research, Vol. 1).
- Sokal, J.E. & Lessmann, E.M. (1960) Effects of cancer chemotherapeutic agents on the human fetus. *J. Am. Med. Assoc.*, **172**, 1765–1771.
- Solymosy, F. (1981) The role of metabolic activation in sister-chromatid exchange inducing activity of ethyl carbamate (urethane) and vinyl carbamate. *Mutat. Res.*, **85**, 262–263.
- Sotomayor, R.E. & Washington, M.C. (1996) Formation of etheno and oxoethyl adducts in liver DNA from rats exposed subchronically to urethane in drinking water and ethanol. *Cancer Lett.*, **100**, 155–161.
- Sotomayor, R.E., Sega, G.A. & Kadlubar, F. (1994) Induction of DNA damage by urethane in mouse testes — DNA binding and unscheduled DNA synthesis. *Environ. Mol. Mutagen.*, **24**, 68–74.
- Sozzi, G., Dragani, T.A., Presutti, M. & Della-Porta, G. (1985) Kinetics of sister-chromatid exchange induction by different carcinogens in C57BL/6J and DBA/2 mice. *Mutat. Res.*, **156**, 177–180.
- Stoewsand, G.S., Anderson, J.L. & Munson, L. (1991) Inhibition by wine of tumorigenesis induced by ethyl carbamate (urethane) in mice. *Food Chem. Toxicol.*, **29**, 291–295.
- Sugihara, K., Kitamura, S. & Tatsumi, K. (1983) Involvement of liver aldehyde oxidase in conversion of *N*-hydroxyurethane to urethane. *J. Pharmacobiodyn.*, **6**, 677–683.
- Sugiyama, T., Ueda, N., Maeda, S., Shiraishi, N., Goto-Mimura, K., Murao, S. & Chattopadhyay, S.C. (1981) Chemical carcinogenesis in the rat: common mode of action of carcinogens at the chromosome level. *J. Natl. Cancer Inst.*, **67**, 831–839.
- Svensson, K. (1988) Alkylation of protein and DNA in mice treated with urethane. *Carcinogenesis*, **9**, 2197–2201.
- Takaori, S., Tanabe, K. & Shimamoto, K. (1966) Developmental abnormalities of skeletal system induced by ethylurethan in the rat. *Jpn. J. Pharmacol.*, **16**, 63–73. As cited by Collins, T.F.X. & Sotomayor, R.E. (1989) Developmental toxicity of urethane. *Toxicol. Ind. Health*, **5**, 1045–1060.
- Thorgeirsson, U.P., Dalgard, D.W., Reeves, J. & Adamson, R.H. (1994) Tumor incidence in a chemical carcinogenesis study of nonhuman primates. *Regul. Toxicol. Pharmacol.*, **19**, 130–151.
- Tomatis, L., Turusov, V., Day, N. & Charles, R.T. (1972) The effect of long-term exposure to DDT on CF-1 mice. *Int. J. Cancer*, **10**, 489–506.
- Toth, B. & Boreisha, I. (1969) Tumorigenesis with isonicotinic acid hydrazide and urethan in the Syrian golden hamsters. *Eur. J. Cancer*, **5**, 164–171.
- Trioli, G. & Ough, C.S. (1989) Cause for inhibition of an acid urease from *Lactobacillus* fermentation. *Am. J. Enol. Viticult.*, **40**, 245–252.
- Trzos, R.J., Petzold, G.L., Brunden, M.N. & Swenberg, J.A. (1978) The evaluation of sixteen carcinogens in the rat using the micronucleus test. *Mutat. Res.*, **58**, 79–86.
- Tsuchimoto, T. & Matter, B.E. (1981) Activity of coded compounds in the micronucleus test. *Prog. Mutat. Res.*, **1**, 705–711.
- Turusov, V.S. & Cardis, E. (1989) Review of experiments on multigeneration carcinogenicity: of design, experimental models and analyses. In: Napalkov, N.P., Rice, J.M., Tomatis, L. & Yamasaki, H., eds., *Perinatal and Multigenerational Carcinogenesis*. Lyon: International Agency for Research on Cancer, pp. 105–120.
- Tutikawa, K. (1968) Lack of effect of urethan on the induction of dominant lethal mutations in male mice. *Annu. Rep. Natl. Inst. Genet.*, **19**, 69–70.

- Tutikawa, K. & Harada, Y. (1972) Teratogenicity and nonmutagenicity of urethane. *Teratology*, **6**, 123 (abstract).
- Tutikawa, K. & Tutikawa, K. (1972) Induction of congenital molar anomalies in mice by urethane. *Teratology*, **6**, 123 (abstract).
- United Kingdom (2003) Submission to the 64th JECFA meeting: ethyl carbamate residues in bread, toast and similar products. United Kingdom Food Standards Agency.
- United Kingdom (2004) Submission to the 64th JECFA meeting: ethyl carbamate residues in 100 liquid products and food samples. United Kingdom Food Standards Agency.
- Uthurry, C.A., Varela, F., Colomo, B., Lepe, J.A.S., Lombardero, J. & del Hierro, J.R.G. (2004) Ethyl carbamate concentrations of typical Spanish red wines. *Food Chem.*, **88**: 329–336.
- Vahl, M. (1993) A survey of ethyl carbamate in beverages, bread and acidified milks sold in Denmark. *Food Addit. Contam.*, **10** (5), 585–592.
- Van Esch, G.J. & Kroes, R. (1972) Long-term toxicity studies of chlorpropham and propham in mice and hamsters. *Food Cosmet. Toxicol.*, **10**, 373–381.
- Venitt, S. & Crofton-Sleigh, C. (1981) Mutagenicity of 42 coded compounds in a bacterial assay using *Escherichia coli* and *Salmonella typhimurium*. In: de Serres, F.J. & Ashby, J., eds., *Evaluation of Short-Term Tests for Carcinogens*. New York: Elsevier/North-Holland, pp. 351–360 (Progress in Mutation Research, Vol. 1).
- Vesselinovitch, S.D., Mihailovich, N. & Pietra, G. (1967) The prenatal exposure of mice to urethane and the consequent development of tumors in various tissues. *Cancer Res.*, **27**, 2333–2337.
- Vesselinovitch, S.D., Mihailovich, N., Rao, K.V.N. & Itze, L. (1971) Perinatal carcinogenesis by urethan. *Cancer Res.*, **31**, 2143–2147.
- Vogt, M. (1948) Mutationsauslösung bei Drosophila durch Aethylurethan. *Experientia*, **4**, 68–69.
- Waddell, W.J., Marlowe, C. & Pierce, W.M.J. (1987) Inhibition of the localization of urethane in mouse tissues by ethanol. *Food Chem. Toxicol.*, **25**, 527–531.
- Walker, G., Winterlin, W., Fonda, H. & Seiber, J. (1974) Gas chromatographic analysis of urethane (ethyl carbamate) in wine. *J. Agric. Food Chem.*, **22**, 944–947.
- Westmoreland, C., Plumstead, M. & Gatehouse, D. (1991) Activity of urethane in rat and mouse micronucleus tests after oral administration. *Mutat. Res.*, **262**, 247–251.
- Whiton, R.S. & Zoecklein, B.W. (2002) Determination of ethyl carbamate in wine by solid-phase microextraction and gas chromatography/mass spectrometry. *Am. J. Enol. Viticult.*, **53** (1), 60–63.
- Wild, D. (1978) Cytogenetic effects in the mouse of 17 chemical mutagens and carcinogens evaluated by the micronucleus test. *Mutat. Res.*, **56**, 319–327.
- Williams, C.V., Fletcher, K., Tinwell, H. & Ashby, J. (1998) Mutagenicity of ethyl carbamate to lacZ⁺ transgenic mice. *Mutagenesis*, **13**, 133–137.
- Wine Institute (2004) Submission to the 64th JECFA meeting: 2001 surveillance data. San Francisco, California: Wine Institute.
- Witt, K.L., Knapton, A., Wehr, C.M., Hook, G.J., Mirsalis, J., Shelby, M.D. & MacGregor, J.T. (2000) Micronucleated erythrocyte frequency in peripheral blood of B6C3F(1) mice from short-term, prechronic, and chronic studies of the NTP carcinogenesis bioassay program. *Environ. Mol. Mutagen.*, **36**, 163–194.
- Yamamoto, T., Pierce, W.M.J., Hurst, H.E., Chen, D. & Waddell, W.J. (1988) Inhibition of the metabolism of urethane by ethanol. *Drug Metab. Dispos.*, **16**, 355–358.

- Yamamoto, T., Pierce, W.M., Hurst, H.E., Chen, D. & Waddell, W.J. (1990) Ethyl carbamate metabolism: in vivo and in vitro enzymatic systems. *Drug Metab. Dispos.*, **18**, 276–280.
- Yoshizawa, K. & Takahashi, K. (1988) Utilization of urease for urea decomposition in sake. *J. Brew. Soc. Jpn.*, **83**, 142–144.
- Yu, W., Sipowicz, M.A., Haines, D.C., Birely, L., Diwan, B.A.S., Riggs, C.W., Kasprzak, K.S. & Anderson, L.M. (1999) Preconception urethane or chromium(III) treatment of male mice: multiple neoplastic and non-neoplastic changes in offspring. *Toxicol. Appl. Pharmacol.*, **158**, 161–176.
- Zeiger, E., Anderson, B., Haworth, S., Lawlor, T. & Mortelmans, K. (1992) *Salmonella* mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ. Mol. Mutagen.*, **19**, 2–141.
- Zimmerli, B. & Schlatter, J. (1991) Ethyl carbamate: analytical methodology, occurrence, formation, biological activity and risk assessment. *Mutat. Res.*, **259**, 325–350.
- Zimmermann, F.K. & Mohr, A. (1992) Formaldehyde, glyoxal, urethane, methyl carbamate, 2,3-butanedione, 2,3-hexanedione, ethyl acrylate, dibromoacetonitrile and 2-hydroxypropionitrile induce chromosome loss in *Saccharomyces cerevisiae*. *Mutat. Res.*, **270**, 151–166.
- Zimmermann, F.K. and Scheel, I. (1981) Induction of mitotic gene conversion in strain D7 of *Saccharomyces cerevisiae* by 42 coded chemicals. In: de Serres, F.J. & Ashby, J., eds., *Evaluation of Short-Term Tests for Carcinogens*. New York: Elsevier/North-Holland, pp. 481–490 (Progress in Mutation Research, Vol. 1).

INORGANIC TIN (addendum)

First draft prepared by

R. Kroes,¹ T. Hambridge² and J. Schlatter³

**¹ Institute for Risk Assessment Sciences, Utrecht University, Soest,
The Netherlands**

² Food Standards Australia New Zealand, Canberra, Australia

³ Swiss Federal Office of Public Health, Zurich, Switzerland

Explanation	318
Biological data	319
Biochemical aspects	319
Absorption, distribution and excretion	319
Biotransformation	319
Effects on enzymes and other biochemical parameters	319
Toxicological studies	320
Acute toxicity	320
Short-term studies of toxicity	320
Special studies	321
Observations in humans	321
Episodes of poisoning	321
Studies with volunteers	321
Irritation and sensitization	323
Levels and patterns of contamination of food commodities	323
Dietary intake assessment	332
Introduction and background to intake estimates	332
Estimated chronic dietary intakes of tin	332
Methods	332
Australia	333
United Kingdom	334
Estimated short-term dietary intakes of tin	335
Methods	335
Australia	338
New Zealand	341
United Kingdom	342
General estimates of short-term intakes based on MLs	342
Conclusions	344
Comments	346
Observations in humans	346
Prevention and control	347
Levels and pattern of food contamination	347
Dietary intake assessment	347
Evaluation	348
References	348

1. **EXPLANATION**

Inorganic tin is found in food in the +2 and +4 oxidation states; it may occur in cationic form (stannous and stannic compounds) or as anions (stannites or stannates). Inorganic tin was evaluated by the Committee at its fourteenth, fifteenth, twenty-second, twenty-sixth, thirty-third and fifty-fifth meetings (Annex 1, references 22, 26, 47, 59, 83 and 149). At its thirty-third meeting, the Committee converted the previously established provisional maximum tolerable daily intake (PMTDI) of 2 mg/kg bw to a provisional tolerable weekly intake (PTWI) of 14 mg/kg bw. At these meetings, the Committee reviewed data from short- and long-term dietary studies and noted that inorganic tin compounds generally have low systemic toxicity in animals because of limited absorption from the gastrointestinal tract, low accumulation in tissues and rapid passage through the gastrointestinal tract. Insoluble tin compounds are less toxic than soluble tin salts.

At its Thirty-first Session, the Codex Committee on Food Additives and Contaminants (CCFAC) asked the Committee to review information on the toxicity of inorganic tin in order to establish an acute reference dose (ARfD) (CAC, 1999). At its fifty-fifth meeting (Annex 1, reference 149), the Committee considered studies of the acute toxic effects seen after consumption of foods containing high concentrations of inorganic compounds of tin. It concluded that the acute toxicity of inorganic tin in animals and humans, however, results from irritation of the mucosa of the gastrointestinal tract, which may lead to vomiting, diarrhoea, anorexia, depression, ataxia and muscular weakness. There was no clear dose-response relationship, and the vehicle in which the tin was administered may have affected its toxicity. The Committee concluded that insufficient data were available to establish an ARfD for inorganic tin. At that meeting, the PTWI previously established for compounds containing inorganic tin was not reconsidered and was retained at its current value. The Committee did not consider studies on organic tin compounds, since it had concluded at its twenty-second meeting (Annex 1, reference 47) that these compounds, which differ considerably from inorganic tin compounds with respect to toxicity, should be considered separately.

At its Thirty-fifth session, CCFAC (CAC, 2003) decided to ask the Committee to evaluate current levels of inorganic tin in "canned food other than beverages" and "canned beverages" and to determine an ARfD, since new data would become available. At its Thirty-sixth session (CAC, 2004), CCFAC asked the Committee, when possible, to take population sensitivity into consideration when considering the new data and to assess the likelihood of the occurrence of effects at the proposed draft maximum levels (MLs) (200 mg/kg in canned beverages and 250 mg/kg in canned foods other than beverages).

At its present meeting, the Committee reconsidered studies of the acute toxic effects seen in humans after consumption of foods containing high concentrations of inorganic compounds of tin and also considered a new study.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

No new data became available after the fifty-fifth meeting (Annex 1, reference 150).

Inorganic tin is poorly absorbed in humans as well as animals and is excreted mainly in the faeces, with additional slow elimination in the urine. Absorption may differ depending on dose, anion and the presence of other substances.

No new animal data on distribution became available after the fifty-fifth meeting (Annex 1, reference 150). Tin is widely distributed in tissues after parenteral injection, especially in the liver and spleen, where it is deposited in the reticuloendothelial system, most being excreted eventually in the urine and a limited amount in the bile (Barnes & Stoner, 1959).

Tin tends to be retained most in the tongue, liver, kidneys and bones and least in the brain, and rats and rabbits accumulate both inorganic and organic tin in their skin and keratinized appendages. Administration of tin to pregnant rats did not lead to detectable levels of tin in fetuses on day 10 of pregnancy, while on days 20–21, small amounts of tin were detected. Overall, only trace amounts of inorganic tin cross the placental barrier, and this placental transfer is of little toxicological significance (Theuer et al., 1971; Hiles, 1974).

In a human volunteer study ($n = 20$), the serum inorganic tin concentrations were not elevated after the volunteers drank tomato juice containing <0.5, 161, 264 or 529 mg tin (as tin(II) chloride, or SnCl_2) per kg bw (Boogaard et al., 2003) and remained below the detection limit of 10 $\mu\text{g/l}$ serum. The authors concluded that the absorption of tin from the gastrointestinal is minimal in humans.

2.1.2 Biotransformation

No new animal data became available after the fifty-fifth meeting (Annex 1, reference 150). In rats, the half-life of inorganic tin in the femur was estimated to be 34 days for Sn(II) and 40 days for Sn(IV) (Hiles, 1974). Half-lives of 85 and 50 days were reported for tin in liver and spleen, respectively (Marciniak, 1981). A biological half-life of approximately 30 days was estimated for inorganic tin in mice by the whole-body counting method (Brown et al., 1977). A review by Magos (1986) stated that in humans, 20% of absorbed tin was cleared with a half-life of 4 days, 20% with a half-life of 25 days and 60% with a half-life of 400 days.

2.1.3 Effects on enzymes and other biochemical parameters

Tin cations have the ability to influence the biodegradation of cytochrome P450. Some data indicate that Sn(II) may be more potent than Sn(IV). In addition, tin seems to have an inhibitory effect on the activity of several other enzymes, including δ -aminolevulinic acid dehydratase, superoxide dismutase, glutathione

peroxidase, glutathione reductase and glucose 6-phosphate dehydrogenase (Westrum & Thomasson, 2002). Thus, tin may alter metabolism. An effect of tin(II) chloride on nerve transmission via altered calcium fluxes is reported (Westrum & Thomasson, 2002).

Tin(II) chloride induced cytotoxicity in several *Escherichia coli* strains. Simultaneous treatment with reactive oxygen species scavengers completely protected the cells against cytotoxic damage (de Silva et al., 2002).

2.2 Toxicological studies

2.2.1 Acute toxicity

As described in the monograph of the fifty-fifth meeting (Annex 1, reference 150), tin metal itself, taken orally, is practically innocuous, but inhaled dust or fumes may cause benign, symptomless pneumoconiosis. The inorganic salts are caustic and of variable toxicity, but some alkyl and aryl derivatives are highly toxic. Inorganic tin compounds and mixed colloidal tin and tin stearate have been used as antistaphylococcal and anthelmintic agents (Kolmer et al., 1931).

Studies in rats provided evidence that the chemical form of inorganic tin is important in determining its toxicity, as concluded by the fifty-fifth meeting (Annex 1, reference 149). Inorganic tin compounds generally have a low systemic toxicity in animals because of limited absorption from the gastrointestinal tract and rapid gastrointestinal passage. The median lethal dose (LD₅₀) of tin(II) chloride varies from 40 to 1200 mg/kg bw in mice and from 700 to 3200 mg/kg bw in rats. Acute toxicity signs include extreme gastrointestinal irritation, anorexia, depression, ataxia and muscular weakness. Mottling, hyperaemia and tubular necrosis in the kidneys of rats have also been described.

Vomiting and diarrhoea were reported in cats given soluble salts of tin, but there was no clear dose–response relationship, and the vehicle in which the tin was administered may have affected its toxicity, as noted by the fifty-fifth meeting (Annex 1, reference 149).

2.2.2 Short-term studies of toxicity

As described in the monograph of the fifty-fifth meeting (Annex 1, reference 150), insoluble tin compounds, such as tin(II) sulfide, had minimal toxic effects in rats when administered for 28 days in the diet at concentrations similar to those at which the soluble tin salts are clearly toxic. In short-term studies with soluble salts in rats, histological changes to the gastrointestinal tract, kidneys, liver and adrenal cortex were observed. Alterations in haematological parameters indicative of anaemia have also been recorded. The toxicity of tin results from irritation of the mucosa of the gastrointestinal tract. In a study (Janssen et al., 1985) not described in the monograph of the fifty-fifth meeting (Annex 1, reference 150), the effects of feeding inorganic tin on the gastrointestinal tract were examined in rats. Three groups of male weanling Wistar rats were fed a diet for 4 weeks to which Sn²⁺ as tin(II) chloride at 0, 250 or 500 mg/kg had been added. A fourth group was

subjected to feed restriction by pair feeding with the 500 mg/kg group. Independent of the reduced feed intake, Sn^{2+} affected the haemoglobin concentration in the blood and had several effects on the small intestine: total length as well as absolute and relative weights were increased. An increase was also observed in the migration of epithelial cells along the villus, as revealed by [^3H]thymidine incorporation and autoradiography in rats fed Sn^{2+} at 500 mg/kg in the feed for 4 weeks. Stereo-light microscopy and scanning electron microscopy revealed the formation of ridge-like villi due to Sn^{2+} feeding and a decreased number of villi per unit surface. These data suggest that an increase in cell turnover in the small intestine due to Sn^{2+} was responsible for these changes.

2.2.3 Special studies

(a) Interaction of tin with trace elements

No new data became available after the fifty-fifth meeting (Annex 1, reference 150). Limited information is available about the biochemistry of the metabolism of inorganic tin in the body or the exact mechanisms by which this element affects physiological processes. It is known, however, that tin can interact with a number of trace elements, many of which have vital functions in the body. In addition to its effects on copper, zinc and iron metabolism, tin has also been shown to interact with calcium.

(b) Effect on bone strength

No new data became available after the fifty-fifth meeting (Annex 1, reference 150). Tin, when administered in drinking-water as tin(II) chloride for 4 weeks, significantly decreased the compressive mechanical strength of the distal epiphysis of the femur at higher dosages (300 and 600 mg/l; Ogoshi et al., 1981).

2.3 Observations in humans

2.3.1 Episodes of poisoning

No new data became available after the fifty-fifth meeting (Annex 1, reference 150). Episodes of human poisoning have been described to be related to the consumption of tin-contaminated foods and drinks. The common symptoms were abdominal distension and pain, vomiting, diarrhoea and headache. These symptoms start within 0.5–3 h after consumption, and recovery occurs within 48 h. The doses of tin ingested in such episodes of poisoning were not estimated, but the symptoms occurred when canned food or drinks were noticed to contain tin concentrations varying from 250 to 2000 mg/kg.

2.3.2 Studies with volunteers

As described in the monograph of the fifty-fifth meeting (Annex 1, reference 150), in one study (Benoy et al., 1971), five volunteers experienced symptoms when they ingested orange juice derived from tinned containers containing tin at

1370 mg/kg (corresponding to a dose of 4.4–6.7 mg/kg bw). Administration of the same dose of the same juice to these individuals 1 month later resulted in symptoms in only one person. Orange juices containing tin at 498, 540 and 730 mg/kg (corresponding to 1.6–3.6 mg/kg bw) did not provoke symptoms in groups of five volunteers.

After the fifty-fifth meeting (Annex 1, reference 150), one publication describing two separate randomized, single-centre, double-blind, crossover studies became available (Boogaard et al., 2003). In study 1, the tolerability of inorganic tin added as tin(II) chloride at concentrations of <0.5, 161, 264 and 529 mg/kg in 250 ml tomato juice (approximately 0, 0.5, 1 and 2 mg/kg bw, assuming an average weight of 60 kg) was compared in 20 volunteers who had fasted for more than 6 h. In study 2, the effects of inorganic tin that migrated from packaging at concentrations of <0.5, 201 and 267 mg/kg in 250 ml tomato soup were investigated in 24 volunteers who had fasted for 6 h. Carry-over effects were prevented by washing-out periods of 48 h between the different treatments. The distribution of tin in solid matter, supernatant and low-molecular-mass tin complexes in supernatant was measured in both the spiked tomato juice and the tomato soup. Adverse events were classified according to the WHO programme for international monitoring of adverse reactions to drugs. The events were graded as mild, moderate or severe, and the investigator assessed the causal relationship to the ingested tin-containing material in this double-blind study as none, unlikely, possible, probable and highly probable.

The distribution of adverse events related and non-related to tin(II) chloride freshly added to tomato juice is given in Table 1. A clear statistically significant dose–response relationship for related adverse events was observed. Fitting the data points to a linear function with a threshold would suggest a threshold of 150 mg tin/kg juice.

Table 1. Distribution of adverse events related and non-related to tin(II) chloride freshly added to tomato juice

Concentration of tin in juice (mg/kg)	Number of subjects in treatment period	Number of related adverse events		Number of non-related adverse events
		Mild	Moderate	
<0.5	18	0	0	2
161	18	1	0	3
264	18	2	5	2
529	5 ^a	5	8	0

^a Treatment at this dose level was discontinued based on the frequency and the number of adverse events reported.

Table 2 shows the distribution of adverse events related and non-related to tin that migrated from packaging into tomato soup. Related adverse events occurred in 4 out of 23 subjects in the higher dose group (267 mg/kg) versus 3 out of 23 in

the controls. At the lower dose (201 mg/kg), no related adverse effects were noticed (Boogaard et al., 2003).

Table 2. Distribution of adverse events related and non-related to inorganic tin that migrated from packaging in tomato soup

Concentration of tin in soup (mg/kg)	Number of subjects in treatment period	Number of related adverse events		Number of non-related adverse events
		Mild	Moderate	
<0.5	23 ^a	2	1	0
201	23 ^a	0	0	1
267	23 ^a	2	2	1

^a One subject was withdrawn for protocol violation.

The distribution studies on tin showed that elemental tin concentrations in solid and liquid phases differed markedly between tomato juice freshly spiked with tin(II) chloride (study 1) and tomato soup (study 2). One hour after mixing, at the moment of consumption in study 1, solid matter contained 15% of the total tin in study 1 versus 52% in study 2. Correspondingly, supernatant concentrations of tin were 85% and 48% in the materials used in study 1 and 2, respectively. The amount of low-molecular-mass tin (<1000 daltons) in the supernatant was 59% versus 31%, respectively. Examination after 24 h revealed that tin in solid matter had increased from 15% to 35%. The significant differences in occurrence of adverse effects in studies 1 and 2 strongly suggest that these effects and their severity are determined by different speciation of tin and not simply by concentration and are likely a result of differences in the concentration of tin and in the nature of tin complexes formed (Blunden & Wallace, 2003).

2.3.3 Irritation and sensitization

Only a limited number of patch test reports are available. The results showed a positive response with tin, but its relevance to contact dermatitis is unclear (Health Council, 2005).

3. LEVELS AND PATTERNS OF CONTAMINATION OF FOOD COMMODITIES

Extensive information on the tin content of foods was reported in the monograph from the last review of tin by the Committee at its fifty-fifth meeting (Annex 1, reference 150). In summary, concentrations of tin in non-canned foods were very low, with a mean value of <2 mg/kg. Mean concentrations of tin in lacquered canned foods were between 0 and 6.9 mg/kg. Food in unlacquered, partially lacquered or unspecified cans had higher concentrations of tin, with mean values ranging from <1 to 1000 mg/kg.

This assessment is an addendum to the report from the fifty-fifth meeting (Annex 1, reference 150). Therefore, data submitted to the Committee for review at this meeting or that have become available since the last review of tin by the Committee that related to tin concentrations in food have been included in this addendum.

The Inter-Industry Tin Strategy Group submitted information on tin concentrations in foods from a number of sources. The submission included a small number of new data on tin concentrations in foods from recent surveys that have become available since the last review of tin by the Committee. Data submitted to the Committee were from Australia, France, Lithuania and the United Kingdom. A second study from the United Kingdom was also located and reviewed. These data are outlined below.

Two Australian Total Diet Surveys have been conducted since the last tin review: the 19th Australian Total Diet Survey (ANZFA, 2001) and the 20th Australian Total Diet Survey (FSANZ, 2002). These surveys analysed a broad range of food collected randomly at the retail level and were representative of the whole Australian diet. Composites of three individual retail samples were analysed, producing a resulting tin concentration that is the average of the tin concentrations of the individual samples. Concentrations of tin in the foods analysed in the Australian Total Diet Surveys are shown in Table 3. A range of mean values is reported for some foods, whether concentrations below the limit of reporting (LOR) are assumed to be equal to zero or the LOR.

Summary information from a tin monitoring study in France in 2003 was submitted to the Committee for review. The submission reported that 73 samples were taken from 11 areas in France, some samples of which were imported. All samples had a concentration of tin lower than the proposed regulatory limits for France (200 mg/kg, 100 mg/kg or 50 mg/kg, depending on the type of canned food). Samples from lacquered cans had concentrations lower than 3 mg/kg (48 samples). Only two foods had concentrations at levels equal to the proposed regulations (canned olives at 100 mg/kg; lychees at 200 mg/kg).

A study of tin in canned milk products in Lithuania (Ramonaitytė, 2001) was also submitted to the Committee for review. The paper looked specifically at the changes that can occur in tin concentrations during storage. The milk was produced in Lithuania, and the cans used were unlacquered. The products investigated were evaporated sterilized milk, concentrated sterilized milk (solids >28.5%, fat 8.5%) and sweetened condensed milk (solids >28.5%, fat 8.5%). From 1989 to 1992, the products were sampled and analysed at 0, 3, 6, 9, 12, 15, 18, 21 and 24 months. The products were stored at room temperature of 18 °C (± 2 °C).

The average contents of tin did not differ greatly over the course of the investigation (see Table 4). Concentrations of tin seemed to increase in the products during sterilization in the cans. This is due to the temperature causing changes in the deeper layers of the tinned plate. Condensed milk has lower concentrations due to the thicker nature of the product, resulting in the concentrations of tin increasing more slowly.

Table 3. Concentrations of tin in composite foods sampled at the retail level from recent Australian Total Diet Surveys

Food	19th ATDS ^a			20th ATDS ^b		
	Number of analyses	Mean ^c (mg/kg)	Maximum (mg/kg)	Number of analyses	Mean ^c (mg/kg)	Maximum (mg/kg)
Alfalfa sprouts	21	0.006–0.022	0.04	–	–	–
Apples, unwashed	21	0.002–0.019	0.02	–	–	–
Apples, washed	21	ND	ND	–	–	–
Baked beans, canned	–	–	–	9	7.0	11.0
Bananas	9	ND	ND	–	–	–
Beef, minced	27	0.014–0.026	0.13	7	0.021–0.015	0.09
Beer, 3.5% alcohol	21	ND	ND	–	–	–
Biscuits, savoury	9	ND	ND	–	–	–
Biscuits, sweet plain	9	ND	ND	–	–	–
Bok choy	21	0.005–0.021	0.04	–	–	–
Bran, wheat processed	9	ND	ND	–	–	–
Bread, white	27	0.007–0.022	0.08	7	0.017–0.014	0.07
Bread, wholemeal	21	0.006–0.02	0.04	–	–	–
Breakfast cereal, mixed grain	9	0.001–0.019	0.01	–	–	–
Capsicum	21	0.004–0.02	0.03	–	–	–
Carrots	9	ND	ND	–	–	–
Cauliflower	21	ND	ND	–	–	–
Cheese, feta, cow's milk	9	ND	ND	–	–	–
Cheese, feta, sheep's milk	9	1.793	7.7	–	–	–

Table 3. (contd)

Food	19th ATDS ^a			20th ATDS ^b		
	Number of analyses	Mean ^c (mg/kg)	Maximum (mg/kg)	Number of analyses	Mean ^c (mg/kg)	Maximum (mg/kg)
Chicken drumsticks	21	0.012–0.027	0.08	–	–	–
Chiko rolls	21	0.003–0.022	0.07	–	–	–
Chocolate cake, iced	9	0.056–0.062	0.16	–	–	–
Cornflakes	9	ND	ND	–	–	–
Crab, canned	9	0.109–0.111	0.34	–	–	–
Crocodile	6	0.002	0.01	–	–	–
Dim Sim	–	–	–	21	0.003–0.012	0.04
Eggs	27	0.018	0.02	7	ND	ND
Fish fillets	21	ND	ND	–	–	–
Grapes, unwashed	18	ND	ND	–	–	–
Grapes, washed	18	ND	ND	–	–	–
Green beans (19th frozen; 20th fresh)	9	ND	ND	9	ND	ND
Ham	21	ND	ND	21	0.005–0.014	0.07
Hamburger	–	–	–	21	0.001–0.011	0.02
Honey	9	0.042	0.07	–	–	–
Infant cereal, mixed	9	0.024–0.04	0.2	–	–	–
Infant custard, chocolate	9	0.114–0.117	0.29	–	–	–
Infant dinner, canned	9	0.082	0.12	–	–	–
Infant formula	9	ND	ND	–	–	–
Lamb loin chops	21	0.011–0.023	0.05	–	–	–

Table 3. (contd)

Food	19th ATDS ^a			20th ATDS ^b		
	Number of analyses	Mean ^c (mg/kg)	Maximum (mg/kg)	Number of analyses	Mean ^c (mg/kg)	Maximum (mg/kg)
Lamb's kidneys	21	0.001–0.02	0.02	–	–	–
Lamb's liver	27	0.004–0.02	0.04	–	–	–
Lettuce	27	0.003–0.021	0.04	–	–	–
Margarine	9	ND	ND	7	0.009–0.011	0.04
Meat pies	21	0.019–0.031	0.18	–	–	–
Milk chocolate	12	0.023–0.033	0.06	–	–	–
Milk, full fat	27	ND	ND	7	ND	ND
Muscatsels, dried	9	ND	ND	–	–	–
Mushrooms	21	0.002–0.021	0.04	21	0.001–0.01	0.01
Mussels	21	ND	ND	–	–	–
Oats, rolled	9	ND	ND	–	–	–
Oil, blended vegetable	9	ND	ND	–	–	–
Onions	21	ND	ND	–	–	–
Orange juice	27	0.000–0.02	0.01	7	ND	ND
Pasta, macaroni	9	0.006–0.023	0.05	–	–	–
Peaches	9	0.006–0.023	0.05	–	–	–
Peanut butter	–	–	–	9	0.002–0.01	0.01
Peanuts, roasted, salted	9	0.012–0.023	0.04	–	–	–
Pears, unwashed	21	0.002–0.02	0.02	–	–	–
Pears, washed	21	0.002–0.02	0.03	–	–	–

Table 3. (contd)

Food	19th ATDS ^a			20th ATDS ^b		
	Number of analyses	Mean ^c (mg/kg)	Maximum (mg/kg)	Number of analyses	Mean ^c (mg/kg)	Maximum (mg/kg)
Peas, canned	9	4.578	6.4	—	—	—
Pineapple, canned	9	51.889	81.0	—	—	—
Pork butterfly steaks	20	0.006–0.021	0.03	—	—	—
Potato	27	ND	ND	7	ND	ND
Red kidney beans, canned	9	0.227	0.41	—	—	—
Rice crackers	9	ND	ND	—	—	—
Rice, jasmine	9	ND	ND	—	—	—
Rockmelon	21	ND	ND	—	—	—
Salmon, red, canned	9	0.059	0.12	—	—	—
Sausages	21	0.06–0.062	0.21	—	—	—
Soya sauce	9	ND	ND	—	—	—
Tahina	9	0.003–0.019	0.02	—	—	—
Tea, brewed from tea bags	9	ND	ND	—	—	—
Tomato salsa	9	0.071	0.11	—	—	—
Tomatoes	21	ND	ND	7	ND	ND
Tuna, canned	—	—	—	9	0.196	0.26
Walnuts	9	0.003–0.021	0.03	—	—	—
Yoghurt, strawberry, full fat	9	0.009–0.027	0.08	—	—	—

ATDS, Australian Total Diet Survey; ND, not detected

^a ANZFA (2001).

^b FSANZ (2002).

^c Range of concentrations where ND = 0 to ND = LOR. LOR = 0.01 mg/kg. Where a single value is reported, all samples were detections. Results presented as ND means all of samples analysed were ND.

Table 4. Concentrations of tin over time in canned milk products from Lithuania

Product	Year	Number of samples	Tin concentration (mg/kg)		
			Mean	Minimum	Maximum
Evaporated sterilized milk	1986–1990	12	97	40	124
	1991–1992	27	114	84	146
	1993–1994	28	53	20	87
	1995–1997	24	—	—	—
	Mean	91	85	20	146
Concentrated sterilized milk	Initial level	NS	74	57	104
	1983–1985	24	73	60	104
	1986–1990	35–46	102	33	148
	1991–1992	40	98	53	161
	1993–1994	35	98	3	161
	Mean	126–157	89	44	161
Sweetened condensed milk	Initial level	NS	49.5	21	67
	1983–1985	34–52	28	9	60
	1986–1990	49	60	24	98
	1991–1992	45	38	16	58
	1993–1994	41	37	19	52
	1995–1997	27	39	12	80
	Mean	196–214	40	9	98

From Ramonaitytė (2001)

NS, not specified

For many foods, the Committee has previously outlined that there tend to be increases in tin in canned foods over time. This study did not demonstrate this. This may be due to the food type that was assessed.

In a study in the United Kingdom, samples of canned foods were analysed for tin between December 2000 and August 2001 (UK FSA, 2002). A summary of these data is shown in Table 5. There were over 1200 individual samples taken for this survey, with results from 400 brand/product combinations reported. Each of the 400 samples usually consisted of three individual cans of the food from the same batch number. Of the 400 samples, only two samples (one of spaghetti in tomato sauce and the other of gooseberries) exceeded the maximum permitted

level specified in the United Kingdom's *Tin in Food Regulations 1992* of 200 mg/kg.

Table 5. Concentrations of tin in canned foods in the United Kingdom

Product type	Number of samples	Tin concentration (mg/kg as sold)		
		Mean ^a	Minimum	Maximum
Tomatoes (plum and chopped)	54	24	<5	196
Spaghetti and other pasta in tomato sauce	54	61	<5	298
Tomato soups	54	77	<5	199
Other tomato-based soups, e.g. minestrone, vegetable, etc.	30	25	<5	141
Baked beans	42	24	<5	76
Cooking sauces containing tomato	30	<5	<5	6
Pineapples	30	61	26	169
Fruit cocktail	18	78	9	167
Grapefruit	15	80	39	123
Apricots	15	68	<5	135
Fruit fillings	12	<5	<5	<5
Mushrooms	10	9	<5	46
Celery	9	<5	<5	<5
Gooseberries	9	106	<5	237
Rhubarb	9	6	<5	13
Asparagus	9	47	<5	139
All samples	400	44	<5	298

From UK FSA (2002)

^a Means are calculated by assuming that not detected results are equal to the limit of detection (LOD) of 5 mg/kg.

The 2000 United Kingdom Total Diet Study also included an analysis of tin in foods (UK FSA, 2004). The tin concentrations determined are shown in Table 6. The samples were analysed as composites. Concentrations are reported as a range where there were not detected results for some foods. The lower-bound mean is where not detected results were assigned a concentration of zero, and upper-bound means were where not detected results were assigned a concentration equal to the limit of detection (LOD) (between 0.0001 and 0.0016 mg/kg, depending on the food).

Table 6. Mean tin concentration for food groups in the 2000 United Kingdom Total Diet Study

Food group	Mean tin concentration (mg/kg)
Bread	0.006
Miscellaneous cereals	0.030
Carcass meat	0.008
Offal	0.007
Meat products (including some canned)	0.130
Poultry	0.003
Fish	0.028
Fats and oils	0.004–0.005
Eggs	0.0002–0.0005
Sugar and preserves	0.055
Green vegetables	0.0009
Potatoes	0.001
Other vegetables	0.008
Canned vegetables	25.000
Fresh fruits	0.012
Fruit products (including some canned)	11.000
Beverages	0.001
Milk	0.0008–0.0009
Dairy products	0.034
Nuts	0.022

From UK FSA (2004)

Overall, concentrations from the 2000 United Kingdom Total Diet Study are similar to or lower than those from the previous 1997 United Kingdom Total Diet Study and were lower than those from the United Kingdom survey outlined above (UK FSA, 2002) specifically on canned fruits and vegetables, particularly for non-canned foods.

The recent data on tin concentrations in foods are consistent with the findings of the last review of tin undertaken by the Committee, the new concentrations ranging between <1 and 300 mg/kg.

4. DIETARY INTAKE ASSESSMENT

4.1 Introduction and background to intake estimates

Inorganic tin (hereafter called tin) was last evaluated by the Committee at its fifty-fifth meeting in 2000 (Annex 1, reference 150). The last evaluation included information on concentrations in foods and estimates of chronic intake. Estimates of intake were compared with the PTWI of 14 mg/kg bw maintained by the Committee at its fifty-fifth meeting.

In summary, the previous evaluation of tin intakes showed that the main source of tin in the diet is from canned foods. Water and air were not significant sources of intake of tin for the general population. Natural concentrations of tin in plant and animal foods were low. Tin-containing food additives contributed little to the intake. There were a number of factors influencing the tin concentrations in canned foods, including the pH of the food, whether the can is lacquered, storage conditions, plant pigments and the presence of reducible organic compounds.

Mean dietary intakes of tin reported from seven countries ranged from <1 mg/day to about 14 mg/day, considerably lower than the tolerable daily intake established at the earlier meeting by the Committee.

This current assessment is an addendum to the report from the fifty-fifth meeting (Annex 1, reference 150). While the focus of this evaluation by the Committee was on acute toxicity and therefore short-term dietary intakes, data submitted to the Committee for review at this meeting or that have become available since the last review of tin by the Committee that related to chronic intakes have also been included in this addendum.

The Inter-Industry Tin Strategy Group submission included some estimates of chronic tin intakes for review by the Committee.

Also included in this assessment are short-term intake estimates for tin (i.e. in a period of 24 h or less). Short-term intake estimates for tin have not previously been considered by the Committee. Short-term intake estimates were submitted for Australia and New Zealand.

4.2 Estimated chronic dietary intakes of tin

Some recent estimates of chronic intakes of tin have become available since the last review of tin by the Committee. Chronic intake estimates for tin were submitted from two Australian total diet studies and a United Kingdom total diet study.

4.2.1 Methods

Individual dietary records were used for intake estimates for the Australian Total Diet Surveys, producing a distribution of estimated intakes from which population summary statistics (e.g. mean intakes) were derived. The 2000 United

Kingdom Total Diet Study estimates of tin intakes were based on a model diet of mean population consumption data.

Apart from reviewing the recent chronic intakes submitted to the Committee, a literature review was also conducted to find recent studies of chronic intake of tin. No other recent estimates were found.

4.2.2 *Australia*

Estimated chronic intakes of tin from two recent Australian Total Diet Surveys have become available since the last review of tin by the Committee: the 19th Australian Total Diet Survey (ANZFA, 2001) and the 20th Australian Total Diet Survey (FSANZ, 2002). Estimated intakes from these two surveys are summarized in Table 7.

Table 7. Estimated mean chronic intakes of tin from recent Australian Total Diet Surveys for all respondents in various age groups

Population group	Estimated tin intake (mg/kg bw per day)	
	19th ATDS ^a	20th ATDS ^b
Adult males 25–34 years	0.0088–0.0094	0.0013–0.0016
Adult females 25–34 years	0.009–0.0096	0.0015–0.0018
Boys 12 years	0.011	0.0017–0.0020
Girls 12 years	0.0091–0.0096	0.00064–0.00093
Toddlers 2 years	0.031–0.032	0.012–0.013
Infants 9 months	0.013–0.015	0.0087–0.0090

ATDS, Australian Total Diet Survey

^a ANZFA (2001).

^b FSANZ (2002).

The estimated intakes for the 19th and 20th Australian Total Diet Surveys were calculated using analytical concentrations of tin (see Table 3) and food consumption data from the 1995 Australian National Nutrition Survey ($n = 13\,858$). The survey respondents were 2 years old and above. The survey used a 24-h recall methodology. Foods consumed in the National Nutrition Survey were “mapped” to the foods analysed in the Australian Total Diet Survey before concentrations of tin were assigned. Individual dietary records and body weights from individuals were used for the intake calculations, resulting in a distribution of intakes, from which population summary statistics were derived. Results for all respondents in the age groups assessed are presented. Lower- and upper-bound estimates were derived, the lower-bound estimate where not detected results were assigned a concentration of zero, and an upper-bound estimate where not detected results were assigned a concentration equal to the LOR (0.02 mg/kg, 19th Australian Total Diet Survey; 0.01 mg/kg, 20th Australian Total Diet Survey).

Intakes for infants 9 months of age were based on a model diet with average food consumption data, extrapolated from a 2-year-old's diet.

These intakes are similar to or slightly lower than those estimated from an earlier total diet study from Australia that was considered in the previous review of tin by the Committee. The differences in the intakes could be attributed to differences in methodology used to estimate the intakes.

4.2.3 *United Kingdom*

When the United Kingdom recently conducted an analytical survey of tin in canned foods (see concentration data in Table 5), intake of tin from the diet was also estimated (UK FSA, 2002). Tin intake from canned foods was estimated, with the additional intake from the rest of the diet added on. The additional intake was the mean intake of tin from the 1997 United Kingdom Total Diet Study. Estimated intakes of tin from this United Kingdom study are shown in Table 8. The estimated intakes are an overestimate; they include a certain amount of double-counting, as canned foods would have been included in the estimation of the mean intake of tin from the 1997 Total Diet Study. Samples less than the LOD were assigned a concentration equal to the LOD.

Table 8. Estimated intakes of tin from United Kingdom survey of tin in canned foods

Intake (mg/person per day)				
From canned fruits and vegetables		From rest of diet (1997 TDS)	Total dietary intake	
Mean	P97.5	Mean	Mean	P97.5
1.7	5.6	1.9	3.6	7.5

From UK FSA (2002)

P97.5, 97.5th percentile; TDS, Total Diet Study

Estimated tin intakes for the United Kingdom from the 2000 Total Diet Study (UK FSA, 2004) were also submitted for consideration by the Committee. Estimated intakes for consumers only are shown in Table 9. The Total Diet Study included 119 foods combined into 20 food groups for analysis. The proportion of each food reflects its relative importance in the United Kingdom diet, based on data from the National Food Survey. The mean concentration of tin for each food was used in the calculations. Concentration data for tin used in the intake estimates were shown previously in Table 6. Food consumption data used for these calculations were from the relevant National Diet and Nutrition Survey. Children had higher intakes on a body weight basis due to their higher food consumption amounts per kilogram of body weight. The foods contributing the most to tin intakes were canned vegetables (61%) and fruit products (37%).

Table 9. Estimated consumer intakes of tin for various population groups from the 2000 United Kingdom Total Diet Study

Population group	Mean intake (mg/kg bw per day)	P97.5 intake (mg/kg bw per day)
Adults	0.020	0.070
Toddlers 1.5–4.5 years	0.070	0.283
Young people 4–18 years	0.038	0.150
Elderly: free-living	0.017	0.076
Elderly: institutionalized	0.017	0.061
Vegetarians ^a	0.026	0.101

From UK FSA (2004)

P97.5, 97.5th percentile

^a Some of these respondents consumed fish.

Estimated intake of tin on a population basis, reported in the 2000 United Kingdom Total Diet Study report (based on consumption data from the National Food Survey), was 1.4 mg/day. While recognizing the different methodologies used to estimate intakes for the total population from United Kingdom Total Diet Studies since 1976 (i.e. different survey methodology, food consumption survey data and analytical methodology), it appears as though tin intakes of the population have reduced from 4.4 mg/day in 1976 to 1.4 mg/day in 2000 (COT, 2003).

4.3 Estimated short-term dietary intakes of tin

Short-term dietary intakes of contaminants from food are defined as those occurring in a period of 24 h or less.

A limited number of short-term intake estimates were available for the Committee to review. Short-term intake estimates were submitted for Australia and New Zealand. A literature search was also conducted; however, no short-term intake estimates for tin were located. Additional short-term intake estimates were made based on United Kingdom tin concentration data from the recent study on tin in canned foods reported above (UK FSA, 2002). Estimated intakes were also calculated based on proposed Codex MLs for tin in canned foods.

4.3.1 Methods

There is no internationally agreed methodology for estimating short-term dietary intakes of contaminants.

International Estimated Short Term Intakes have been calculated for pesticide residues for a number of years and have been included in the assessments conducted by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and also considered at the Codex Committee on Pesticide Residues. Some national

agencies also conduct short-term intake assessments for agricultural residues and veterinary chemicals.

Equations for estimating short-term intakes for pesticide residues are defined by JMPR (WHO, 2005). Some of the basic principles of the methodology used for estimating short-term dietary intakes for pesticide residues can be applied to contaminants and were therefore used to develop the method for calculating short-term intakes of tin.

The main data sets required to calculate short-term intakes are information on food consumption and chemical concentrations. Information on body weights is also required.

Estimated short-term dietary intakes of pesticide residues are based on 97.5th-percentile consumption amounts. This was deemed appropriate to use for short-term intake estimates for the tin assessment. Thirty-nine or more consumers of a food are needed to ensure a reliable 97.5th-percentile consumption figure.

In relation to concentration data, the calculations of short-term intake for pesticide residues take account of the high variability in residue levels between individual commodity units (e.g. individual apples) within a composite sample, by the incorporation of a variability factor, where relevant. It is assumed that for commodities where the unit weight is smaller than the total high consumption amount, the first unit consumed has a concentration equal to the highest residue of any composite sample from data from supervised trials for use of the chemical, multiplied by the variability factor. The rest of the consumption is assigned a concentration equal to the highest residue. This is based on the fact that the treatment of crops, from sprayed applications, for example, can result in one unit being sprayed with a lot of the chemical, whereas another unit, which may have been more protected from the spray by leaves or other units, has not had the same level of application of the chemical.

Tin contamination is generally not expected to result in residues that follow a pattern similar to pesticide residues. Therefore, in the short-term intake estimates for tin, no consideration of serving size or varying concentrations in different proportions of the total consumption have been taken into account.

Mean or median concentrations of contaminants are generally used in calculations of intake estimates for contaminants on a long-term basis; however, different concentration data are needed for conducting short-term intake estimates. Resulting residues from pesticide trials would not be expected to be above a certain concentration if the chemical has been applied and used in the specified way. Contaminants that are naturally occurring generally have a positively skewed distribution of concentration values. Within the naturally occurring distribution, there can be some high concentrations. This may occur in "spikes" for some contaminants such as toxins. Naturally occurring tin concentrations in foods are very low, with none of the high values seen in canned foods. Tin concentrations in canned foods probably vary in distribution, depending on the food.

Based on the general approach for calculating short-term intake estimates for pesticide residues, the following method has been determined and used for the short-term intake estimates for tin:

Short-term intake (mg/kg bw per day)

$$= \frac{\text{P97.5th consumption (kg)} \times \text{representative high tin concentration (mg/kg)}}{\text{Mean body weight (kg)}}$$

Short-term intakes based on 90th-percentile tin concentration data and the maximum concentration of tin for a food group are presented in Tables 11 and 13 below. The 90th percentile is reflective of representative high concentrations of tin in a canned food. The maximum concentrations were, for the majority of samples, below the proposed Codex MLs (200 mg/kg for canned beverages and 250 mg/kg for canned foods other than beverages) and, if not, were not largely exceeding this value. The maximum concentrations for the countries for which short-term intakes were calculated were a lot lower than concentrations reported in the previous review by the Committee (up to 1000 mg/kg); therefore, it was not unreasonable to estimate short-term intakes based on maximum concentrations as well.

Short-term dietary intakes of tin were calculated only for canned foods, as these contribute the most to chronic intakes, and as concentrations in foods from naturally occurring sources are much lower and do not warrant investigation for acute health risks.

Due to the varying nature of tin concentrations in different canned foods, which depends on the food type, there is a need to look at some foods separately. Therefore, short-term intakes were calculated for single foods. Intakes of tin from short-term assessments cannot be summed to obtain a total short-term intake of tin from all foods because they are based on high-level consumption data, and it is unrealistic to assume that people have consumed all foods at a high level of consumption. In addition, high-level food consumption figures are derived for “consumers only” for each food group. There is a different population of consumers for each food, which means that estimated short-term intakes cannot be summed across the whole diet.

Short-term intakes were also calculated for tin based on proposed Codex MLs to determine whether concentrations of tin at the current proposed regulatory limits would pose an undue acute health risk to humans.

Short-term estimates from consumption of water have not been estimated due to the low contribution of water to chronic intakes of tin and low tin concentrations in water (0.01 mg/l quoted previously by the Committee; Annex 1, reference 150).

Short-term intake estimates have been calculated on a “whole population” basis only; no population subgroups were assessed.

Concentration data used for the Australian and New Zealand short-term intake estimates were sourced from a range of Australian and New Zealand national surveys, including the 19th and 20th Australian Total Diet Surveys mentioned above. It was assumed that concentrations of tin in canned foods are the same for Australia and New Zealand. Results from individual and composite samples were

pooled for each food to boost sample numbers to derive concentrations for use in estimating short-term intakes. Concentration data are shown in Table 10. Microsoft Excel was used to derive the 90th-percentile figures. Nine samples or more for a food are needed to ensure a reliable 90th-percentile concentration figure. Despite this, estimated intakes based on fewer than nine samples are presented below for completeness, although they have been highlighted as being not as reliable.

Table 10. Tin concentrations used for short-term intake assessments for Australia and New Zealand

Food	Tin concentration (mg/kg)		Number of samples
	90th-percentile tin concentration	Maximum tin concentration	
Pineapple, canned	87.0	270.0	52
Peaches, canned	107.9	130.0	12
Fruit salad, canned	86.4	116.0	9
Asparagus, canned	186.5 ^a	230.0	4
Kidney beans, canned	1.1	1.1	9
Baked beans, canned	172.8	230.0	27
Beetroot, canned	5.1	9.0	19
Corn, sweet and creamed, canned	1.4	2.0	11
Mushrooms, canned	197.0	260.0	84
Tomatoes, canned in juice	112.0	190.0	71
Water chestnuts, canned	5.2 ^a	5.4	4
Spaghetti in tomato sauce, canned	169.2 ^a	190.0	3
Seafood, canned (including crab, salmon, tuna, abalone)	2.0	2.0	38

^a Not as reliable due to fewer than nine samples.

4.3.2 Australia

Consumption figures for canned foods for Australia at the 97.5th-percentile level are based on food consumption for a 24-h period based on data from the 1995 Australian National Nutrition Survey and include consumption of the food on its own (e.g. a can of diced peaches as a snack) as well as use of the item in a mixed food (e.g. canned peaches in a trifle dessert).

Estimated short-term intakes for Australia for tin from canned foods are presented in Table 11. Intakes ranged from 0.004 to 1.34 mg/kg bw per day when based on 90th-percentile tin concentrations and from 0.005 to 1.53 mg/kg bw per day based on maximum tin concentrations. There was little difference between the estimated intakes based on the different concentration levels, because there was

Table 11. Estimated short-term intakes of tin for Australia based on analytical data

Food	Number of consumers	97.5th-percentile consumption (kg)	Source of concentration data	Short-term intake: 90th-percentile concentrations (mg/kg bw per day) ^a	Short-term intake: maximum concentrations (mg/kg bw per day) ^a
Tropical fruit, canned, including pineapple	331	0.236	Pineapple, canned	0.310	0.950
Peaches, canned	233	0.496	Peaches, canned	0.800	0.960
Stone fruits, except peaches	87	0.275	Peaches, canned	0.440	0.530
Fruit salad, canned	148	0.526	Fruit salad, canned	0.680	0.910
Two fruits, canned	41	0.521	Fruit salad, canned	0.670	0.900
Pome fruits (including apple and pear), canned	83	0.475	Fruit salad, canned	0.610	0.820
Stalk vegetables (including asparagus)	140	0.253	Asparagus, canned	0.700 ^b	0.870
Legumes and pulses (including kidney beans, lentils, chickpeas, soya beans)	119	0.310	Kidney beans, canned	0.005	0.005
Baked beans, canned	376	0.446	Baked beans, canned	1.150	1.530
Root vegetables, including beetroot and carrot	963	0.099	Beetroot, canned	0.008	0.013
Corn, sweet and creamed, canned	293	0.192	Corn, sweet and creamed, canned	0.004	0.006
Mushrooms, canned	673	0.192	Mushrooms, canned	0.570	0.750
Tomatoes, canned in juice	80	0.439	Tomatoes, canned	0.730	1.250

Table 11. (contd)

Food	Number of consumers	97.5th-percentile consumption (kg)	Source of concentration data	Short-term intake: 90th-percentile concentrations (mg/kg bw per day) ^a	Short-term intake: maximum concentrations (mg/kg bw per day) ^a
Artichoke/water chestnuts, canned	23	0.094 ^b	Water chestnuts, canned	0.007 ^b	0.008 ^b
Pasta in tomato sauce, canned	151	0.530	Spaghetti, canned	1.340 ^b	1.500
Seafood, canned	0.559	0.225	All canned seafood	0.007	0.007

^a Mean body weight of all respondents, 67 kg.

^b Not as reliable due to either fewer than 39 consumers for the food or fewer than 9 samples for the analytical data.

often very little difference between the 90th-percentile and maximum tin concentrations for a food.

Based on the ML of tin in canned foods in Australia, 250 mg/kg, estimated short-term intakes of tin, using the same consumption data as used in previous estimates, are shown in Table 12. Short-term intakes range from 0.35 to 1.98 mg/kg bw per day.

Table 12. Estimated short-term intakes of tin for Australia based on national MLs

Canned food	Short-term intake based on Australian ML, 250 mg/kg (mg/kg bw per day) ^a
Tropical fruit, canned, including pineapple	0.88
Peaches, canned	1.85
Stone fruits, except peaches	1.03
Fruit salad, canned	1.96
Two fruits, canned	1.94
Pome fruits (including apple and pear), canned	1.77
Stalk vegetables (including asparagus)	0.94
Legumes and pulses (including kidney beans, lentils, chickpeas, soybeans)	1.16
Baked beans, canned	1.66
Root vegetables, including beetroot and carrot	0.37
Corn, sweet and creamed, canned	0.72
Mushrooms, canned	0.72
Tomatoes, canned in juice	1.64
Artichoke/water chestnuts, canned	0.35 ^b
Pasta in tomato sauce, canned	1.98
Seafood, canned	0.84

^a Mean body weight of all respondents, 67 kg.

^b Not as reliable due to fewer than 39 consumers for the food.

4.3.3 New Zealand

Consumption figures for canned foods for New Zealand at the 97.5th-percentile level are based on food consumption for a 24-h period based on data from the 1997 New Zealand National Nutrition Survey and include consumption of the food on its own (e.g. a can of diced peaches as a snack) as well as use of the item in a mixed food (e.g. canned peaches in a trifle dessert).

Estimated short-term intakes for New Zealand for tin from canned foods are presented in Table 13. Intakes ranged from 0.005 to 1.37 mg/kg bw per day when based on 90th-percentile tin concentrations and from 0.005 to 1.55 mg/kg bw per day based on maximum tin concentrations. There was little difference between the estimated intakes based on the different concentration levels because there was often very little difference between the 90th-percentile and maximum tin concentrations for a food.

Based on the ML of tin in canned foods in New Zealand, 250 mg/kg, estimated short-term intakes of tin, using the same consumption data as used in the estimates above, are shown in Table 14. Short-term intakes range from 0.39 to 3.34 mg/kg bw per day.

The same concentration values were used for the Australian and New Zealand estimates; therefore, the similarity in the short-term intake estimates between the two countries can be attributed to similar consumption of canned foods.

4.3.4 United Kingdom

From the 2002 United Kingdom survey of tin in canned fruits and vegetables (UK FSA, 2002), there were over 1200 individual cans of food analysed. The raw data were compiled, and the 90th-percentile and maximum tin concentrations were determined from the 1200 samples for use in the short-term intake estimates. These are shown in Table 15.

In the absence of high-percentile food consumption data for canned foods for the United Kingdom, a single can size was used as a representative high-percentile consumption amount (approximately 440 g). Using this as approximating a 97.5th-percentile consumption does not appear to be unreasonable when comparing it with the 97.5th-percentile consumption figures derived for Australia and New Zealand. It may be an overestimate of the amount of food consumed for some foods that are generally eaten in smaller quantities (e.g. peas compared with peaches); however, this means the short-term intake estimate is likely to be a worst-case scenario.

Estimated short-term intakes for tin for the United Kingdom based on the 90th-percentile tin concentrations range between 0.037 and 1.3 mg/kg bw per day; based on maximum tin concentrations, short-term intakes range between 0.037 and 2.19 mg/kg bw per day.

4.4 General estimates of short-term intakes based on MLs

Various MLs are specified for tin, usually in canned foods, in regulations both internationally, by Codex, and at the national level. Assessments of intake of tin based on Codex-proposed MLs and high-level consumption data have also been made to determine if there are acute health concerns associated with these levels.

Proposed MLs by Codex were 200 mg/kg in canned beverages and 250 mg/kg in canned foods other than beverages.

Table 13. Estimated short-term intakes of tin for New Zealand based on analytical data

Food	Number of consumers	97.5th-percentile consumption (kg)	Source of concentration data	Short-term intake: 90th-percentile concentrations (mg/kg bw per day) ^a	Short-term intake: maximum concentrations (mg/kg bw per day) ^a
Tropical fruit, canned, including pineapple	113	0.234	Pineapple, canned	0.280	0.890
Stone fruits, including peaches, apricots, canned	175	0.416	Peaches, canned	0.630	0.760
Fruit salad, canned	88	0.474	Fruit salad, canned	0.580	0.770
Pome fruits (including apple and pear), canned	31	0.950 ^b	Fruit salad, canned	1.160 ^b	1.550 ^b
Asparagus, canned	15	0.300 ^b	Asparagus, canned	0.790 ^b	0.970 ^b
Beans mix, canned	119	0.310	Kidney beans, canned	0.005	0.005
Baked beans, canned	101	0.425	Baked beans, canned	1.030	1.380
Beetroot, canned	145	0.111	Beetroot, canned	0.008	0.014
Corn, sweet and creamed, canned	56	0.425	Corn, sweet and creamed, canned	0.008	0.012
Mushrooms, canned	13	0.220 ^b	Mushrooms, canned	0.610 ^b	0.810 ^b
Tomatoes, canned in juice	88	0.408	Tomatoes, canned	0.640	1.090
Spaghetti in tomato sauce, canned	102	0.576	Spaghetti, canned	1.370 ^b	1.540
Seafood, canned	195	0.289	All canned seafood	0.008	0.008

^a Mean body weight of all respondents, 71 kg.^b Not as reliable due to either fewer than 39 consumers for the food or fewer than 9 samples for the analytical data.

Table 14. Estimated short-term intake of tin for New Zealand based on national MLs

Canned food	Short-term intake based on New Zealand ML, 250 mg/kg (mg/kg bw per day) ^a
Tropical fruit, canned, including pineapple	0.820
Stone fruits, including peaches, apricots	1.470
Fruit salad, canned	1.670
Pome fruits (including apple and pear), canned	3.340 ^b
Asparagus, canned	1.060 ^b
Beans mix, canned	1.090
Baked beans, canned	1.500
Beetroot, canned	0.390
Corn, sweet and creamed, canned	1.500
Mushrooms, canned	0.780 ^b
Tomatoes, canned in juice	1.440
Spaghetti in tomato sauce, canned	2.030
Seafood, canned	1.020

^a Mean body weight of all respondents, 71 kg.

^b Not as reliable due to fewer than 39 consumers for the food.

The estimated short-term intake of tin based on consumption of canned foods of a large can size (440 g) and the highest proposed ML of 250 mg/kg would be 1.8 mg/kg bw per day (Table 16).

4.5 Conclusions

The concentrations of tin in foods from recent studies are similar to those previously evaluated by the Committee, with the new data ranging between <1 and 300 mg/kg.

These recent chronic intake estimates of tin from Australia and the United Kingdom were in the same range of reported intakes presented in the previous review of tin by the Committee (which were from <1 to 14 mg/day). All estimated chronic intakes were lower than the tolerable limit that was previously established by the Committee. The major contributor to tin intake is canned foods.

Estimated short-term dietary intakes range between 0.004 and 3.3 mg/kg bw per day. Intakes depend on the concentration in the food, but also the type of food. These short-term intakes can be regarded as preliminary only due to small sample numbers for either the food consumption data or concentration data from which they were derived and the assumptions made for the calculations. They are a

Table 15. Concentrations of tin in canned foods from the United Kingdom and short-term intake estimates

Food	Number of samples	90th percentile		Maximum	
		Tin concentration (mg/kg)	Short-term intake (mg/kg bw per day) ^a	Tin concentration (mg/kg)	Short-term intake (mg/kg bw per day) ^a
Canned tomato products	161	79	0.580	196	1.440
Canned pasta products	162	119	0.870	298	2.190
Canned tomato soup products	162	139	1.020	196	1.440
Canned tomato-based soup products	90	54	0.400	141	1.034
Baked beans	126	46	0.340	76	0.560
Canned cooking sauces	90	5	0.037	6	0.044
Canned pineapple	90	96	0.710	168	1.230
Canned fruit cocktail	54	147	1.080	166	1.220
Canned grapefruit	45	100	0.730	123	0.900
Canned apricot	45	109	0.800	135	0.990
Canned fruit fillings	36	5	0.037	5	0.037
Canned mushrooms	30	9	0.065	46	0.340
Canned celery	27	5	0.037	5	0.037
Canned gooseberries	27	178	1.300	236	1.730
Canned rhubarb	27	9	0.065	12	0.088
Canned asparagus	29	108	0.790	139	1.020

^a Based on a canned food consumption amount of 440 g and a mean body weight of 60 kg.

Table 16. Short-term intake estimates for tin, based on can size and MLs

Can size (g)	ML (mg/kg)	Body weight (kg)	Short-term intake (mg/kg bw per day)
440	250	60	1.83
	200	60	1.47
220	250	60	0.92
	200	60	0.73

guide to likely short-term dietary intakes only and are not representative of international intakes.

No short-term intakes of tin specifically from beverages, such as canned fruit juices, were available, as submitters had no data on either consumption or concentrations of tin in these foods. Beverages are consumed in larger amounts than non-beverage foods, which may potentially lead to higher short-term intakes of tin from beverages, and even more so if high tin concentrations occur in this food group.

No short-term estimates of tin intake by children were available due to a lack of information on consumption of canned foods for this age group. Children are likely to have higher short-term intakes of tin per kilogram of body weight compared with adults, due to their lower body weights and higher food consumption per kilogram of body weight than adults.

The Committee noted that the data on acute effects available indicated that it is inappropriate to establish an ARfD for inorganic tin, since whether or not acute effects of gastric irritation occur after ingesting a food containing tin depends on the concentration and nature of tin in the product, rather than on the dose ingested on a body weight basis. Therefore, the Committee concluded that the short-term dietary intake estimates were not particularly relevant for the assessment, as they were estimated likely doses of total inorganic tin.

5. COMMENTS

5.1 Observations in humans

Episodes of human poisoning resulting from consumption of food and drink contaminated with inorganic tin have resulted in abdominal distension and pain, vomiting, diarrhoea and headache. Symptoms commonly start within 0.5–3 h, and recovery occurs within 48 h. The doses of inorganic tin ingested in such episodes of poisoning were not estimated, but the symptoms occurred when canned food or beverages were found to contain tin at concentrations varying from 250 to 2000 mg/kg.

In one study, all five volunteers experienced symptoms when they ingested orange juice containing inorganic tin at a concentration of 1370 mg/kg (equal to a

dose of 4.4–6.7 mg/kg bw). Orange juice containing inorganic tin at concentrations of 498, 540 or 730 mg/kg (equal to a dose range of 1.6–3.6 mg/kg bw) did not provoke any symptoms in groups of five volunteers. Administration of the same amount of the same juice (containing tin at 1370 mg/kg) to these individuals 1 month later resulted in symptoms in only one person. Although this was explained by the authors as development of tolerance, another possible explanation might be that the longer storage of the juice led to a different speciation.

A newly available study (Boogaard et al., 2003) showed that tomato juice freshly spiked with tin(II) chloride at a concentration of ≥ 161 mg/kg causes gastrointestinal disorders in humans in a concentration-related manner. The concentration–response relationship indicated a threshold for acute effects caused by inorganic tin at a concentration of about 150 mg/kg of juice. In the second part of this study, volunteers receiving 250 ml of a tomato soup contaminated with inorganic tin that had migrated from packaging at concentrations of <0.5, 201 and 267 mg/kg did not experience an increased incidence of adverse effects compared with controls. The results of distribution studies of tin in the soup and juice consumed supported the view that both complexation and adsorption of tin onto solid matter reduce its irritant effect on the gastrointestinal tract.

Overall, the information available showed that gastrointestinal irritation from inorganic tin in canned foods is more closely related to the concentration and nature of tin in the product than to the dose of tin ingested on a body weight basis. No information was available regarding subpopulations such as children or people with gastrointestinal disorders.

5.2 *Prevention and control*

The lacquering of tin-plated cans prevents the migration of inorganic tin into food and beverages. Food and beverages should not be stored in opened tin-plated cans.

5.3 *Levels and pattern of food contamination*

Data on the concentrations of inorganic tin in a range of foods from four countries (Australia, France, Lithuania and the United Kingdom) had become available since the last review of inorganic tin by the Committee and were reviewed at this meeting. The Committee noted that the reported concentrations of inorganic tin were in the same range as those previously assessed by the Committee, the new values ranging from not detected to 300 mg/kg.

5.4 *Dietary intake assessment*

The major dietary source of inorganic tin is food packaged in unlacquered or partially lacquered tin-plated cans. The migration of inorganic tin from tin plate into foods is greater in highly acidic foods such as pineapples and tomatoes; with increased time and temperature of food storage; and in foods, such as fruit juice, in opened cans. The inorganic tin content of canned foods is variable, and some

foods may have concentrations high enough to cause an acute toxic reaction. Information previously evaluated by the Committee and additional data from Australia and the United Kingdom indicated that the mean long-term dietary intakes of inorganic tin by individuals ranged from <1 to about 14 mg/person per day. Population groups with higher intakes of canned foods may have higher intakes of inorganic tin. A small number of estimates of short-term dietary intake (i.e. in a period of 24 h or less) were assessed by the Committee. Based on limited data, preliminary short-term intakes of inorganic tin were estimated to be between 0.004 and 3.3 mg/kg bw per day, depending on the food considered.

6. EVALUATION

The Committee concluded that the data available indicated that it is inappropriate to establish an ARfD for inorganic tin, since whether or not irritation of the gastrointestinal tract occurs after ingestion of a food containing tin depends on the concentration and nature of tin in the product, rather than on the dose ingested on a body weight basis. Therefore, the Committee concluded that the short-term intake estimates were not particularly relevant for the assessment, as they were estimated likely doses of total inorganic tin. The Committee reiterated its opinion, expressed at its thirty-third and fifty-fifth meetings, that the available data for humans indicated that inorganic tin at concentrations of >150 mg/kg in canned beverages or >250 mg/kg in canned foods may produce acute manifestations of gastric irritation in certain individuals. Therefore, ingestion of reasonably sized portions of food containing inorganic tin at concentrations equal to the proposed standard for canned beverages (200 mg/kg) may lead to adverse reactions. No information was available as to whether there are subpopulations that are particularly sensitive for such adverse reactions. The Committee reiterated its advice that consumers should not store food and beverages in opened tin-plated cans.

In addition, the Committee noted that the basis for the PMTDI and PTWI established at its twenty-sixth and thirty-third meetings was unclear and that these values may have been derived from intakes associated with acute effects. The Committee concluded that it was desirable to (re)assess the toxicokinetics and effects of inorganic tin after long-term exposure to dietary doses of inorganic tin at concentrations that did not elicit acute effects.

7. REFERENCES

- ANZFA (2001) *The 19th Australian Total Diet Survey*. Canberra: Australia New Zealand Food Authority (now Food Standards Australia New Zealand) (<http://www.foodstandards.gov.au/mediareleasespublications/publications/19thaustralia%20totaldietsurveyapril2001/index.cfm>).
- Barnes, J.M. & Stoner, H.B. (1959) The toxicology of tin compounds. *Pharmacol. Rev.*, **11**, 211–231.
- Benoy, C.J., Hooper, P.A. & Schneider, R. (1971) The toxicity of tin in canned fruit juices and solid foods. *Food Cosmet. Toxicol.*, **9**, 645–656.
- Blunden, S. & Wallace, T. (2003) Tin in canned food: a review and understanding of occurrence and effect. *Food Chem. Toxicol.*, **41**, 1651–1662.

- Boogaard, P.J., Boisset, M., Blunden, S., Davies, S., Ong, T.J. & Taverne, J.P. (2003) Comparative assessment of gastrointestinal irritant potency in man of tin(II) chloride and tin migrated from packaging. *Food Chem. Toxicol.*, **41**, 1663–1670.
- Brown, R.A., Nazario, C.M., de Tirado, R.S., Castrillon, J. & Agard, E.T. (1977) A comparison of the half-life of inorganic and organic tin in the mouse. *Environ. Res.*, **13**, 56–61.
- CAC (1999) *Report of the Thirty-first Session of the Codex Committee on Food Additives and Contaminants, The Hague, The Netherlands, 22–26 March 1999*. Rome: Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ALINORM 99/12A; <http://www.codexalimentarius.net/web/archives.jsp?year=99>).
- CAC (2003) *Report of the Thirty-fifth Session of the Codex Committee on Food Additives and Contaminants, Arusha, Tanzania, 17–21 March 2003*. Rome: Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ALINORM 03/12A; <http://www.codexalimentarius.net/web/archives.jsp?year=03>).
- CAC (2004) *Report of the Thirty-sixth Session of the Codex Committee on Food Additives and Contaminants, Rotterdam, The Netherlands, 22–26 March 2004*. Rome: Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ALINORM 04/27/12; <http://www.codexalimentarius.net/web/archives.jsp?lang=en>).
- COT (2003) *COT Statement on Twelve Metals and Other Elements in the 2000 Total Diet Study*. London: United Kingdom Food Standards Agency, Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT statement 2003/07; <http://www.food.gov.uk/multimedia/pdfs/cotstatements2004metals.pdf>).
- de Silva, C.R., Oliveira, M.B., Melo, S.F., Dantas, F.J., de Mattos, J.C., Bezerra, R.J., Caldeira-de-Araujo, A., Duatti, A. & Bernardo-Filho, M. (2002) Biological effects of stannous chloride, a substance that can produce stimulation or depression of the central nervous system. *Brain Res. Bull.*, **59** (3), 213–216.
- FSANZ (2002) *20th Australian Total Diet Survey*. Canberra: Food Standards Australia New Zealand (<http://www.foodstandards.gov.au/mediareleasespublications/publications/20thaustralia nttotaldietsurveyjanuary2003/index.cfm>).
- Health Council (2005) *Tin and Inorganic Tin Compounds: Health-Based Recommended Occupational Exposure Limit*. The Hague: Health Council of the Netherlands, Dutch Expert Committee on Occupational Standards (Publication No. 2005/06OSH).
- Hiles, R.A. (1974) Absorption, distribution and excretion of inorganic tin in rats. *Toxicol. Appl. Pharmacol.*, **27**, 366–379.
- Janssen, P.J., Bosland, M.C., van Hees, J.P., Spit, B.J., Willems, M.I. & Kuper, C.F. (1985) Effects of feeding stannous chloride on different parts of the gastrointestinal tract of the rat. *Toxicol. Appl. Pharmacol.*, **78** (1), 19–28.
- Kolmer, J.A., Brown, H. & Harkins, M.J. (1931) A note on tin compounds with chemotherapy of experimental staphylococcus infection. *J. Pharmacol.*, **43**, 515–519.
- Magos, L. (1986) Tin. In: Friberg, L., Nordberg, G. & Vouk, V., eds., *Handbook on the Toxicology of Metals*. Vol. 2. Amsterdam: Elsevier, pp. 568–593.
- Marciniak, M. (1981) Bivalent tin metabolism and toxicity after intravenous injection in rat. *Acta Physiol. Pol.*, **32**, 193–204.
- Ogoshi, K., Kurumatani, N., Aoki, Y., Moriyama, T. & Nanzai, T. (1981) Decrease in compressive strength of the femoral bone in rats administered stannous chloride for a short period. *Toxicol. Appl. Pharmacol.*, **58**, 331–332.
- Ramonaitytė, D.T. (2001) Copper, zinc, tin and lead in canned evaporated milk, produced in Lithuania: the initial content and its change at storage. *Food Addit. Contam.*, **18** (1), 31–37.

- Theuer, R.C., Mahoney, A.W. & Sarett, H.P. (1971) Placental transfer of fluoride and tin in rats given various fluoride and tin salts. *J. Nutr.*, **101**, 525–532.
- The Tin in Food Regulations 1992* (S.I. [1992] No. 496). London: The Stationery Office.
- UK FSA (2002) *Tin in Canned Fruit and Vegetables*. London: United Kingdom Food Standards Agency (Food Safety Information Sheet 29/02; <http://www.food.gov.uk/science/surveillance/fsis-2002/tinincannedfruitandveg>).
- UK FSA (2004) *2000 Total Diet Study of 12 Elements — Aluminium, Arsenic, Cadmium, Chromium, Copper, Lead, Manganese, Mercury, Nickel, Selenium, Tin and Zinc*. London: United Kingdom Food Standards Agency (Food Safety Information Sheet 48/04; <http://www.food.gov.uk/multimedia/pdfs/fsismetals.pdf>).
- Westrum, B. & Thomasson, Y. (2002) *Tin and Inorganic Tin Compounds*. Prepared by the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals and the Dutch Expert Committee on Occupational Standards. Published on behalf of the Nordic Council of Ministers by the National Institute for Working Life, Stockholm (Report No. 130; Arbete och Hälsa 2002:10; <http://www.arbetslivsinstitutet.se/publikationer/en/detaljerad.asp?ID=1123>).
- WHO (2005) *Acute Hazard Exposure Assessment for Pesticide Residues in Food*. Geneva: World Health Organization (http://www.who.int/foodsafety/chem/acute_data/en/).

POLYBROMINATED DIPHENYL ETHERS

First draft prepared by

**L. Barraj,¹ M. van den Berg,² P.O. Darnerud,³ M. Feeley,⁴
H. Lilienthal,⁵ O. Päpke,⁶ M. Bolger⁷ and C. Tohyama⁸**

¹ **Exponent, Washington, DC, USA**

² **Utrecht University, Utrecht, The Netherlands**

³ **National Food Administration, Uppsala, Sweden**

⁴ **Food Directorate, Health Canada, Ottawa, Canada**

⁵ **Berufsgenossenschaftliches Forschungsinstitut für Arbeitsmedizin
(BGFA), Klinische Arbeitsmedizin, Bochum, Germany**

⁶ **Ergo Research, Hamburg, Germany**

⁷ **Food and Drug Administration, College Park, MD, USA**

⁸ **University of Tokyo, Bunkyo-ku, Tokyo, Japan**

Explanation.....	353
Introduction	353
Uses	355
General considerations on exposure sources of PBDEs.....	355
Biological data	356
Biochemical aspects	356
Absorption, distribution, metabolism and excretion	356
Biochemical effects	367
Toxicological studies	369
Acute toxicity	369
Short-term studies of toxicity	372
Long-term studies of toxicity and carcinogenicity	379
Genotoxicity	382
Reproductive/developmental toxicity	384
Special studies	387
Observations in humans.....	404
Biomarkers of effect	404
Clinical observations	404
Epidemiological studies.....	405
Analytical methods	406
Commercial PBDE production.....	406
Description of analytical methods.....	406
Introduction	406
Congeners analysed	406
Screening tests	406
Quantitative methods	407
Sampling protocols.....	420
Personnel	420
Representative sample.....	420
Packaging, transport and storage of aggregate and laboratory samples.....	420

Human milk samples	421
Sealing and labelling	421
Edible parts	421
Effects of processing	421
Levels and patterns of contamination of food commodities	422
Surveillance data	422
Canada	422
Finland	425
Germany	425
Japan	425
The Netherlands	432
Spain	433
United Kingdom	433
United States	433
Other data	435
Distribution curves	446
Dietary intake assessment	453
Introduction and background	453
Methods	453
Definitions	453
Intake calculations	460
Compounds	461
Distributions of concentrations	461
National diets	462
International diets	462
Estimates of dietary intake	463
National estimates for adults	463
Regional estimates	472
Dietary intake for infants	475
Potential sources of intake other than food	475
Prevention and control	476
Levels and patterns of contamination of humans	476
Comparison of analytical data in different tissues	476
Different countries	479
Australia	479
Belgium	480
Canada	481
Czech Republic	481
Faroe Islands	482
Finland	482
Germany	482
Italy	487
Japan	487
Mexico	488
The Netherlands	489
Norway	489
Republic of Korea	490
Spain	491

Sweden	492
United Kingdom	493
United States	495
Summary	500
Data on time trends for PBDEs	500
Dose-response analysis and estimation of risk	504
Contribution of above data to assessment of risk	504
Pivotal data from biochemical and toxicological studies	504
Pivotal data from human clinical/epidemiological studies	508
Biomarker studies	508
General modelling considerations	510
Selection of data	510
Measure of exposure	510
Measure of response	523
Potency estimates	523
Potency estimates in humans based on epidemiological data	523
Potency estimates in humans based on biomarkers	523
Potency estimates in test species and basis for extrapolation to humans	523
Comments	525
Absorption, distribution, metabolism and excretion	525
Toxicological data	525
Special studies	527
Observations in humans	529
Analytical methods	530
Effects of processing	530
Prevention and control	531
Levels and pattern of food contamination	531
Dietary intake assessment	531
Dose-response analysis	538
Evaluation	539
Recommendations	540
References	540
Appendix 1	560

1. **EXPLANATION**

1.1 **Introduction**

Polybrominated diphenyl ethers (PBDEs) are anthropogenic chemicals that are added to a wide variety of consumer/commercial products (e.g. plastics, polyurethane foam, textiles) in order to improve their fire resistance. PBDEs have been produced since the 1960s, primarily as three main commercial products (indicated with initial capital letters): Pentabromodiphenyl Oxide or Ether (PentaBDE), Octabromodiphenyl Oxide or Ether (OctaBDE) and Decabromodiphenyl Oxide or Ether (DecaBDE). Some variability in composition is known to exist

between products from different manufacturers, but each technical product can be approximately described by its congener compositions, given in Table 1.

Table 1. General composition of commercial PBDE flame retardants and substitution pattern of selected congeners

PBDE	
<i>Mixture</i>	<i>Congener composition (% of total)</i>
Penta	24–38% tetraBDEs, 50–60% pentaBDEs, 4–8% hexaBDEs
Octa	10–12% hexaBDEs, 44% heptaBDEs, 31–35% octaBDEs, 10–11% nonaBDEs, <1% decaBDEs
Deca	<3% nonaBDEs, 97–98% decaBDE
<i>Individual congeners^a</i>	<i>Substitution pattern</i>
BDE-47	2,2',4,4'-tetraBDE
BDE-99	2,2',4,4',5-pentaBDE
BDE-153	2,2',4,4',5,5'-hexaBDE
BDE-209	2,2',3,3',4,4',5,5',6,6'-decaBDE

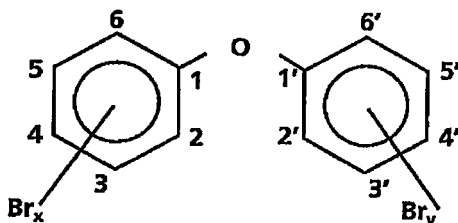
^a See Appendix 1 for a list of common PBDE congeners.

PBDEs belong to a class of brominated aromatic compounds that are structurally similar to polychlorinated biphenyls (PCBs) and therefore share the same nomenclature, as proposed by Ballschmitter & Zell (1980) (Figure 1). Theoretically, as with PCBs, 209 distinct PBDE isomers are possible; however, each commercial mixture usually contains only a limited number of congeners from each homologue group. For example, Bromkal 70-5-DE, a commercial PentaBDE product, is 70% by weight BDE-47 and BDE-99, with approximately equal contributions from each isomer (Sjödén et al., 1998). Additional congeners detected include BDE-100 (6.8%), BDE-153 (3.9%) and BDE-154 (2.5%). For the commercial OctaBDE formulations, BDE-153, BDE-183 and BDE-203 are major congeners that have been identified (Alaee & Wenning, 2002). Earlier formulations of OctaBDE may have contained up to 12% pentaBDE isomers, in particular BDE-99, but data from more recent productions suggest that the concentration is now less than 0.5% of the total (EU, 2003).

PBDEs have not been evaluated previously by the Committee. In 1994, WHO published an Environmental Health Criteria document on brominated diphenyl ethers (IPCS, 1994), as part of an overview on the possible environmental and human health impacts of flame retardants. Recent analysis of archived samples collected over the last three to four decades has demonstrated significant increases in concentrations of PBDEs in samples from the environment and in certain samples from humans in Europe and North America. This has led to both voluntary and formal bans on the production and use of certain formulations of PBDEs. Limited national food surveys have identified diet as one of the possible main sources of human exposure. The present evaluation was undertaken in

response to a request from the Codex Committee on Food Additives and Contaminants (CCFAC), most recently at its Thirty-fifth Session (CAC, 2003), to evaluate the potential risks associated with the presence of PBDEs in food.

Figure 1. General structural formula for PBDEs, where $x + y = 1-10$



1.2 Uses

Flame retardants are anthropogenic chemicals that are either physically blended (additive) or chemically bonded (reactive) to a variety of combustible products in order to improve or increase their ignition and fire resistance. Products treated with flame retardants include electronic equipment (circuit boards, computers, monitors, etc.), textiles, commercial and residential construction materials, insulation, furniture and carpets. Various reviews of the global production, applications and mechanism of action of flame retardants are available (IPCS, 1997; Rahman et al., 2001; de Wit, 2002; Alaei et al., 2003).

PBDEs are primarily used as additive flame retardants, specific to applications as defined by their physical-chemical properties. Commercial PentaBDE mixtures are mainly used in polyester and flexible polyurethane foam formulations in amounts that can result in the finished material being composed of up to 30% by weight of flame retardant. The main use of OctaBDE is in a variety of thermoplastic resins, in particular ABS (acrylonitrile–butadiene–styrene) plastic, which can contain up to 12% by weight OctaBDE. DecaBDE is used in various plastic polymers such as polyvinyl chloride, polycarbonates and high-impact polystyrene, as well as back coating for textiles (commercial furniture, automobile fabrics, carpets, etc.).

1.3 General considerations on exposure sources of PBDEs

The worldwide demand for PBDEs in 2001 was estimated to be almost 70 000 tonnes (BSEF, 2003), with DecaBDE accounting for almost 80% of the total market. This is in comparison with an estimated production figure of 40 000 tonnes in 1990 (cited in de Wit, 2002). The major commercial product currently in use, as noted above, is the DecaBDE formulation, which accounts for over 80% of the total market demand. From a regional perspective, the Western Hemisphere and Asia represent the largest users of PBDEs (85% of market), with almost 95%

of the PentaBDE total of 7500 tonnes used in the Americas. The Organisation for Economic Co-operation and Development (OECD) classifies PBDEs as high production volume chemicals (annual production of greater than 1000 tonnes). Main sources of PBDE input to the environment include emissions from manufacturing and production facilities (air and surface waters) and release during the life cycle of consumer products treated with PBDEs (degradation, recycling, disposal). For example, it has been estimated that up to 43 tonnes of PentaBDE per year are released to the environment in Europe by volatilization from polyurethane foam used in a variety of consumer products (Prevedouros et al., 2004). Airborne emissions of lower brominated PBDE congeners (up to hexa-substituted) are expected to exist in both the vapour and particulate phases and therefore be subject to long-range atmospheric transport processes. An additional source of PBDEs entering the environment is the use of municipal sewage treatment sludge as fertilizer (Hale et al., 2001; Öberg et al., 2002). Based on their low vapour pressure and high log octanol–water partition coefficients (log K_{ow} range of 5.0–9.9), PBDEs are expected to strongly adsorb to soil and suspended organic material in the water column, facilitating their transfer to aquatic organisms. Current abiotic and biotic PBDE residues, including those detected in food and human tissues, are typically dominated by a limited number of congeners, namely BDE-47, BDE-99, BDE-153 and BDE-100. Environmental monitoring programmes have documented the ubiquitous nature of PBDE contamination, including increased temporal trends in sediments, biota and humans (Hites, 2004).

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution, metabolism and excretion

(a) Introduction

A large amount of information is available on the occurrence of PBDEs in wildlife and human biological matrices, such as blood and milk. However, specific information about uptake, distribution, metabolism and excretion of PBDEs in experimental animals is significantly less than what is known about structurally related chlorinated biphenyls (PCBs) and dibenzo-*p*-dioxins (PCDDs). Nevertheless, the number of experimental studies done with rodents and PBDEs allows us to draw some conclusions regarding the fundamental toxicokinetic and metabolic aspects of this type of flame retardant. Recently, several detailed review papers dealing with these aspects have been published, and this section contains much information that has already been presented in these publications (Darnerud et al., 2001; de Wit, 2002; Hakk & Letcher, 2003; Gill et al., 2004). In addition, this information has been cross-checked with the very recent European Union (EU) risk assessment reports for octaBDE and decaBDE (EU, 2001, 2003, 2004) and, if applicable, adjusted and updated. Although several PBDEs accumulate in wildlife, including fish and invertebrates, and can enter the human food-chain through this pathway, this section deals only with the mammalian toxicokinetics and metabolism of these compounds, mainly derived from laboratory studies.

(b) *Uptake*

The toxicokinetics and metabolism of one of the most persistent PBDEs, BDE-47, have been studied in rats and mice (Örn, 1997; Örn & Klasson-Wehler, 1998).

A significant part (86%) of the oral administered dose in rats was retained in the body of male Sprague-Dawley rats 5 days after administration of a single gavage dose of 14.4 mg/kg bw. The highest concentration was found in adipose tissue and consisted mainly of the parent compound. In addition, only the parent compound could be detected in most other lipid-rich tissues, with the exception of liver and plasma, in which trace amounts of hydroxylated metabolites were also found (Örn & Klasson-Wehler, 1998). A similar absorption value was seen when female C57BL/6J mice ($n = 4-6$) were given a single oral dose (gavage) of BDE-47 (0, 0.1, 1.0, 10.0 or 100 mg/kg bw). Based on excreta analysis 1 day following dosing, over 80% of BDE-47 was shown to be absorbed (Staskal et al., 2005).

In adult male Sprague-Dawley rats ($n = 3-4$) exposed to a single oral dose of BDE-99 (15 $\mu\text{mol/kg}$ bw or 8.5 mg/kg bw), analysis of urine and faeces indicated that approximately 82% of the original dose was retained after 24 h (Klasson-Wehler et al., 2001).

Except for decaBDE, no toxicokinetic studies have been performed with individual PBDEs that allow identification of the absorption rate from the gastrointestinal tract. However, a few studies have been done with technical mixtures that identified the absorption of either total PBDEs or a distinct isomeric group. In two studies, the gastrointestinal absorption of various PBDEs present in the technical mixtures DE-71, a commercial pentaBDE product dominated by BDE-47 and BDE-99, and DE-79 (dominated by hexa- through nonaBDE congeners) was determined in male Sprague-Dawley rats that were fed for 21 days with 33 ng/day each. Based on tissue analysis at day 21, the minimal absorption of the PBDE mixture in DE-71 and DE-79 was estimated to be 36.7% and 32.3% of the dose, respectively (Hakk et al., 2001; Huwe et al., 2002).

Several experimental studies with rodents have addressed the bioavailability of decaBDE. Absorption of the fully brominated congener from the gastrointestinal tract appears to be very low compared with that of the lower brominated PBDEs, as was estimated from analysis of excretions (urine and faeces), which ranged between 90% and >99% a few days after oral dosing (Norris et al., 1975a; NTP, 1986; el Dareer et al., 1987; Mörck & Klasson-Wehler, 2001). In addition to its low bioavailability, it was also observed that intestinal absorption of decaBDE in male rats did not depend on the dietary concentration over 2 orders of magnitude (0.025–5.0%) (NTP, 1986; el Dareer et al., 1987). In contrast with these earlier studies, very recent rat studies by Mörck et al. (2003) have specifically addressed the bioavailability and metabolism of decaBDE. In these experiments, different solvents were used to maximize the solubility of this compound in the test vehicle. Solvents were dimethyl sulfoxide/peanut oil (50:50 mixture), anisole/peanut oil (30:70 mixture) and a solution of soya phospholipone/Lutrol (16:34 w/w) in water (concentration 0.11 g/l). Approximately 90% of the [^{14}C]decaBDE dose (3 $\mu\text{mol/kg}$ bw or 2.9 mg/kg bw) was excreted by male Sprague-Dawley rats via the faeces

within 3 days. These specific experiments showed that absorption of decaBDE can be at least 10%. In additional experiments, it was suggested that this bioavailability could be even higher (Sandholm, 2003; Sandholm et al., 2003), and 26% was recently suggested for risk assessment purposes (EU, 2001, 2004). Oral administration of a single dose of BDE-209 (2 $\mu\text{mol/kg}$ bw) in a combination dimethylamide/polyethylene glycol/water (4:4:1) vehicle to male Sprague-Dawley rats indicated that a maximum plasma concentration of 264 pmol/ml was reached approximately 6 h after dosing. The estimated bioavailability was 26% (Sandholm et al., 2003). The question remains if these special vehicle formulations in the latter experiments are representative of the real-life uptake situation of decaBDE for humans, when compared with the much lower absorptions found in earlier animal studies using administration through the diet.

The majority of results from these experiments suggest that intestinal uptake of decaBDE is not efficient when administered in the diet; consequently, this congener is expected to have a low bioaccumulation potential (Därnerud et al., 2001; Hakk & Letcher, 2003). However, these experiments also showed that absorption from the intestinal tract after oral exposure may display a dosing vehicle dependency. The bioavailability of representative PBDEs, as a function of excretion, is given in Table 2 (Hakk & Letcher, 2003).

Besides uptake from the gastrointestinal tract, percutaneous absorption has also been addressed in two recent EU risk assessment studies for octa- and decaBDE (EU, 2001, 2003, 2004). Although no information is available about the percutaneous absorption of penta-, octa- or decaBDE, the authors refer to the physicochemical properties of PCBs, which are assumed to be similar to those of PBDEs. For these types of lipophilic compounds, it is assumed that the stratum corneum is the crucial barrier and the rate-limiting step in the uptake. This will determine i) diffusion into and through the lipid-rich intercellular matrix of the stratum corneum or ii) diffusion out of the stratum corneum into and through the relatively aqueous viable epidermis below. This process depends on the lipophilicity or lipid solubility of the compound (Jackson et al., 1993). In view of these considerations and given the physicochemical properties of octaBDE — a high $\log K_{ow}$ (6.29), poor water solubility (<0.5 $\mu\text{g/l}$) and high relative molecular mass (801) — the dermal absorption is expected to be low and estimated to be 4.5%. Using a similar approach for decaBDE and data from studies with PCBs (Garner & Matthews, 1998), a percutaneous absorption of a maximum of 1% is estimated for this compound. However, in spite of the low estimated percutaneous absorption for octa- and decaBDE, the EU risk assessment studies (EU, 2001, 2003, 2004) suggested that the percutaneous absorption may be associated with a likely trend towards accumulation in the stratum corneum, which by itself might behave as a storage site (Leung & Paustenbach, 1994). Analogous with PCBs, it is postulated that this could lead to a slow systemic release over time (EU, 2001, 2003). With respect to this suggested role of the stratum corneum, it should, however, be noted that at present, there is no indication that this actually occurs for either the octa- or decaBDE congeners in humans.

Table 2. Excretion and bioavailability results from studies in rats administered various PBDE congeners

	Percentage of administered dose							
	Male SD rat, 14.46 mg/kg po, 5 days, BDE-47	Male SD rat, 8.1 mg/kg po, 72 h, BDE-99	Male SD rat, 9.2 mg/kg po, 72 h, BDE-99	Male SD rat, 1.09 mg/kg po, 16 days, BDE-209	Male F344 rat, 0.0277% diet, days 1–7, 9– 11 cold BDE- 209, at 8 days ¹⁴ C BDE-209	Male F344 rat, 4.8% diet, days 1–7, 9–11 cold BDE-209, at 8 days ¹⁴ C BDE- 209	Male F344 rat, 1.07 mg/kg iv, 72 h, BDE-209	Male SD rat, 3.0 mg/kg po, 72 h, BDE- 209
<i>Urine</i>	<0.05	0.9	0.35	<1.0	0.012	0.008	0.129	<0.05
<i>Faeces</i>								
Day 1	5.7	22.3	52.5	90.6				
Day 2	5.4	14.8	30.4	>8.4				
Day 3	1.2	6.0	3.6	–				
Day 4	0.9	–	–	–				
Day 5	0.5	–	–	–				
Total	13.7	43.1	86.5	>99	82.5 (0–72 h)	85.1 (0–72 h)	70.0	>90
<i>Bile</i>								
Day 1			0.7					
Day 2			1.8					
Day 3			1.4					
Total			3.9					9.5
References ^a	[1]	[2]	[2]	[3]	[4]	[4]	[4]	[5]

From Hakk & Letcher (2003)

iv, intravenous; po, per os (by mouth)

^a [1] Örn & Klasson-Wehler (1998); [2] Hakk et al. (2002); [3] Norris et al. (1975b); [4] el Dareer et al. (1987); [5] Mörck & Klasson-Wehler (2001).

(c) *Tissue distribution*

The tissue distribution of a variety of lower and higher brominated PBDEs has been studied in rats and mice. Studies with BDE-47 and rodents have shown that adipose tissue is the major storage site in the body, but concentrations on a lipid-adjusted basis were comparable for adipose tissue, liver, lung and kidney. This study also showed a marked species difference between rat and mice in tissue retention. In rats, 86% of a single oral dose (approximately 14.6 mg/kg bw) was retained in the body 5 days following dosing, while for mice, it was 47%. In addition, the radioactivity was about 3 times higher in the adipose tissue of the rat than in liver, while levels in both tissues in the mice were comparable (Örn & Klasson-Wehler, 1998). When female C57BL/6J mice were dosed by gavage with BDE-47 (single dose, 0.1–100 mg/kg bw), the tissue distribution determined 5 days later was mainly based on lipid content. Adipose tissue had the highest concentration (8–14% of dose), followed by skin, liver and muscle (0.9–2.6%) (Staskal et al., 2005).

A similar body distribution has been observed for BDE-99, with preference for the lipid-rich tissues, including adipose tissue, adrenals, gastrointestinal tract and skin, which contained more than 50% of the dose after 72 h (Hakk et al., 2002). Using whole-body autoradiography, the distribution of ^{14}C -labelled BDE-47, BDE-85 and BDE-99 was determined in C57BL mice. Shortly after efficient uptake from the gastrointestinal tract, radioactivity for these congeners was highest in the adipose tissue, liver, adrenals, ovaries, lung and brain. In most tissues, the concentrations decreased significantly after a longer post-injection time, but radioactivity was present longest in white and brown adipose tissue. For these PBDE congeners, transfers to the fetus and to the offspring via the milk were also studied in mice. It was found that there was a low fetal uptake, with no significant differences between the three PBDEs. However, a significant maternal transfer via the milk of approximately 20% of the administered dose was found for BDE-85 and BDE-99 in the suckling offspring after 4 days. At this time, plasma levels in the neonates were more than 2 times those of the mothers (Darnerud & Risberg, 2005).

For other higher brominated PBDEs, such as octa-substituted congeners, there are no detailed toxicokinetic studies available that would allow specific conclusions about tissue retention and accumulation to be drawn (EU, 2001, 2003, 2004).

A number of older studies conducted during the 1970s by the Great Lakes Chemical Company with octaBDE have been recently evaluated in the EU risk assessment report (EU, 2003). Four weeks after dietary treatment with 100 or 1000 mg/kg of a commercial octaBDE mixture (8 or 88 mg/kg bw per day, respectively), a dose-related increase in total bromine content in the liver was reported in Charles River CD rats. These levels were 6–40 times higher than those found in controls. There was a slow decline of bromine levels in the tissues, indicating some capacity for bioaccumulation after repeated exposure at both doses. Since only total bromine content was determined, it is not known if the retained bromine represented parent compounds and/or metabolites.

A 2-year dietary accumulation study was done with rats that received technical decaBDE (77.4% decaBDE, 21.8% nonaBDE and 0.8% octaBDE) at doses of up to approximately 1.0 mg/kg bw per day. A variety of tissues were analysed (serum, liver, kidneys, skeletal muscle and testes); in most, bromine content was not above background (control values). In the adipose tissue, there was a dose- and time-dependent increase in bromine levels observed in rats ingesting decaBDE at 0.1 and 1.0 mg/kg bw per day. In the 0.1 mg/kg bw per day dose group, the bromine concentration was 3-fold higher than that of controls. The bromine content in the adipose tissue did not change during a recovery period of 90 days, which is in contrast to the liver, in which the bromine was eliminated within 10 days following the end of dosing (Kociba et al., 1975). In another study, the tissue retention of decaBDE after a single oral dose of 1 mg/kg bw was studied in female Sprague-Dawley rats 16 days after dosage. Based on radioactivity, measurable levels were found only in the adrenal glands (0.01% of the dose) and spleen (0.06% of the dose), but not in any other tissues (Norris et al., 1973, 1975a).

Viberg et al. (2003b) recently investigated the tissue retention of decaBDE in neonatal NMRI mice. After a single oral dose of ^{14}C -labelled decaBDE (purity >98%; 1500 kBq/kg bw or approximately 13.8 nmol/kg bw) on postnatal days 3, 10 or 19 ($n = 4-7$), low levels of radioactivity were detected in the brain, heart and liver 24 h and 7 days after dosing. Results from this study show that ^{14}C label was taken up into the brain at 1% or less of the administered dose and that there were age-dependent differences in retention of ^{14}C label in the brain (greater amounts on days 3 and 10 of dosing compared with day 19). Based on ^{14}C radioactivity only, it cannot be concluded if this is parent decaBDE or one or more of its metabolites (Viberg et al., 2003b). In response to this paper, Vijverberg & van den Berg (2004) pointed to some inconsistencies regarding calculations in tissue retention and brain levels in these mice, which were estimated to be approximately 3 orders of magnitude higher than the highest levels of decaBDE found in humans.

Thus, with respect to the bioaccumulation potential of PBDEs, it can be concluded that there is definitely significant potential for some of the lower brominated congeners, such as BDE-47, BDE-99 and BDE-154, to bioaccumulate. However, the bioaccumulation potential of the higher brominated congeners, especially that of decaBDE, appears to be low, although measurable low concentrations in lipophilic tissues and blood of decaBDE can occur in humans (Hakk & Letcher, 2003; Sjödin et al., 2003).

In Table 3, the retentions of different PBDE congeners in the rat are summarized (Hakk & Letcher, 2003).

(d) *Metabolism*

The metabolism of only a very limited number of PBDE congeners, primarily BDE-47, BDE-99 and BDE-209, has been studied in some detail. Information regarding this metabolism has been recently summarized by Darnerud et al. (2001) and Hakk & Letcher (2003).

Table 3. Tissue recoveries from male rats administered various PBDE congeners

	Male SD rat, 8.1 mg/kg po, 72 h, BDE-99	Male SD rat, 9.2 mg/kg po, 72 h, BDE-99	Male SD rat, 1.09 mg/kg po, 16 days, BDE- 209	Male F344 rat, 0.0277% diet, days 1–7, 9–11 cold BDE-209, at 8 days ¹⁴ C BDE-209	Male F344 rat, 4.8% diet, days 1–7, 9–11 cold BDE-209, at 8 days ¹⁴ C BDE- 209	Male F344 rat, 1.07 mg/kg iv, 72 h, BDE-209	Male SD rat, 2.0 mg/kg po, 72 h, BDE-209
Liver	0.9	0.3		0.109	0.016	4.27	0.9
Kidney	0.1	0.03		0.013	<0.001	0.697	0.05
Lungs	0.1	0.04		0.004	0.001	0.361	<0.1
Spleen	<0.1	–	0.06	0.001	<0.001	0.027	<0.1
Pancreas	–	–	–	–	–	–	–
Adrenals	0.1	0.01	0.01	–	–	–	<0.1
Heart	0.03	0.01		–	–	–	<0.1
Brain	–	–		<0.001	<0.001	0.047	–
GI	6.1	1.5		0.09	0.60	5.063	3.5
Muscle	0.7	–		0.248	0.008	12.9	0.7
Skin	0.4	–		0.136	0.036	7.25	0.4
Fat	3.8	0.8		0.048	0.012	2.99	0.3
Blood	0.03	0.007		0.026	0.006	0.763	0.05
References ^a	[1]	[1]	[2]	[3]	[3]	[3]	[4]

From Hakk & Letcher (2003)

GI, gastrointestinal tract; iv, intravenous; po, per os (by mouth)

^a [1] Hakk et al. (2002); [2] Norris et al. (1975a); [3] el Dareer et al. (1987); [4] Mörck & Klasson-Wehler (2001).

(i) BDE-47

Studies with rats and mice have shown that hydroxylated metabolites of BDE-47 are the major metabolic products (Örn & Klasson-Wehler, 1998), with noticeable differences in metabolism and excretion between both species. No debromination products were found (Därnerud et al., 2001). The metabolism of this congener in the rat is rather slow, and the parent compound was the major residue found in all tissues, including liver, adipose tissue, brain, kidney and lung. Trace amounts (<1%) of ^{14}C radioactivity in the form of hydroxylated metabolites were detected in liver and lung. In the faeces of the rat, six metabolites were found, including five tentatively assigned as two *ortho*-OH-tetraBDE metabolites, one *meta*-OH-tetraBDE metabolite and two *para*-OH-tetraBDE metabolites. Results from this study suggest that *ortho*- and *para*-OH-metabolites can be formed in the rat as a result of an NIH shift, with evidence for arene oxide as an intermediate. In addition, a very small amount of thiol-tetraBDE metabolite was found in the faeces, but its assignment also remains tentative due to the absence of reference compounds (Örn & Klasson-Wehler, 1998). A similar experiment with mice showed that BDE-47 is metabolized faster in this species than in the rat, with formation of five mono-OH-tetraBDEs and two mono-OH-triBDEs. In addition, evidence was obtained that BDE-47 could be metabolized to reactive intermediates, as suggested by the presence of non-extractable radioactivity in several organs, including liver, lung and kidney (Örn & Klasson-Wehler, 1998).

(ii) BDE-99

The metabolism of this BDE has been reported from only one study (Hakk et al., 2002). Metabolism in the rat was low, resulting in slow excretion. Only small amounts of monohydroxylated metabolites of penta- and tetraBDEs were detected in the faeces. The presence of tetraBDE metabolites indicates that at least in the rat, debromination can occur in vivo. In the bile, mono- and dihydroxy-pentaBDEs as well as two thio-substituted pentaBDEs were found. For BDE-99, evidence for the formation of reactive intermediates was also found, as a significant amount of labelled compound was apparently covalently bound in the faeces and non-extractable.

(iii) BDE-209 (decaBDE)

This BDE congener has been the subject of detailed metabolism studies. Using ^{14}C -labelled decaBDE, its metabolism was studied in conventional and bile duct-cannulated rats (Klasson-Wehler et al., 2001; Mörrck & Klasson-Wehler, 2001). Following a single oral dose of ^{14}C -labelled decaBDE (3 $\mu\text{mol/kg}$ bw) administered to male Sprague-Dawley rats, approximately 22–45% of the radioactivity in the faeces found from day 1 to day 3 consisted of eight phenolic metabolites. Metabolites of BDE-209 in the faeces included debrominated mono-OH- and *ortho*-MeO-OH-BDEs (Klasson-Wehler et al., 2001; Mörrck & Klasson-Wehler, 2001). The methyl ester group was probably introduced by a catechol-O-methyl transferase of an *ortho* catechol substrate, but the route of formation is not known (Hakk & Letcher, 2003). It was deduced that decaBDE is metabolized via an

oxidative debromination pathway due to the presence of debrominated dihydroxy-BDEs and that dehydroxylation always occurs on the same aromatic ring. This oxidation process likely produces an epoxide as an intermediate metabolite, with further metabolism to a diol, which could explain the observed metabolites. Debromination of decaBDE to other PBDEs does not appear to be a major metabolic pathway from a quantitative point of view, as trace amounts of only three nona-BDEs were found in the faeces. The formation of reactive intermediates cannot be excluded, as a significant amount of the radioactivity in the jejunum wall and liver was non-extractable. With respect to metabolism of decaBDE in the rat, it is noteworthy that in plasma at days 3 and 7, the levels of radioactivity were approximately 4 times higher in the phenolic fraction than in the neutral fraction. This indicates significant retention of the metabolites of decaBDE compared with the parent compound. The actual cause of the plasma retention of these decaBDE metabolites could not be determined, as the nature of these metabolites in the phenolic fraction is unknown. However, it was suggested by the authors that the high plasma concentrations of hydroxy-decaBDE metabolites could be caused by reversible binding of these metabolites to transthyretin (TTR), the thyroxine (T4) hormone transporting protein in rodents (Mörck et al., 2003).

It has also been suggested that extensive metabolism of decaBDE could occur in the gastrointestinal tract after oral administration in rats (el Dareer et al., 1987). However, it should be noted that after oral or intravenous administration of decaBDE, the occurrence of the same three metabolites was noted, independent of the route of administration. This indicates a distinct role for hepatic metabolism (EU, 2004).

In general, it can be concluded that decaBDE is metabolized faster than the more biologically persistent BDE-47 and BDE-99. Several metabolic products have been described, including phenolic, neutral, non-extractable, water-soluble and lipid-bound compounds, but no glutathione metabolites have been observed so far. Based on these metabolism studies, it can also be concluded that decaBDE is not biotransformed to the lower, more persistent BDE-47 or BDE-99. However, the structure of the hydroxylated metabolites that are retained in plasma needs to be further elucidated in order to determine if these metabolites could possibly cause biological or toxicological effects (EU, 2004).

In Figure 2, an overview is given of the proposed metabolic pathways in the rat for BDE-47, BDE-99 and BDE-209 (Hakk & Letcher, 2003).

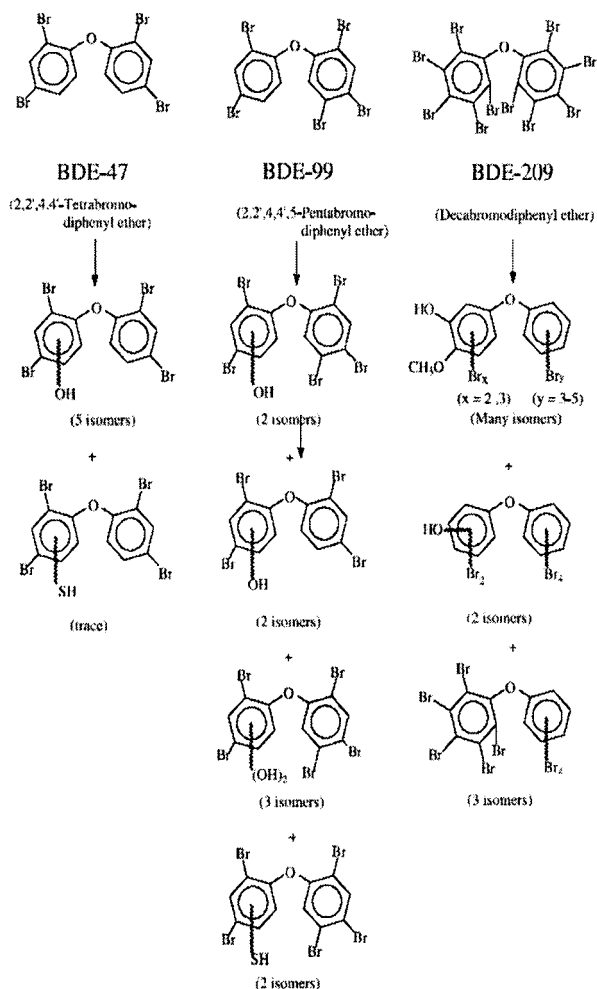
(e) *Elimination*

Information about congener-specific half-lives of PBDEs is scarce, while there are some limited data on commercial PBDE mixtures.

A toxicokinetic study using Bromkal 70, a commercial pentaBDE product, determined the elimination of tetra-, penta- and hexaBDEs in the rat. Wistar rats ($n = 4$ per time point) were given a single oral Bromkal 70 dose of 300 mg/kg bw, and perirenal fat samples were analysed every week for 10 weeks. Half-lives depended on the degree of bromination (von Meyerinck et al., 1990) and are given

in Table 4. Congener-specific information was not provided in this study; however, based on detailed gas chromatographic–mass spectrometric (GC-MS) analysis of Bromkal 70 by Sjödin et al. (1998), the tetra congener was most likely BDE-47, the two penta congeners BDE-99 and BDE-100, and the two hexa congeners BDE-153 and BDE-154. Except for the proposed BDE-47 congener, no statistical difference was found between the sexes, and half-lives for the tetra- to hexaBDEs ranged between 25 and 91 days for females and between 19 and 119 days for males.

Figure 2. Proposed metabolic pathways of BDE-47, BDE-99 and BDE-209 in the rat



From Hakk & Letcher (2003)

It has been suggested (Hakk & Letcher, 2003) that the large oral dose administered in the latter study (300 mg/kg bw) was in great excess of the minimal dose required for induction of cytochrome P450 by Bromkal 70 (3–10 mg/kg bw). Therefore, it cannot be excluded that at lower environmental exposures, a different and lower elimination rate might occur. This suggestion would be in agreement with the results of a study done with male Sprague-Dawley rats orally administered a much lower dose of BDE-47 (14.5 mg/kg bw), in which only 14% and <0.5% of the dose were eliminated in faeces and urine, respectively, during the first 5 days (Örn & Klasson-Wehler, 1998). In the same experiment conducted with male C57BL mice and this PBDE congener, it was shown that this species is capable of eliminating BDE-47 more rapidly, with 33% of the dose excreted in the urine and 20% via the faeces during the first 5 days after dosage (Örn & Klasson-Wehler, 1998). A recent study by Staskal et al. (2005) determined the elimination kinetics and half-life of BDE-47 in female C57BL/6J mice. It was observed that excretion via the urine following a single oral dose of 0.1–100 mg/kg bw had a major influence on the initial whole-body half-life of 1.5 days. From a quantitative point of view, retention was stronger in lipophilic tissue such as adipose and skin. In these tissues, elimination followed a biphasic pattern, with initial half-lives between 1 and 3 days but much longer terminal half-lives (estimated by the authors to be 30–40 days in adipose), indicating the potential for bioaccumulation.

Table 4. Half-lives of individual pentaBDE components in Wistar rats^a

PentaBDE HPLC peak	Half-lives in female rats (days)	Confidence interval, $P = 0.05$	Half-lives in male rats (days)	Confidence interval, $P = 0.05$
Br ₄ DE	29.9	26.8–33.1	19.1*	16.5–21.7
Br ₅ DE1	47.4	42.5–52.4	36.8	33.7–40.0
Br ₅ DE2	25.4	22.6–28.4	24.9	22.6–27.1
Br ₆ DE1	44.6	37.4–51.9	55.1	48.4–61.7
Br ₆ DE2	90.9	78.7–103.6	119.1	102.8–136.1

From von Meyerinck et al. (1990)

Br₄DE, tetraBDE; Br₅DE1, pentaBDE; Br₅DE2, pentaBDE; Br₆DE1, penta- and hexaBDE; Br₆DE2, hexaBDE; HPLC, high-performance liquid chromatography

* $P = 0.01$ significant difference between sexes

^a Single oral Bromkal 70 dose of 300 mg/kg bw dissolved in peanut oil. Groups of four rats were sacrificed consecutively until the 10th week. Concentrations of pentaBDE components in the perirenal fat were determined by HPLC, and data were corrected for the body weight of the rats.

The elimination of BDE-99 was studied in both bile-cannulated and uncannulated male Sprague-Dawley rats after an oral dose of 2.2 mg/kg bw (Hakk et al., 2002). Elimination via the faeces was the major route of excretion in both groups of rats. After 72 h, 43% of the administered dose in uncannulated and greater than

86% in cannulated rats were excreted in the faeces. This indicates a half-life in the rat of this compound of approximately 3 days or less.

Several studies have addressed the elimination of BDE-209 in the rat using different routes of administration. These studies indicated that decaBDE is metabolized faster than some lower brominated PBDEs, such as BDE-47. In several experiments, it was found that after oral dosage, 80–90% of the compound was eliminated via the faeces within 3 days (el Dareer et al., 1987; Mörck & Klasson-Wehler, 2001). The low intestinal absorption of decaBDE is thought to influence the fast elimination rates from the rat in these experiments. In male Sprague-Dawley rats ($n = 8$) treated with a single oral dose of ^{14}C -labelled decaBDE (3 $\mu\text{mol/kg}$ bw, 555 GBq/mol), approximately 90% of the dose was eliminated via the faeces after 3 days (Mörck et al., 2003). Only a minor additional amount (1%) was eliminated after 7 days. An experiment with male Fischer rats using an intravenous BDE-209 dose of 1.07 mg/kg bw in rats showed that 74% of the dose was eliminated in the faeces within 72 h. These results indicate that the rat is capable of metabolizing this compound rather effectively (el Dareer et al., 1987). In occupationally exposed workers, the elimination of BDE-209 and BDE-183 was also estimated based on serum measurements. The estimated half-lives were 6.8 days for BDE-209 and 86 days for BDE-183 (Hagmar et al., 2000). These results indicate that the half-life for BDE-209 may be longer in humans than in rats, but the human half-life is still relatively short for such a highly halogenated aromatic compound compared with higher chlorinated biphenyls and dibenzo-*p*-dioxins.

2.1.2 Biochemical effects

Darnerud et al. (2001) and de Wit (2002) recently summarized the possible hepatic enzyme induction by PBDEs.

Several studies have shown that commercial PBDE mixtures are capable of inducing phase I and phase II xenobiotic metabolizing enzymes. In Wistar rats and in rat hepatoma H4-IIIE cells, Bromkal 70 was able to induce CYP1A1 and CYP1A2 as measured by increased activity of hepatic 7-ethoxyresorufin *O*-deethylase (EROD) activity (Hanberg et al., 1991; von Meyerinck et al., 1990). Hepatic enzyme induction has also been studied in female weanling Long-Evans rats with three technical PBDE mixtures, including DE-71 (tetra- and pentaBDEs), DE-79 (hepta- and octaBDEs) and DE-83R (98% decaBDE). In the rats treated with DE-71 (0.3–300 mg/kg bw per day for 4 days) and DE-79 (0.3–100 mg/kg bw per day for 4 days), a dose-dependent 10- to 20-fold induction in EROD and 30- to 40-fold induction in 7-pentoxoresorufin *O*-depentylase (PROD) were found, which indicates an induction of CYP1A1 and CYP2B by these technical PBDE mixtures (Zhou et al., 2001). Benchmark dose (BMD) estimates indicated that hepatic PROD induction was the more sensitive parameter for either DE-71 or DE-79 (0.54 and 0.40 mg/kg bw per day, respectively, for the 95% lower confidence limits). DE-83R had no effect on any measured hepatic enzyme.

In addition, other phase I enzyme activities, such as benzphetamine *N*-demethylase, *p*-nitroanisole demethylase, arylhydrocarbon hydroxylase and benzo[a]pyrene hydroxylase, have also been induced in vivo by technical penta- and

octaBDE mixtures in the rat (Carlson, 1980a, 1980b; von Meyerinck et al., 1990). However, decaBDE has been found to have a low enzyme-inducing potency. In Sprague-Dawley rats, the enzyme-inducing potency of BDE-47 has been compared with that of the commercial PCB mixture Aroclor 1254 (Hallgren & Darnerud, 1998). The induction of CYP1A1 (EROD) and 7-methoxyresorufin O-deethylase (MROD) by BDE-47 was limited and much lower than that observed in rats treated with equivalent doses of Aroclor 1254. In contrast, the inductions of CYP2B measured as PROD by BDE-47 and Aroclor 1254 were similar. Carlson (1980b) also specifically examined the hepatic enzyme induction of a commercial formulation of decaBDE. Although liver enlargement was found at a dose of 95 mg/kg bw per day (14-day dosing by gavage), no enzyme induction was observed for *O*-ethyl-*O*-*p*-nitrophenyl phenylphosphonothioate detoxification, *p*-nitroanisole demethylation, benzo[*a*]pyrene hydroxylase, uridine diphosphate glucuronosyl-transferase (UDPGT), NADPH cytochrome *c* reductase and cytochrome P450. Thus, it can be concluded that at this relatively low dose of decaBDE, no hepatic enzyme induction occurs in male Sprague-Dawley rats.

Phase II enzyme induction by commercial PBDE mixtures containing various amounts of tetra-, penta-, hepta- and octabrominated congeners (14 days, 0.1 mmol/kg bw) has also been tested. With the exception of decaBDE, all of these compounds were capable of inducing prolonged UDPGT activity in rats (Carlson, 1980a). Short-term exposure (4 days) of weanling Long-Evans rats to commercial PBDEs (0.3–300 mg/kg bw per day) also produced significant induction of hepatic UDPGT activity by DE-71 and DE-79, albeit at higher doses than required for EROD and PROD induction (Zhou et al., 2001).

The issue of possible CYP1A1 induction by PBDE congeners is also highly relevant from a toxicological point of view. This type of cytochrome P450 induction is mediated via the aryl hydrocarbon (Ah) receptor, which has a high binding affinity for planar halogenated polyaromatics such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and some planar PCBs. In addition, it is also considered to be one of the most sensitive biological effects of these dioxin-like compounds (Safe, 1990). Thus, if some persistent PBDE congeners would cause induction of CYP1A1 activity, this might indicate that PBDEs could be considered in the toxic equivalency factor (TEF) concept for dioxin-like compounds (Van den Berg et al., 1998). To determine possible affinity for the Ah receptor, several *in vitro* studies with PBDEs have been performed using CYP1A1 (EROD) induction as an end-point of Ah receptor-mediated biological activity. Some of the earlier studies indicated that the closely related chlorinated diphenyl ethers (PCDEs) can be weak inducers of CYP1A1 activities, depending on the number and position of the chlorine atoms (Chui et al., 1985; Howie et al., 1990; Safe, 1990; Rozman, 1991). However, in contrast with these studies, another study showed that polychlorinated dibenzofuran (PCDF) impurities lower than 1% could be responsible for the *in vitro* induction of EROD activity of almost all of the 29 tested PCDEs in H4IIE rat hepatoma cells (Koistinen et al., 1996). The results of the latter study are in agreement with the outcome of a quantitative structure–activity relationship (QSAR) study for binding to the Ah receptor by halogenated diphenyl ethers, which would predict that due to the non-planarity of these compounds, a low binding affinity to

the Ah receptor should exist (Gillner & Jakobsson, 1996). These results illustrate that even at low concentrations, these impurities can induce a considerable Ah receptor-mediated response, including the induction of some specific enzymes such as CYP1A1 (EROD) (Darnierud et al., 2004). In this respect, it is fair to conclude that at least in the studies using technical PBDE mixtures, the observed induction of CYP1A1 activities can very likely be attributed to the presence of known impurities in these technical formulations, such as brominated dibenzofurans. A few *in vitro* studies have also tested individual PBDE congeners for CYP1A1 induction or other Ah receptor-related activities. Using a recombinant rat hepatoma cell line H4IIE with a luciferase reporter gene, it was reported that several PBDE congeners might act as Ah receptor agonists, and the potencies were in the same range as those reported for some mono-*ortho* PCBs (Meerts et al., 1998). Another study with primary rat hepatocytes and individual PBDE congeners also reported congener-specific induction of both CYP1A1 messenger RNA (mRNA) and protein levels. BDE-77 and BDE-126 induced responses that were 3–5 orders of magnitude less than that of TCDD in this *in vitro* system. Although these PBDEs are not environmentally relevant, these results suggest that those congeners that most closely resemble TCDD or dioxin-like PCBs have the highest agonistic activity. In contrast, the environmentally common and persistent BDE-47 and BDE-99 were not active in inducing CYP1A1, which indicates no agonistic properties for the Ah receptor (Chen et al., 2001; Chen & Bunce, 2003). A recent study by Peters et al. (2004) more or less confirmed the latter results. After a rigorous cleanup of environmentally relevant PBDEs (BDE-47, BDE-99, BDE-100, BDE-153, BDE-183) for possible impurities with dioxin-like activities, no induction of CYP1A1 activity could be determined in three Ah receptor responsive cell lines — the rat hepatoma H4IIE, the human hepatoma HepG2 and breast carcinoma MCF7 cells — when tested at concentrations up to 10 $\mu\text{mol/l}$. Based on the combined results of these *in vitro* studies with PBDE congeners, it must be concluded that those PBDEs that are environmentally relevant and bioaccumulate do not possess dioxin-like activity. Interestingly, the latter three studies all reported antagonistic effects of PBDEs on Ah receptor-mediated activities, including CYP1A1 induction (Meerts et al., 1998; Chen & Bunce, 2003; Peters et al., 2004). This raises the question whether these antagonistic effects of PBDEs measured on this enzyme induction may also apply for toxicological end-points that are Ah receptor-mediated.

2.2 Toxicological studies

The toxicity of PBDEs has been covered in several review books and articles (IPCS, 1994; Darnierud et al., 2001; de Wit, 2002; ATSDR, 2004; Gill et al., 2004). The present text on PBDE toxicity has been based on these compilations as well as on new results published thereafter.

2.2.1 Acute toxicity

Results of studies on the acute toxicity of PBDE commercial mixtures are summarized in Table 5.

Table 5. Acute toxicity of PBDE commercial mixtures

Dosing regimen	Strain, species	End-point	Effects	LD ₅₀ (oral) (mg/kg bw)	Reference
DecaBDE technical grade, single oral dose	SD rats, female	Mortality, gross pathological changes	No deaths	>2000	Norris et al. (1975b)
DecaBDE technical grade, single oral dose	Spartan rats, male	Mortality, weight gain	No deaths	>5000	Great Lakes (1974)
DE-79 (OctaBDE), single oral dose	Charles River rats, male	Mortality, weight gain	No deaths	>5000	Great Lakes (1987, 1990)
Saytex 111 (OctaBDE), single oral dose	Rats, sex not specified	Mortality, weight gain, gross pathological changes	No deaths	>10 000	Ethyl Corporation (1985)
PentaBDE, technical grade, single oral dose	Charles River rats, male	Mortality, weight gain	5000 mg/kg bw: 4/5 dead; 0, 50, 500 mg/kg bw: no deaths	500–5000	Great Lakes (1975)
PentaBDE, technical grade, single oral dose	Rats, male and female	Mortality, weight gain, gross pathological changes	Decreased growth and activity, diarrhoea, postmortem effects, liver and stomach	male: 7400 female: 5800	Great Lakes (1975)
Saytex 115 (PentaBDE), single oral dose	Rats, sex not specified	Mortality	Lack of information	5000	BIBRA (1977)
DE-71 (PentaBDE), single oral dose	Rats, sex not specified	Mortality	Lack of information	6200	PAI (1984)
Bromkal 70 (PentaBDE), single oral dose	Wistar rats, male	Liver weight and microsomal enzyme content/activity	Induction of EROD at threshold dose 3 mg/kg bw	—	von Meyerinck et al. (1990)

(a) *DecaBDE*

(i) *Rat*

In female Sprague-Dawley rats ($n = 5$), gastric intubation of a single DecaBDE (77.4% decaBDE congener, 21.8% nonaBDE congeners, 0.8% octaBDE congeners; in 10% corn oil suspension) dose of 0, 126, 252, 500, 1000 or 2000 mg/kg bw did not result in any indication of toxicity or gross pathological changes during a 14-day observation period (Norris et al., 1975b).

In male Spartan rats ($n = 5$) receiving DecaBDE at up to 5000 mg/kg bw by a single gavage dose in corn oil suspension, no deaths occurred, and the weight gain was normal during a 14-day observation period (Great Lakes, 1974).

(b) *OctaBDE*

(i) *Rat*

Male Charles River CD rats ($n = 5$) were intubated with a single dose of OctaBDE (DE-79; in corn oil suspension) at 0, 50, 500 or 5000 mg/kg bw, followed by an observation period of 14 days. The rats showed normal weight gain, and no mortality was observed (Great Lakes, 1987, 1990).

Rats were intubated with a single dose of Saytex 111, a PBDE mixture containing several congeners from penta- to decaBDE, of which the hepta- and octaBDEs are most abundant (45% and 34% of all congeners, respectively), at 0, 500, 2500, 7500 or 10 000 mg/kg bw in corn oil and studied for effects during a 72-h period (Ethyl Corporation, 1985). None of the animals died during this study, and no signs of toxicity were observed immediately after the dosing period. No effect on weight gain and no gross pathological changes were observed. The LD₅₀ was greater than 10 000 mg/kg bw.

Acute oral LD₅₀ doses for OctaBDE in rats were reported to be >28 g/kg bw (Kalk, 1982).

(c) *PentaBDE*

(i) *Rat*

Groups of male albino Charles River CD rats ($n = 5$) were given PentaBDE at 0, 50, 500 or 5000 mg/kg bw by gavage in corn oil and observed for 14 days. The rats receiving 50 and 500 mg/kg bw survived and had normal body weight gain, whereas four of five rats dosed with 5000 mg/kg bw died within 5 days. The remaining rats survived and had a normal weight gain (Great Lakes, 1975).

Groups of male and female Wistar rats ($n = 5$) were given PentaBDE in single doses of 0, 2400, 4800, 7621 or 9600 mg/kg bw (in corn oil) and subsequently observed for 44 days. From this study, the estimated LD₅₀ was 7400 mg/kg bw for male rats and 5800 mg/kg bw for female rats. Observed symptoms included decreased growth, diarrhoea, piloerection, reduced activity, tremors of the forelimbs, red staining around eyes and nose and a continual chewing movement of

the jaws. Examination postmortem showed enlarged, mottled and necrotic livers and multiple small ulcers of the gastric mucosa (Great Lakes, 1975).

Single-dose oral LD₅₀ values for PentaBDE, in studies with rats during 14 days of observation, were reported to be 5000 mg/kg bw (BIBRA, 1977) and 6200 mg/kg bw (PAI, 1984) for Saytex 115 and DE-71, respectively.

Male Wistar rats ($n = 3$) were administered a single oral Bromkal 70 dose of 0, 3, 10, 30 or 100 mg/kg bw and killed 3 days after dosing (von Meyerinck et al., 1990). The Bromkal 70 treatment increased the relative liver weight, the content of cytochrome P450 and the activity of microsomal liver enzymes in a dose-dependent manner. The EROD activity was induced at the lowest dose tested, 3 mg/kg bw, which the authors concluded was the threshold dose for induction of this enzyme.

(d) *BDE congeners*

No information is available on the acute toxicity of any specific BDE congeners.

2.2.2 Short-term studies of toxicity

Studies on the short-term toxicity of PBDEs are summarized in Table 6.

(a) *DecaBDE*

(i) *Mouse*

In an oral 14-day study, groups of B6C3F1 mice ($n = 5$, both sexes) were exposed to BDE-209 in the diet at concentrations of 0, 5, 10, 20, 50 or 100 g/kg diet. No effects were observed on health, survival or body weights, and no compound-related clinical signs or gross pathological effects were reported (NTP, 1986).

A 13-week study was performed in which B6C3F1 mice of both sexes ($n = 10$) were given DecaBDE (two different lots: >97% and 99% purity, respectively) in the diet at concentrations of 0, 3.1, 6.2, 12.5, 25 or 50 g/kg diet. No evidence was found for compound-related effects on the studied parameters, including body weight gain, survival, physical appearance and gross and microscopic pathology (NTP, 1986).

(ii) *Rat*

In a 14-day study, DecaBDE was administered in the diet to Fischer 344/N rats of both sexes ($n = 5$) at doses of 0, 5, 10, 20, 50 or 100 g/kg diet. No compound-related clinical signs or gross pathological effects were observed (NTP, 1986).

In 28-day feeding studies with Charles River CD rats ($n = 10$, both sexes, three separate studies), DecaBDE was given in the diet at doses of 0, 100 or 1000

mg/kg. In these studies, adverse effects or lesions associated with DecaBDE administration were not found (observation of appearance, mortality, food consumption, body weight gain, organ weights, gross pathological and microscopic examination) (Great Lakes, 1974).

Table 6. Short-term toxicity data for PBDEs

Dosing regimen	Species, strain, sex	End-point	NOEL ^a	LOEL	Reference
DecaBDE, technical grade, in diet, 14 days	Mice, B6C3F1, both sexes	Survival, body weight, clinical and gross pathology	100 g/kg diet males: 13.3 g/kg bw females: 15.6 g/kg bw	>100 g/kg diet	NTP (1986)
DecaBDE, technical grade, in diet, 13 weeks	Mice, B6C3F1, both sexes	Survival, body weight gain, gross/microscopic pathology	50 g/kg diet males: 6.65 g/kg bw females: 7.78 g/kg bw	>50 g/kg diet	NTP (1986)
DecaBDE, technical grade, in diet, 14 days	Rats, Fischer 344/N, both sexes	Clinical signs, gross pathology	100 g/kg diet males: 4.5 g/kg bw females: 5.1 g/kg bw	>100 g/kg diet	NTP (1986)
DecaBDE, technical grade, in diet, 28 days	Rats, Sprague-Dawley, male	Liver enlargement, thyroid hyperplasia	8 mg/kg bw per day	80 mg/kg bw per day	Norris et al. (1973, 1975b)
DecaBDE, technical grade, in diet, 90 days	Rats, Fischer 344/N, both sexes	Liver enlargement, male rats	12.5 g/kg diet males: 560 mg/kg bw females: 600 mg/kg bw	25 g/kg diet males: 1120 mg/kg bw females: 1200 mg/kg bw	NTP (1986)
OctaBDE, technical grade, in diet, 28 days	Rats, Charles River, both sexes	Liver enlargement and histopathology	—	100 mg/kg diet	Great Lakes (1987)
OctaBDE, technical grade, in diet, 28 days	Rats	Liver histopathology lesions	—	100 mg/kg diet	Great Lakes (1975)

Table 6. (contd)

Dosing regimen	Species, strain, sex	End-point	NOEL ^a	LOEL	Reference
OctaBDE, technical grade, in diet, 30 days	Rats, Sprague-Dawley, male	Liver enlargement, thyroid hyperplasia, histology liver and kidney lesions	—	8 mg/kg bw per day	Norris et al. (1975b)
OctaBDE, technical grade, in diet, 13 weeks	Rats, Sprague-Dawley, both sexes	Increased liver weight, microscopic hepatic changes	—	8 mg/kg bw per day	Great Lakes (1987)
DE-71 (PentaBDE), single or repeated gavage	Mice, C57BL, female	Relative liver and thymus weight (thyroid effects reported separately)	36 mg/kg bw per day (14 days)	72 mg/kg bw per day (14 days)	Fowles et al. (1994)
PentaBDE, technical grade, in diet, 28 days	Rats, Charles River CD, both sexes	Increased liver weight, liver lesions	—	10 mg/kg bw per day	Great Lakes (1975)
DE-71 (PentaBDE), repeated gavage, 28 days	Rats, Sprague-Dawley, both sexes	Increased liver weight, increased serum glucose and cholesterol levels, decreased LDH levels (thyroid effects reported separately)	5 mg/kg bw per day	25 mg/kg bw per day	Rowse et al. (2004)
DE-71 (PentaBDE), in diet, up to 90 days	Rats, Sprague-Dawley, both sexes	Decreased food consumption and body weight, increased cholesterol levels	10 mg/kg bw per day	100 mg/kg bw per day	Great Lakes (1975)
		Relative liver weight increase	2 mg/kg bw per day	10 mg/kg bw per day	

Table 6. (contd)

Dosing regimen	Species, strain, sex	End-point	NOEL ^a	LOEL	Reference
(contd)		Slight liver cell degeneration and necrosis (females)	—	2 mg/kg bw per day	
Bromkal 70-5-DE (PentaBDE), repeated gavage, 14 days	Rats, Sprague-Dawley, female	Decreased hepatic vitamin A levels	—	18 mg/kg bw per day	Hallgren et al. (2001)
Bromkal 70-5-DE, gavage, 28 days	Rats, Sprague-Dawley, both sexes	Decreased hepatic vitamin A levels, increased hepatic EROD activity	2.5 mg/kg bw per day	25 mg/kg bw per day	Fattore et al. (2001)

EROD, 7-ethoxyresorufin O-deethylase; LDH, lactate dehydrogenase; LOEL, lowest-observed-effect level; NOEL, no-observed-effect level

^a For the NTP (1986) studies, per kg bw daily intake estimations are based on body weight and feed intake data as indicated in the reference.

Male Sprague-Dawley rats ($n = 5$) were given diets consisting of 0, 0.01, 0.1 or 1% DecaBDE for 30 days (roughly equivalent to 0, 8, 80 or 800 mg/kg bw per day). The DecaBDE product contained 77% deca, 22% nona and some extent of octa congeners. Food intake and body weight gain were not different between the groups. No difference was noted in heart, testes, brain and kidney weights or in haematology and urinalysis parameters. The livers of rats receiving DecaBDE at 80 and 800 mg/kg bw per day were enlarged, and liver lesions consisted of centrilobular cytoplasmic vacuolization (at 800 mg/kg bw per day). In addition, degenerative changes in the kidney (at 800 mg/kg bw per day) and thyroid hyperplasia (at 80 and 800 mg/kg bw per day) were found (Norris et al., 1973, 1975a, 1975b).

No toxic effects were observed in a 90-day study with Fischer 344/N rats of both sexes ($n = 10$) when DecaBDE (containing >97% BDE-209) was given in the diet (0, 3.1, 6.2, 12.5, 25 or 50 g/kg diet), but increased liver weight was suggested in male rats consuming the two highest doses (NTP, 1986).

(b) OctaBDE

(i) Rat

Charles River CD rats (both sexes, $n = 10$) were given OctaBDE in the diet at various concentrations for 28 days. In sub-study I, the dietary doses were 0, 100

or 1000 mg/kg diet. Most studied parameters did not change between the groups. However, liver weights (both absolute and relative) were significantly increased in female rats at 100 mg/kg and in rats of both sexes at 1000 mg/kg. Moreover, compound-related histopathological liver lesions, consisting of enlarged centrilobular and midzonal liver parenchymal cells containing eosinophilic "round bodies," were seen at both dose levels. The incidence and severity of the liver lesions were dose-related and more severe in the male animals. In addition, rats at the 1000 mg/kg dose exhibited hyperplasia of the thyroid, but it was unclear whether this effect was compound-related (Great Lakes, 1987).

In sub-study II with OctaBDE in rats, the doses were 0, 100, 1000 or 10 000 mg/kg diet. The control group consisted of 35 animals from a 90-day feeding study (see below). At the end of the 28-day study, five animals per group were sacrificed, whereas the other five were maintained on control diet for an additional 4 weeks. No changes in behaviour, appearance or mortality were seen. The food intake and weight gain were slightly higher in the control group than in the OctaBDE exposure groups. Serum urea nitrogen levels were slightly higher in some of the rats on the 10 000 mg/kg diet. Increases in absolute and relative liver weights were observed in rats given the 1000 and 10 000 mg/kg diets. The histopathology of liver from all three dose levels showed enlargement of the centrilobular and midzonal hepatocytes, with the presence of "round bodies" in cytoplasm. In the highest dose group, vacuolization of hepatocytes and necrosis of individual hepatocytes were seen. Generally, the liver lesions were less severe after the 4-week recovery period. In addition, an increase in bromine levels in the liver was seen in rats in all treated groups, but the levels decreased in the recovery period (Great Lakes, 1975).

In a 30-day study with male Sprague-Dawley rats given diets containing 0, 0.01, 0.1 or 1.0% OctaBDE (corresponding to 0, 8, 80 and 800 mg/kg bw per day), the authors reported liver enlargement, thyroid hyperplasia and histological lesions in liver and kidney (hyaline degenerative cytoplasmic changes) at all dose levels and decreased packed cell volume, decreased total red blood cell count and increased kidney weight at the highest dose level (Norris et al., 1973, 1975b).

The same strain of rat was given commercial OctaBDE in the feed at dietary levels of 0, 100, 1000 or 10 000 mg/kg for 13 weeks ($n = 35$, male and female animals in separate dose groups) (Great Lakes, 1987). Behaviour, appearance, body weight and other important parameters were studied after 1 and 2 months and after 13 weeks, i.e. the end of the feeding period (five animals per sex per group). The remaining animals were studied 13 and 21 weeks and 6 months after withdrawal of the OctaBDE exposure. A few animals died during the study, but without any apparent dose relationship. In the 100 mg/kg diet group (corresponding to about 8 mg/kg bw per day), the only effect seen was an increase in absolute and relative liver weights, coupled to microscopic hepatic changes (granular cytoplasm) in some of the rats. At the 1000 mg/kg diet level (about 80 mg/kg bw per day), there was a decrease in body weight, in spite of normal blood chemistry, urine and haematology parameters. There was also an increase in absolute and relative liver and thyroid weights. Microscopic hepatic lesions (including vacuolization and hyaline inclusions) were observed in centrilobular and midzonal hepato-

cytes. After administration of the 10 000 mg/kg diet (about 800 mg/kg bw per day), the animals had a decrease in body weight gain, which persisted during the withdrawal period. Decreases in haemoglobin, haematocrit and erythrocyte counts were also observed, along with significant increases in the absolute and relative weights of the liver, kidney and thyroid. In the liver, autopsy revealed accentuated lobulation and yellowish mottling of the liver and brownish discoloration of the liver and kidney. After the recovery phase (1 year), no such changes were observed. Microscopic examination of the liver revealed granular cytoplasmic changes, cytoplasmic vacuolization, necrosis of parenchymal and centrilobular cells, centrilobular fibrosis and pigmented Kupffer cells. In the kidney, there was an occurrence of small to moderate numbers of cortical regenerative tubules, with one rat having severe tubular necrosis. In the thyroid, the observed cellular changes — a tall columnar epithelium instead of the normal cuboidal type (seen in 4/35 males and in 1/35 females at the highest dose) — were described by the authors as being compound-related. The observed histological changes decreased in severity and frequency during the recovery period. A hyperplastic nodule was found, after 6 months' withdrawal, in one rat from each of the 1000 and 10 000 mg/kg dose groups (Great Lakes, 1987).

(c) *PentaBDE*

(i) *Mouse*

In a study by Fowles and co-workers (Fowles et al., 1994) on immunological and endocrine effects of the PentaBDE mixture DE-71 in mice, organ and body weights were also measured. Female C57BL/6J mice ($n = 6$) were dosed either once by gavage with DE-71 at 0, 0.8, 4, 20, 100 or 500 mg/kg bw or by repeated gavage at daily oral doses of 0, 18, 36 or 72 mg/kg bw during 14 days. After an undefined survival time, the animals were killed, and spleen, thymus, liver and body weights were measured. The relative liver weight was dose-dependently increased compared with controls following subchronic exposure. After acute exposure, the highest dose (500 mg/kg bw) gave a similar increase in relative liver weight. The relative thymus weight was increased only at the highest subchronic exposure (72 mg/kg bw per day for 14 days). Neither the relative spleen weights nor the absolute body weights were significantly changed following DE-71 treatment.

(ii) *Rat*

In a 28-day study, Charles River CD rats ($n = 10$ per sex) were given PentaBDE in the diet at 0, 100 or 1000 mg/kg (roughly equivalent to 0, 10 and 100 mg/kg bw per day). Liver weights were significantly increased in female rats at 100 mg/kg diet and in both female and male rats at 1000 mg/kg diet. No gross pathological lesions were seen. Liver lesions were observed, were more prevalent in males and increased with dose. At the highest dose, a significant decrease in the relative weights of the pituitary and adrenal glands was observed. Microscopic studies revealed enlargement of parenchymal liver cells (centrilobular and mid-zonal) and the presence of granular structures and eosinophilic "round bodies" in

the cytoplasm at both dose levels. Hyperplasia of the thyroid was seen in both dose groups and in control animals. Therefore, whether these changes were considered compound-related is not clear (Great Lakes, 1975).

In a recent study (Rowse et al., 2004), a 28-day gavage study was performed with a technical PentaBDE mixture (DE-71) mainly containing tetra- (46%) and penta- (49%) BDE congeners. Male and female Sprague-Dawley rats ($n = 10$ per sex) were given daily doses by gavage of 0, 0.05, 0.5, 5 or 25 mg/kg bw in corn oil, and the animals were sacrificed on the 29th day. The liver and selected other organs were weighed, and a liver sample was taken for analysis of microsomal enzyme activities. Blood was collected, and serum chemistry analysis was performed for a number of parameters. In addition, serum was also analysed for total and free thyroid hormone levels — total triiodothyronine (TT3), total thyroxine (TT4), free triiodothyronine (FT3) and free thyroxine (FT4). No clinical sign of toxicity was seen, and the growth rates between control and treated groups did not differ. Liver weights were increased in both males and females in the 25 mg/kg bw per day group. Hepatic microsomal enzyme induction (benzyloxyresorufin O-deethylase [BROD], EROD, PROD) was seen in animals from the two highest dose groups. TT4 and FT4 levels were significantly lower in the 25 mg/kg bw per day male and female groups. TT3, but not FT3, levels were significantly reduced in the 25 mg/kg bw per day males only. Regarding clinical chemistry, female animals in the highest dose group had significantly higher levels of serum cholesterol and increased levels of glucose. Both males and females in the highest dose group had decreased lactate dehydrogenase (LDH) levels ($P < 0.05$).

PentaBDE (DE-71) was given in the diet to Sprague-Dawley rats ($n = 30$ per dose per sex) at dose levels of 0, 2, 10 or 100 mg/kg bw per day for a maximum of 90 days. The animals were killed after 4 weeks (10 per sex), 90 days (10), 90 + 6 weeks of recovery (5) and 90 + 24 weeks of recovery (5). Decreases in food consumption (highest dose, females) and body weight (highest dose, both males and females) were observed. No increased mortality or clinical effects were obvious. Increased cholesterol values were seen in the 100 mg/kg bw per day dose group animals, whereas T4 levels were decreased in animals exposed to 10 and 100 mg/kg bw per day. Relative liver weights were increased in the 10 and 100 mg/kg bw per day groups, but the remaining increase at 6 weeks of recovery had disappeared after 24 weeks of control diet. Urine and liver porphyrin levels were increased in the highest dose group after 90 days, the urine values being about 10 times higher and the liver levels almost 400 times higher than the control levels. Under microscopic examination, hepatocytomegaly and thyroid hyperplasia were seen, of which the thyroid effects were reversible after 24 weeks of recovery, but the liver effects partially persisted (slight hepatocytomegaly in the 10 and 100 mg/kg bw per day groups). After the 24-week recovery period, the lowest dose (2 mg/kg bw per day) resulted in slight liver cell degeneration and necrosis in female but not in male rats (Great Lakes, 1975).

Effects on vitamin A were followed in female Sprague-Dawley rats (approximately 175 g at start) after daily gavage of PentaBDE (Bromkal 70-5-DE; 0, 18 or 36 mg/kg bw per day) for 14 days (Hallgren et al., 2001). Twenty-four hours after the last gavage, the animals ($n = 8$ –12 per dose) were anaesthetized and killed by

exsanguination. Liver samples were collected and stored at -70°C before vitamin A analysis. Results showed that Bromkal 70 exposure significantly decreased vitamin A levels at both doses (approximately 75% of control) and that the effects were seen irrespective of presenting results as concentrations or as amounts in whole liver. No dose relation in vitamin A effects could be observed. In female C57BL mice studied under the same experimental conditions, no obvious PBDE effects on hepatic vitamin A levels were observed.

(d) *BDE congeners*

No information is available on short-term toxicity of individual BDE congeners.

2.2.3 Long-term studies of toxicity and carcinogenicity

The long-term toxicity/carcinogenicity studies on PBDEs are summarized in Table 7.

(a) *DecaBDE*

Rodent carcinogenicity bioassays have been carried out only for DecaBDE. A mouse study and a rat study have been reported by the United States National Toxicology Program (NTP, 1986), and a rat study has been conducted by the Dow Chemical Company (Kociba et al., 1975).

(i) *Mouse*

In the NTP mouse study (NTP, 1986), DecaBDE (purity 94–99%; no brominated dioxins or furans were found) mixed in diet was given to groups of 50 male and 50 female B6C3F1 mice for 103 weeks, and all the survivors were killed at 112–113 weeks of age. The concentration of DecaBDE in the diet was 0, 25 and 50 g/kg diet, with average daily exposure to DecaBDE estimated to be 3200 and 6650 mg/kg bw in low- and high-dose males and 3760 and 7780 mg/kg bw in low- and high-dose females, respectively. Body weight development and survival of DecaBDE-treated mice were comparable to controls. Stomach ulcers were reported at an increased incidence in high-dose female mice. Liver granulomas were observed in low-dose males, and liver hypertrophy was seen in low- and high-dose males. A significantly increased combined incidence of hepatocellular adenomas and carcinomas was observed in male mice (8/50 in controls, 22/50 in low-dose and 18/50 in high-dose males; trend not significant), whereas the combined incidences of thyroid follicular cell adenomas and carcinomas in males (0/50 in controls, 4/50 in low-dose and 3/50 in high-dose males) and females (1/50 in controls, 3/50 in low-dose and 3/50 in high-dose females) were only non-significantly increased. Furthermore, thyroid follicular cell hyperplasia was increased at both dose levels in males and females, and the response was stronger in male animals (high-dose males 19/50; high-dose females 7/49).

Table 7. Chronic toxicity/carcinogenicity and reproductive/developmental toxicity

Dosing regimen	Species, strain, sex	End-point	Effect level	Reference
DecaBDE, technical grade, in diet, 103 weeks	Mice, B6C3F1, both sexes	Hepatocellular adenoma/carcinoma	Male: 8/50, 22/50, 18/50	NTP (1986)
Dose: 0, 3200 mg/kg bw per day (LD), 6650 mg/kg bw per day (HD)		Thyroid follicular cell adenoma/carcinoma	Male: 0/50, 4/50, 3/50; female: 1/50, 3/50, 3/50	
		Thyroid follicular cell hyperplasia	Male HD: 19/50; female HD: 7/49	
DecaBDE, technical grade (77% decaBDE congener), in diet, 100–105 weeks	Rats, Sprague-Dawley, both sexes	Tumour development, survival, body weight, clinical chemistry parameters	No observed toxic effects	Kociba et al. (1975)
Dose: 0, 0.01, 0.1, 1 mg/kg bw per day				
DecaBDE, technical grade, in diet, 103 weeks	Rats, Fischer 344/N, both sexes	Liver adenoma	Male: 1/50, 7/50, 15/49 ($P < 0.001$ for trend); female: 1/50, 3/49, 9/50 ($P = 0.002$ for trend)	NTP (1986)
Dose: 0, 1120, 2240 mg/kg bw per day		Pancreas adenoma	Male: 0/49, 0/50, 4/49 ($P = 0.017$ for trend)	
		Hepatocellular carcinoma	No dose-related effect	
DecaBDE, technical grade, in diet, 60 days prior to mating to end of lactation	Rats, Sprague-Dawley, both sexes	Reproductive performance, pup maturation	No observed effects	Norris et al. (1975b)
DecaBDE, technical grade, oral gavage, GD 6–15	Rats, Sprague-Dawley, pregnant	Increased frequency of resorptions	10 mg/kg bw	Norris et al. (1975b)
		Increased number of subcutaneous oedema and delayed ossification	1000 mg/kg bw	
DecaBDE, technical grade, oral gavage, GD 0–19	Rats, Sprague-Dawley, pregnant	Maternal toxicity, fertility, gestation or fetal development	No observed effects	Hardy et al. (2002)

Table 7. (contd)

Dosing regimen	Species, strain, sex	End-point	Effect level	Reference
DE-79 (OctaBDE), oral gavage, GD 6–15	Rats, Charles River COBS CD, pregnant	Fetus: reduced weight, oedema, reduced ossification, bent rib bones	50 mg/kg bw (NOEL 15 mg/kg bw)	Great Lakes (1986)
		Mother: Reduced weight gain	50 mg/kg bw (NOEL 25 mg/kg bw)	
Saytex 111 (OctaBDE), oral gavage, GD 6–15	Rats, Charles River SD, pregnant	Fetus: Body weight	10 mg/kg bw (dose dependent)	US EPA (1986)
		Delayed ossification, fetal malformations	25 mg/kg bw	
		Mother: Reduced weight gain	25 mg/kg bw	
Saytex 111 (OctaBDE), GD 7–19	Rabbits, New Zealand White, pregnant	Fetus: Delayed ossification	2 mg/kg bw	Breslin et al. (1989)
		Fetus: Retrocaval ureter and fused sternbrae	5 mg/kg bw	
		Mother: Reduced weight gain, enlarged liver	15 mg/kg bw	
PentaBDE, technical grade, GD 6–15	Rats, pregnant	Maternal weight gain	100 mg/kg bw	BFRIP (1990)
DE-71 (PentaBDE), 31 days: PND 22–41 (females), PND 23–53 (males)	Rats, Wistar, post-weaning	Delay in vaginal opening	30 mg/kg bw	Stoker et al. (2004a)
		Delay in preputial separation	60 mg/kg bw	
		Ventral prostate and seminal vesicle weight decrease	60 mg/kg bw	
BDE-99, single oral dose, GD 6	Rats, Wistar, female off-spring (killed PND 90)	Lesion in ovarian tissues (electron microscopic study)	60 µg/kg bw	Talsness et al. (2003)
BDE-99, single oral dose, GD 6	Rats, Wistar, male off-spring (assessed PND 140–160)	Spermatid, sperm number and sperm production decrease; decrease in ejaculation frequency	60 µg/kg bw	Kuriyama et al. (2004a)

GD, gestation day; HD, high dose; LD, low dose; NOEL, no-observed-effect level; PND, postnatal day

(ii) *Rat*

In a study by the Dow Chemical Company (Kociba et al., 1975), groups of 25 male and 25 female Sprague-Dawley rats were given "decaBDE" (containing decaBDE 77.4%, nonaBDE 21.8% and octaBDE 0.8%) in the diet for 100–105 weeks. The dose levels were 0, 0.01, 0.1 or 1 mg/kg bw per day. The treatment did not have any influence on survival rates, appearance, body weights, feed consumption, haematology, urinalysis or organ weights. There were no other discernible toxic effects and no significant differences in the number of rats developing tumours between the groups. The International Agency for Research on Cancer (IARC) Working Group noted that the dose levels were very low (IARC, 1990).

In the NTP rat study (NTP, 1986), groups of 50 male and 50 female Fischer 344/N rats received DecaBDE (purity 94–99%; no brominated dioxins or furans were found) mixed in diet for 103 weeks, with all survivors killed at 111–112 weeks of age. The concentration of DecaBDE in the diet was 0, 25 and 50 g/kg diet, and the estimated average daily dose of DecaBDE was 1120 and 2240 mg/kg bw per day in low- and high-dose males and 1200 and 2550 mg/kg bw per day in low- and high-dose females, respectively. Body weights of the DecaBDE-treated rats were not significantly different from those of controls throughout the study. After week 102, low-dose male rat survival was significantly lower than controls, but the author suggested that this decreased survival may not have been compound-related. In high-dose males, thrombosis and degeneration of the liver, fibrosis of the spleen, lymphoid hyperplasia and acanthosis of the forestomach were observed. The incidences of neoplastic nodules of the liver (adenomas) were significantly increased in both males (1/50 in controls, 7/50 in low-dose and 15/49 in high-dose males; $P < 0.001$, incidental tumour test for trend) and females (1/50 in controls, 3/49 in low-dose and 9/50 in high-dose females; $P = 0.002$, incidental tumour test for trend). However, no differences in the incidence of hepatocellular carcinomas were detected between the groups. A significantly increased incidence of acinar cell adenomas of the pancreas was observed in males (0/49 in controls, 0/50 in low-dose and 4/49 in high-dose rats; $P = 0.017$, incidental tumour test for trend). Additionally, a high incidence of mononuclear cell leukaemia was observed in both treated and control rats of both sexes.

In summary, it can be concluded from these studies that there is limited evidence for the carcinogenicity of DecaBDE in experimental animals. In the IARC assessment of DecaBDE, this compound was considered not classifiable as to its carcinogenicity to humans (Group 3) (IARC, 1990). The lack of genotoxicity (see next section) suggests that the mechanism of the possible carcinogenic potency of decaBDE would be epigenetic.

2.2.4 Genotoxicity

The *in vivo* genotoxic potency of DecaBDE has been studied by cytogenetic examination of bone marrow cells from maternal Sprague-Dawley rats and their offspring, following exposure 60 days prior to mating as well as during mating, gestation and lactation (0, 3, 30 or 100 mg/kg bw per day; DecaBDE mixture

containing 77% decaBDE congener and 22% nonaBDE congener) (Norris et al., 1975b). No increase in chromosomal aberrations in maternal or neonatal rats was seen at any of the doses. Mutagenicity tests with DecaBDE were negative in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) (NTP, 1986) and in a yeast (*Saccharomyces cerevisiae*) model (Great Lakes, 1974) when tested both with and without metabolic activation at doses up to 10 000 µg/plate. Similarly, studies on DecaBDE in a eukaryotic cell model utilizing the TK locus of the mouse lymphoma cell line L5178Y, as well as chromosomal aberrations or sister chromatid exchanges in Chinese hamster ovary cells (both with and without metabolic activation), were all negative (NTP, 1986).

A commercial OctaBDE preparation, at dose levels of 60–300 µg/ml, was found to be negative in the unscheduled DNA synthesis assay in the human fibroblast cell line WI-38 with and without metabolic activation. It also did not induce mutations in *S. typhimurium* or *S. cerevisiae* or cause sister chromatid exchanges in Chinese hamster ovary cells (exposed to 7.5–750 µg/ml of OctaBDE for 2 h) with or without metabolic activation (Great Lakes, 1987). Also, in an assay of cytogenicity with human lymphocytes, cells were exposed to OctaBDE at 125–500 µg/ml or 32–125 µg/ml in the absence and presence of metabolic activation, respectively. No significant increases in structural and numerical chromosome aberrations were observed with or without metabolic activation relative to the solvent control group (Great Lakes, 1999).

Ames tests were performed on the PBDE mixtures Muster 13, 82 and 84, defined by the United States Environmental Protection Agency (US EPA) as OctaBDEs (US EPA, 1990a, 1990b, 1990c). Neither Muster 13 nor Muster 84 induced an increase in the number of revertant colonies in *S. typhimurium* strains TA98, TA100 or TA1535, with or without an exogenous S9 metabolic activation system at concentrations up to 5000 µg/plate. However, Muster 82 exhibited evidence of weak mutagenicity without metabolic activation in strain TA100. Muster 82 was tested at concentrations ranging from 2500 to 10 000 µg/plate and in a repeated test on TA100 without activation at concentrations from 2500 to 10 000 µg/plate.

Mutagenicity studies with a commercial PentaBDE preparation (doses unknown) in four strains of *S. typhimurium* (TA98, TA100, TA1535 and TA1537) and in *S. cerevisiae*, with and without metabolic activation, were all negative (Great Lakes, 1975). Mutagenicity tests with PentaBDE (1.6–1000 µg/plate) in the above-mentioned *Salmonella* strains, with or without metabolic activation, were also negative (Dead Sea Bromide Works, 1984). Moreover, negative results with PentaBDEs (100–10 000 µg/plate) in the same *Salmonella* strains were shown by Zeiger and co-workers (Zeiger et al., 1987) and Chemische Fabrik Kalk GmbH (Kalk, 1978). However, in one study of PentaBDE using *S. typhimurium* strains TA100, TA1535, TA1536 and TA1537, induction of point mutations (3-fold increase in number of revertant colonies) was seen at the highest dose (10 000 µg/plate) in strains TA1535 and TA1538, in the absence of metabolic activation (ISCCCL, 1977). This was considered a chance finding in the EU risk assessment on PentaBDE (EU, 2001).

Negative results were shown in a cytogenetic assay measuring structural chromosomal aberrations with human lymphocytes exposed to PentaBDE at concentrations up to 3759 µg/ml, both with and without metabolic activation (CMA, 1996). The tetraBDE congener BDE-47 (present in technical PentaBDE mixture) and lower brominated PBDE congeners (2-monoBDE and 3,4-diBDE) were tested (dose range 0–40 µg/ml) in two assays for intragenic recombination at an endogenous mammalian cell locus (SPD8 and Sp5). In the SPD8 assay, all three BDE congeners significantly increased the recombination frequency, whereas in the Sp5 assay, only the lower brominated congeners (2-monoBDE and 3,4-diBDE) caused significant increases in recombination frequency (Helleday et al., 1999). The possible role of this type of increased intragenic recombination in human diseases remains to be clarified.

By using Chinese hamster ovary cell lines with different defects in DNA repair, a screening method for the detection of genotoxic substances has been proposed (Johansson et al., 2004). According to the authors, depending on the cell line (in this case, EM9, UV4 and UV5), different kinds of DNA lesions could be suggested and screened for. Among the studied substances, BDE-47 was tested; in this model, it was found to be inactive and therefore suggested by the authors not to be genotoxic.

BDE-99 was assessed for mutagenicity and clastogenicity in vitro by use of bacterial reverse mutation assays in *S. typhimurium* strains TA98 and TA100 and in *Escherichia coli* WP2 uvrA and with the *Allium cepa* chromosome aberration test (Evandri et al., 2003). In the bacterial assays, the concentrations were 12.5–200 µmol/l (greater doses were not soluble, according to the authors); in the *Allium* test, doses from 1 to 100 µmol/l were used. Results showed that BDE-99 was negative in the bacterial mutagenicity assay, with or without S9 mix. Also, the frequency of chromosomal aberration was not significantly higher than that of the control, and no signs of cytotoxicity were observed in BDE-99-treated *A. cepa*.

2.2.5 Reproductive/developmental toxicity

The reproductive toxicity of PBDEs has been studied using technical Deca-, Octa- and PentaBDE preparations, including Saytex 111. All except the first study under this heading are teratogenicity studies in rats, and only Saytex 111 has been studied in both rats and rabbits. The reproductive/developmental toxicity studies are summarized in Table 7.

(a) Rat

Effects of DecaBDE on reproductive performance were studied in male and female Sprague-Dawley (Spartan) rats given commercial DecaBDE in the diet at dose levels of 0, 3, 30 or 100 mg/kg bw per day (Norris et al., 1975a, 1975b). The group sizes were 20 males and 40 females (control group), 10 males and 20 females (the low and middle dose groups) and 15 males and 30 females (the highest dose group). The treatment was commenced 60 days prior to mating and

continued throughout gestation and lactation. No treatment-related changes were reported in reproductive performance or maturation of pups.

In a teratogenicity study with Sprague-Dawley (Spartan) rats, commercial DecaBDE (77.4% decaBDE, 21.8% nonaBDE, 0.8% octaBDE) was given at dose levels of 0, 10, 100 or 1000 mg/kg bw per day by oral gavage on gestation days (GD) 6–15, and fetuses were collected by caesarean section on GD 21 (Norris et al., 1975b). No maternal toxic effects, in terms of clinical signs, body weight gain, food consumption or liver weights, were observed. Similarly, the number of corpora lutea, position and number of fetuses in utero, individual fetal weight, crown–rump length and sex ratio were not affected by the treatment. However, significantly increased incidences in resorptions were observed at the lower dose levels, but not at 1000 mg/kg bw per day. In the absence of numerical as well as historical control data, the possibility of embryoletality cannot, therefore, be ruled out. No external abnormalities were observed in fetuses, but soft tissue and skeletal examinations revealed increased numbers of litters with subcutaneous oedema and delayed ossification of normally developed bones of the skull at the dose level of 1000 mg/kg bw per day. Analysis of maternal and fetal livers for bromine concentration (reflecting liver concentration of DecaBDE) showed significantly increased concentrations only in maternal livers at the highest dose. Although this study is inadequately reported, it suggests that DecaBDE is not teratogenic, but it is clearly fetotoxic at dose levels that are not maternally toxic.

Female Sprague-Dawley rats ($n = 25$ per dose) were treated by gavage with DecaBDE (composite of three commercial lots; purity 97.3%) at doses of 0, 100, 300 or 1000 mg/kg bw from GD 0 to GD 19. Fetuses were collected on GD 20 and assessed for external, visceral and skeletal anomalies/defects. No effects were observed with respect to maternal toxicity, fertility, gestation or fetal development (Hardy et al., 2002).

The teratogenicity of a commercial OctaBDE preparation (DE-79) was studied in Charles River COBS CD rats ($n = 10$) receiving the test compound by gavage at 0, 2.5, 10, 15, 25 or 50 mg/kg bw per day on GD 6–15 (Great Lakes, 1986). Reduced maternal body weight gain and slightly increased cholesterol levels, but no histopathological changes in livers or kidneys, were observed at 50 mg/kg bw per day. These maternal effects were associated with marked fetal toxicity, as indicated by increased numbers of late resorptions, significantly reduced mean fetal weights, severe generalized oedema (anasarca), reduced ossification of the skull and various unossified bones. In addition, developmental variations, such as bent limb bones and bent ribs, were reported at 50 mg/kg bw per day. No treatment-related effects were observed at 15 mg/kg bw per day or lower, but the results at 25 mg/kg bw per day were not reported. Based on these findings, suggested no-observed-effect levels (NOELs) are 25 mg/kg bw per day for maternal toxicity and 15 mg/kg bw per day for fetal effects.

The teratogenicity of the commercial OctaBDE mixture Saytex 111 was studied in four groups of 25 Charles River (SD) rats (US EPA, 1986). They were administered corn oil suspensions of the test substance by gavage at 0, 2.5, 10 or 25 mg/kg bw per day on GD 6–15. Fetuses were examined on day 20 for gross

visceral and skeletal abnormalities. The test substance was found to be more toxic to the fetuses than to the dams, as shown by a dose-dependent reduction of fetal body weight at the two highest dose levels. At 25 mg/kg bw per day, Saytex 111 also increased the number of early and late resorptions, delayed skeletal ossification and induced fetal malformations, such as enlarged heart and rear limb malformations (type of malformation not specified). The only maternal effect noted was a reduced body weight gain in the high-dose animals.

A teratogenicity study with a commercial PentaBDE preparation was carried out in rats (strain and number of animals not specified) (BFRIP, 1990). The test compound suspended in corn oil was given by gavage at 0, 10, 100 or 200 mg/kg bw per day on GD 6–15. Maternal body weight gain was decreased at 100 and 200 mg/kg bw per day, and a slight (non-significant) reduction of fetal body weight was observed at 200 mg/kg bw per day.

(b) Rabbit

The teratogenicity of Saytex 111 was also studied in groups of 26 New Zealand White rabbits by the Dow Chemical Company (Breslin et al., 1989). The rabbits were administered the test substance by gavage at 0, 2, 5 or 15 mg/kg bw per day on GD 7–19, and the fetuses were collected on GD 28. Approximately half of the fetuses in each litter were randomly assigned for soft tissue examination, in addition to all the fetuses being examined for skeletal alterations. Maternal body weight showed a dose-dependent decrease compared with the control group, which was statistically significant only at 15 mg/kg bw per day (93% of control weight). Also, the absolute and body weight-related maternal liver weights were increased at this dose level. One rabbit at 5 mg/kg bw per day and two rabbits at 15 mg/kg bw per day delivered their litters prior to GD 28. In addition, one rabbit at 15 mg/kg bw per day was killed after exhibiting signs of abortion. This animal had multiple resorption sites in the uterus. Excluding these animals, the number of resorptions was not affected by the treatment. Signs of fetal toxicity included slight (non-significant) decreases in fetal body weights at 5 and 15 mg/kg bw per day and increased incidences of delayed ossification of the hyoid, dental process at 5 mg/kg bw per day only and sternebrae at 2, 5 and 15 mg/kg bw per day (statistically significant only at 15 mg/kg bw per day). Treatment-related fetal anomalies included increased incidences of retrocaval ureter and fused sternebrae at all dose levels of Saytex 111, with the maximum incidence at 5 mg/kg bw per day (statistically significant). These variants were absent from the concurrent controls, but they were reported to have occurred at relatively high incidence in some historical controls. This outcome and the lower incidence at 15 mg/kg bw per day compared with 5 mg/kg bw per day led the authors (Breslin et al., 1989) to consider them as spontaneous. To conclude, Saytex 111 caused fetal toxicity and may also induce fetal anomalies at maternally non-toxic dose levels.

The reproductive/developmental toxicity studies illustrate that, in general, fetuses are more sensitive than mothers and that the increased incidence of developmental variants/anomalies is a frequent fetal effect observed with commercial Octa- and PentaBDE formulations. Although it is known that maternal toxicity

can influence fetal ossification (Khera, 1984), the fetal effects seem to appear at lower doses than those indicative of maternal toxicity.

(c) *Multigeneration reproductive toxicity*

No information is available on multigeneration reproductive toxicity studies involving PBDEs.

2.2.6 *Special studies*

(a) *Thyroid hormone system*

(i) *Mixtures*

Mouse

Depending on the dose, effects of PBDEs on the thyroid hormone system may occur, as the structure of thyroid hormones is very similar to that of halogenated diphenyl ethers, but with iodine instead of bromine substituents. Acute (0, 0.8, 4, 20, 100 or 500 mg/kg bw; $n = 6$ per group) or subchronic (0, 18, 36 or 72 mg/kg bw per day for 14 days, relating to total doses of 0, 250, 500 and 1000 mg/kg bw; $n = 6-8$ per group) oral exposure by gavage to the technical mixture DE-71 was tested in adult female C57BL/6J mice. Acute exposure resulted in decreased serum TT4 concentration at all doses tested, with the exception of 100 mg/kg bw. The maximum reduction measured was approximately 50% at the 20 mg/kg bw dose. Dose-dependent reductions in circulating TT4 and FT4 were caused by subchronic treatment at 18 mg/kg bw per day or higher doses, resulting in maximum reduction of about 40% and 60% for TT4 and FT4 at the highest dose, respectively (Fowles et al., 1994).

Juvenile C57BL/6N mice ($n = 8$ per group, controls $n = 12$) and Sprague-Dawley rats ($n = 6$ per group, controls $n = 10$) were exposed to the technical mixture Bromkal 70-5-DE (18 or 36 mg/kg bw per day by gavage for 14 days, resulting in total doses of 250 or 500 mg/kg bw, respectively). Animals were examined for altered thyroid hormone concentrations in plasma. In both species, a dose-dependent depression of TT4 and FT4 was observed, with decreases to about 50% and 20% of control values for TT4 and FT4, respectively, in rats at the highest dose of Bromkal 70 and of about 60% for both TT4 and FT4 in mice. Plasma thyroid stimulating hormone (TSH) levels were not changed in any species. Induction of the phase II metabolizing enzyme UDPGT was found at the highest dose in rats, while increases in activity of this enzyme were not significant in mice (Hallgren et al., 2001). The activities of phase I enzymes EROD and MROD were significantly induced at both doses of Bromkal 70 in rats and mice, whereas induction of PROD was seen only in rats exposed to 18 or 36 mg/kg bw per day.

In a recent study by Skarman et al. (2005), NMRI dams ($n = 13$ per treated group and $n = 22$ controls) were exposed by gavage to Bromkal 70-5-DE (0 or 80 $\mu\text{mol/kg}$ bw on every third day from GD 4 to postnatal day [PND] 17, resulting in 10 applications). The main constituents of this mixture are BDE-47 (35%) and

BDE-99 (37%). All dams delivered in the Bromkal 70 group, and 20 of 22 in controls. Four dams per group were sacrificed on GD 17, and the remaining dams on PND 20. Differences in FT4 and TT4 were not significant in dams at either time point. TT4 and FT4 plasma levels were decreased in exposed offspring ($n = 6-16$; sex not given) to approximately 70% of control levels on PND 11, but not on PND 18. Hepatic UDPGT activity did not show exposure-related effects on PND 11, while there was a borderline significance on PND 18 (Skarman et al., 2005). However, treatment with the same molar dose of BDE-99 did not result in any significant changes in dams and offspring.

Rat

In a study in weanling female Long-Evans rats, effects of short-term exposure to three different technical mixtures (DE-71, DE-79 and DE-83R, representing penta-, octa- and decaBDE, respectively) were examined for TT4 and TT3 concentrations in serum, TSH and activity of hepatic enzymes (UDPGT, EROD, PROD) 24 h after the last treatment. Following gavage dosing with 0, 0.3, 1, 3, 10, 30, 60 or 100 mg/kg bw per day (for DE-71, substitute 60 mg/kg bw per day with 300 mg/kg bw per day; $n = 8$ per group, except for low dose of DE-71, where $n = 4$), there were dose-dependent decreases in serum TT4 by DE-71 and DE-79 at daily doses greater than 3 mg/kg bw, together with a 2.5- to 5-fold increase in hepatic UDPGT activity. Serum TT3 concentrations were significantly reduced at doses ≥ 60 mg/kg bw per day by both the penta- and octaBDE mixtures. Maximum reductions were 80% and about 30% for serum TT4 and TT3, respectively. BMD calculations revealed influences on thyroid hormones and hepatic enzyme activity at comparable levels (see Tables 85 and 86 in section 10 below for the BMD and lower confidence limit on the BMD [BMDL] of thyroid hormones). Circulating TSH concentrations were not affected. Relative liver weights were significantly increased at DE-71 and DE-79 at doses above 10 mg/kg bw per day. Also, DE-83R (0–100 mg/kg bw per day) did not alter any of the end-points studied, thus indicating that decaBDE is much less effective than lower brominated congeners (Zhou et al., 2001).

In a follow-up of this study, developmental treatment of Long-Evans rats with DE-71 (0, 1, 10 or 30 mg/kg bw per day by gavage; $n = 47-55$ per group) was used from GD 6 to PND 21. The PBDE mixture decreased serum T4 by about 50% at the highest dose in dams on GD 20 and on PND 22 ($n \geq 8$ per group). Reductions in serum T4 (down to 70% of control values at the highest dose) were also found in pups on PND 4 and 14, at doses of 10 and 30 mg/kg bw per day ($n \geq 8$ litters per group). T4 values in the offspring recovered by PND 36. UDPGT activity was induced by DE-71 in dams and pups at the highest dose level. Elevations in hepatic EROD and PROD activities were observed at doses above 1 mg/kg bw per day in both dams and offspring. Serum triiodothyronine (T3), maternal and offspring body weights and time of eye opening were not affected (Zhou et al., 2002). The BMD and BMDL for T4 and hepatic enzymes are shown in Tables 85 and 86 in section 10 below.

Using pubertal protocols, oral exposure by gavage of Wistar rats to DE-71 (0, 3, 30 or 60 mg/kg bw per day) from PND 23 to PND 53 in male rat pups and from

PND 22 to PND 41 in female rat pups or for 5 days only (males: PND 23–27, females: PND 22–26) resulted in decreases in circulating serum TT4 at the two highest dose levels and both exposure durations in females (levels decreased to about 30% of control values). Similar effects were observed in the males, except all three doses of DE-71 significantly reduced total serum T4 following exposure for 31 days, with the maximum reduction at the highest dose to about 20–25% of the control level. Values of T3 were reduced by 35% only in males exposed for 31 days at both higher dose levels. In these groups, there was also an elevation of TSH by up to a factor of 2 at the highest dose. Relative liver weights were also significantly increased in the 30 and 60 mg/kg bw per day dose groups (both sexes) for both exposure periods. Morphological changes in the thyroid gland were found in both sexes exposed to the highest dose of DE-71 for 21 or 31 days. These results indicate that the 31-day protocol is more sensitive at detecting thyrotoxicity (Stoker et al., 2004a). The BMD and BMDL for serum T4 are shown in Tables 85 and 86 in section 10 below.

In a similar study, developmental exposure of Long-Evans rats to DE-71 from GD 6 to PND 21 (gavage with 0, 5, 30 or 100 mg/kg bw per day) caused a dose-dependent decline of serum TT4 in the early postnatal period. Maximal decreases to less than 20% of control levels at the highest dose on PND 14 were observed, with recovery to control values by PND 36 (Gilbert et al., 2004). However, this recovery of hormone levels does not necessarily imply that secondary effects of thyroid hormones (e.g. on the developing nervous system) are reversible, since a lack of hormone supply during a critical developmental period may result in long-lasting changes that persist after normalization of circulating hormone concentrations.

Thyroid hyperplasia is a common sign of subchronic and chronic PBDE exposure. Feeding decaBDE (BDE-209) to male Sprague-Dawley rats for 30 days (0, 100, 1000 or 10 000 mg/kg diet, relating to an average intake of 0, 8, 80 or 800 mg/kg bw per day, respectively) caused thyroid hyperplasia at concentrations of 1000 mg/kg diet (equivalent to about 80 mg/kg bw per day; total dose 2400 mg/kg bw) or higher. The corresponding NOEL was 100 mg/kg diet, or 8 mg/kg bw (total dose 240 mg/kg bw). However, the decaBDE formulation had a purity of approximately 77% and included about 22% nonaBDE and 1% octaBDE (Norris et al., 1975b). DE-79, a technical PBDE mixture containing mainly octaBDE, also caused slight to moderate thyroid hyperplasia in another study in Charles River CD rats, which used dietary exposure (0, 100 or 1000 mg/kg diet for 28 days or 0, 100, 1000 or 10 000 mg/kg diet for 90 days; IRDC, 1976, 1977; reviewed in Gill et al., 2004). The NOEL in these studies was 100 mg/kg diet for both exposure durations. Increased thyroid weights were reported in male Sprague-Dawley rats after dietary exposure to the pentaBDE mixture DE-71 (average daily intake of 2, 10 or 100 mg/kg bw for 90 days, relating to total doses of 180, 900 or 9000 mg/kg bw, respectively). The NOEL for thyroid weight change was 10 mg/kg bw per day, with the increased weight persisting after an exposure-free interval of 168 days (WIL Research Laboratories, 1984; reviewed in Gill et al., 2004).

*(ii) Single congeners and metabolites**Mouse*

Thyroid hyperplasia has been detected in mice after exposure to decaBDE with a purity of 95%. Diets containing decaBDE at 0, 25 or 50 g/kg diet were fed to B6C3F1 mice ($n = 50$) for 2 years, leading to an average daily intake of 3.2 or 6.65 g/kg bw in males and 3.76 or 7.78 g/kg bw in females. Mice from both dose groups developed follicular cell hyperplasia. In both sexes, a marginal occurrence (not statistically significant) of follicular cell adenoma was seen (NTP, 1986; reviewed in Gill et al., 2004).

In the study by Hallgren et al. (2001), daily oral exposure (gavage) of female C57BL/6N mice to BDE-47 at 18 mg/kg bw per day for 14 days (total 250 mg/kg bw; $n = 8$) resulted in reductions of TT4 and FT4 in plasma, measuring about 70% and 60% of control values, respectively. There were no significant effects on TSH levels and hepatic UDPGT activity.

A group of NMRI dams exposed to BDE-99 at 80 $\mu\text{mol/kg}$ bw (45.2 mg/kg bw) on every 3rd day from GD 4 to PND 17 ($n = 13$, controls $n = 22$) was also included in the experiment by Skarman et al. (2005). Ten and 20 dams delivered in the BDE-99 and control group, respectively. Neither TT4 nor FT4 plasma levels differed significantly from control values in dams or offspring, suggesting that other constituents of the Bromkal 70-5-DE mixture might have caused the reported serum T4 differences in offspring on PND 11. Also, UDPGT activity was not enhanced by BDE-99.

Rat

In a subsequent study by Hallgren & Darnerud (2002), the same experimental design was used in 7-week-old female Sprague-Dawley rats (gavage with BDE-47 at 0, 1, 6 or 18 mg/kg bw per day for 14 days, equivalent to total doses of 0, 14, 84 or 250 mg/kg bw; $n = 6$ per group). Groups of rats were also exposed to daily doses of technical PCB mixture Aroclor 1254 (4 mg/kg bw per day) and a mixture of the chlorinated paraffin Witacolor 171P (6.8 mg/kg bw per day) by gavage for 14 days. In addition, rats were exposed to all the possible two- or three-substance mixtures of BDE-47, Aroclor 1254 and Witacolor 171P. Ex vivo binding of [^{125}I]TT4 to the serum transport protein TTR and morphology of the thyroid were examined, in addition to hormone levels and hepatic enzyme activities, 1 day following the last dose. FT4, but not TT4, was decreased by about 35% after exposure to the highest dose of BDE-47. UDPGT activity showed a dose-dependent increase (by 25% at the highest dose), and TTR-bound radioactivity exhibited a dose-dependent decrease (to 70% of control values at the highest dose). TSH and thyroid weights were not affected by BDE-47, and morphology of the thyroid did not reveal signs of glandular activation. However, thyroid glandular activation was found in the group with combined exposure to BDE-47 and the technical PCB mixture Aroclor 1254 alone or in combination with a mixture of chlorinated paraffins (Witacolor 171P). The combination of BDE-47 and PCBs generally led to additive effects; however, synergistic effects were indicated by effects of combined

exposure to BDE-47 and chlorinated paraffins on FT4 (Hallgren & Darnerud, 2002). The authors concluded that the effects of exposure to these halogenated compounds on circulating thyroid hormones are mainly due to the disturbed serum transport of thyroid hormones, caused by binding of metabolites or parent compounds of PBDEs or PCBs to TTR. Additional reductions may occur by the induction of metabolizing enzymes, leading to increased metabolism of thyroid hormones (Hallgren & Darnerud, 2002). The conclusion that competition for binding sites on the transport proteins of thyroid hormones is the main cause for decreases in circulating levels in rats is supported by a comparative study in two additional rat strains, Wistar and Gunn, which demonstrated similar reductions of serum FT4 and TT4 in both strains by PCBs, despite the absence of hepatic UDPGT enzymes due to a genetic mutation in Gunn rats (Kato et al., 2004). However, these authors concluded that the cause of the reduction in circulating T4 concentration remains unclear, since both mechanisms, competition for TTR and induction of UDPGT, do not apply for all species and other mechanisms, such as effects on the hypothalamus–pituitary–thyroid axis, sulfation of iodothyronines and changes in activity of deiodinases, have not been sufficiently examined.

Binding of PBDEs, in particular hydroxylated PBDEs, to TTR (Meerts et al., 2000) and thyroid hormone receptors (Marsh et al., 1998) has been demonstrated *in vitro*. In rats, hydroxylated metabolites of BDE-47, BDE-99 and BDE-209 have been identified, while in human plasma, a potential hydroxylated derivative of BDE-47 has been found (reviewed in Hakke & Letcher, 2003). In humans, the major transport protein is not TTR, but thyroxine binding globulin (TBG), which is reported to have low affinities to hydroxylated PBDEs (Cheek et al., 1999).

When female juvenile Long-Evans rats were dosed with BDE-47 ($n = 8$ – 14) for 4 consecutive days (0, 0.3, 1, 3, 10, 30 or 100 mg/kg bw per day by gavage), dose-related decreases in TT3 and TT4 (by 25% and 75% at the highest dose, respectively) were observed in serum, in the absence of altered TSH concentrations (Hedge et al., 2004).

In a recent study, Wistar rats ($n = 10$) were treated by gavage with low doses of BDE-99 (0, 60 or 300 $\mu\text{g/kg}$ bw) on GD 6. Total serum T4 was reduced in dams of both dose groups (approximately 65% of control value) on PND 1, while offspring of both sexes from the high dose group exhibited significant reductions in circulating T4 (free and total) on PND 22, but not PND 1 (Kuriyama et al., 2004a). TSH levels were also reduced in the low-dose-group offspring on PND 1. Treatment of Wistar rat dams with BDE-47 on GD 6 (0, 140 or 700 $\mu\text{g/kg}$ bw; $n = 7$, 10 and 9, respectively) caused a reduction in serum TT3 to approximately 50% of control values in male offspring on PND 1. On PND 14, decreases were found in both exposed groups, but the effect was slightly stronger at the low dose level (to about 60% of control values) than at the highest dose (about 70%). TSH was also decreased at this age, but the reduction was significant only at the lower dose (by about 10%). On PND 22, TT4 was dose-dependently elevated (by about 10% in the high dose group) and TSH significantly depressed to about 65% of control values at the lower dose. The reduction was smaller at the high dose, measuring about 80% of control values. Body weights of the high-dose male offspring were significantly decreased (approximately 12%) at PND 22. In addition, there was a

decrease of follicle stimulating hormone (FSH) to approximately 65% of control levels in the high dose group (Andrade et al., 2004). In dams, significant decreases in TT4 (to about 80% of control values) and TSH (to nearly 30% of control values) were found only at the high dose level on PND 1. All effects had normalized by PND 22 (Kuriyama et al., 2004b).

Dose-related decreases in thyroid weights, down to 60–65% of control values, were observed in adult male and female Long-Evans rats ($n = 8$ per group) after maternal exposure to BDE-99 (1 or 10 mg/kg bw per day) from GD 10 to GD 18 (Lilienthal et al., 2004).

(iii) Summary of thyroid effects from rodent studies

From the available data in rodent studies, it appears that TT4 is one of the more sensitive parameters associated with PBDE exposure. FT4 may be as sensitive, but it was usually determined in fewer studies. In contrast, TT3 seems to be less sensitive by factors of 3–6 (Zhou et al., 2001; Stoker et al. 2004a). In one report (Stoker et al., 2004a), limited decreases in serum TT3 were seen only in males following 31 days of exposure to DE-71. Also, elevation of serum TSH was found only in males in this experiment, whereas effects on TT4 do not show a clear sex difference in pubertal protocols (Stoker et al., 2004a). From short-term exposures in post-weanling female rats, it seems that the higher brominated octaBDE mixture DE-79 is more potent than the lower brominated DE-71 (Zhou et al., 2001), which may be due to greater accumulation of higher brominated congeners. Of single congeners, only BDE-47 and BDE-99 (and technical-grade decaBDE) have been studied so far; thus, the data are not sufficient for conclusions about differential potencies. However, it appears that decaBDE is much less potent than lower brominated congeners in altering thyroid hormones. In one study, effects on TT4 were reported in offspring after a single gestational treatment with a very low dose of BDE-99 (Kuriyama et al., 2004a). Reductions in circulating thyroid hormone concentrations were observed in dams and developing animals, but the data indicate that thyroid hormone levels recovered by PND 39. In another study, recovery to normal hormone levels was observed 2 weeks after termination of perinatal treatment (Gilbert et al., 2004). Since induction of EROD has been found at similar doses, which caused decreases in thyroid hormone concentrations in many studies using PBDE mixtures or congeners, a contribution of dioxin-like contaminants is likely, as purified PBDEs were reported not to induce EROD in vitro. Several mechanisms may be responsible for decreased thyroid hormones in serum. Competition of hydroxylated PBDEs for TTR binding and induction of UDPGT cannot explain effects in all species, and there is a lack of knowledge about PBDE effects on the hypothalamus–pituitary–thyroid axis, sulfation of iodothyronines and altered activities of deiodinases. Thus, the cause for reduction of thyroid hormones remains unclear (Kato et al., 2004). In contrast to reduced hormone levels, thyroid hyperplasia was found at much higher exposure levels (Norris et al., 1975a, 1975b; Gill et al., 2004). However, long-lasting decreases in thyroid weights have been detected after maternal exposure to BDE-99 (Lilienthal et al., 2004).

(b) *Steroid hormones*

(i) *Mixtures*

Mouse

In the study by Fowles et al. (1994), subchronic exposure to the technical mixture DE-71 elevated serum concentrations of corticosterone. Female C57BL/6 mice ($n = 6$) were treated by gavage with DE-71 (0, 18, 36 or 72 mg/kg bw per day) for 14 consecutive days and then assessed for changes in serum corticosterone. The results suggested that an interaction between the acute stress of necropsy procedures (repeated cage disruptions) and DE-71 exposure resulted in increased corticosterone levels in all dosed groups compared with controls.

Rat

Oral exposure of Wistar rats to DE-71 (0, 3, 30 or 60 mg/kg bw per day by gavage) from PND 22 to PND 41 in females and from PND 23 to PND 53 in males resulted in decreased weights of seminal vesicles (about 20% reduction) and ventral prostate (about 15% reduction) in males at the highest dose tested, whereas testes and epididymis weights were unaltered ($n = 15$ per group). Preputial separation was slightly delayed (1.7–2.1 days) at 30 and 60 mg/kg bw per day, while in females, a delay of vaginal opening (1.8 days) was detected at 60 mg/kg bw per day (Stoker et al., 2004a). No significant differences were noted in body weight increase over the dosing period. Serum testosterone, serum and pituitary luteinizing hormone (LH) and pituitary prolactin were not altered by exposure in males, in contrast to serum prolactin, which was increased 2-fold at the highest dose. No reproductive hormones were measured in female animals. Effects in males may be caused by interference of PBDEs with androgenic stimulation, while vaginal opening is estrogen-dependent. However, secondary effects due to reduced thyroid hormones could not be entirely excluded.

(ii) *Single congeners and metabolites*

In vitro

Estrogenicity of hydroxylated PBDE and parent compounds was studied in different cell lines using an estrogen receptor-based reporter gene assay (ER-CALUX). In human T47D breast cancer cells, several PBDEs exerted estrogenic responses, but with relative potencies (EC_{50}) 6 orders of magnitude lower (2.5–7.3 $\mu\text{mol/l}$) than that of estradiol. Several PBDE congeners exhibited more than 50% maximum luciferase induction, with the highest value found for BDE-30 (114%). Potencies of hydroxylated PBDE were generally higher. The metabolite 2-bromo-4-(2,4,6-tribromophenoxy)phenol caused an induction response exceeding that of estradiol, but at concentrations 50 000 times higher. In an estrogen receptor ($ER\alpha$) specific transfected human embryonic kidney cell line, the hydroxylated congener 4-(2,4,6-tribromophenoxy)phenol showed an induction similar to that of estradiol, with an EC_{50} of $<0.1 \mu\text{mol/l}$. In the analogous $ER\beta$ -specific cell line, the same compound showed 50% of the estrogenic potency of estradiol, with an EC_{50} value

of ≤ 5 $\mu\text{mol/l}$ (Meerts et al., 2001). These results demonstrate that parent PBDEs and, in particular, hydroxylated derivatives have the ability to induce estrogenic responses, albeit at relative potencies orders of magnitude lower than that of estradiol. Metabolism of PBDEs to hydroxylated derivatives *in vivo* is suggested to result in increased potencies (Meerts et al., 2001).

BDE-47, BDE-99, BDE-100 and BDE-154 were examined in a rat ventral prostate assay for their ability to compete with R1881 (synthetic androgen) androgen receptor (AR) binding. The results indicated that BDE-47 and BDE-100, in particular, are more potent at inhibiting the binding of labelled R1881 to the AR, showing 60% and 98% inhibition, respectively, at a concentration of 33 $\mu\text{mol/l}$ (explicit IC_{50} values were not given). These two congeners also inhibited dihydrotestosterone (DHT) induced human androgen receptor (hAR) transcriptional activation in the MDA-kb2/luciferase cell line, with IC_{50} values of about 5 $\mu\text{mol/l}$ (Stoker et al., 2004b). According to the results of inhibition constant K_i determinations, BDE-100 appears to be a competitive inhibitor of ^3H -labelled R1881 binding to hAR. In addition, rat ventral prostate cytosol was incubated overnight at 4 °C with increasing concentrations of labelled R1881 in the presence of BDE-100 at 0, 6, 9 or 18 $\mu\text{mol/l}$. Suppression of R1881 binding was observed, with an IC_{50} of approximately 5 $\mu\text{mol/l}$.

In vivo: rat

In a developmental toxicity study, daily exposure of Sprague-Dawley rats during gestation (GD 0–19) to BDE-209 (0, 100, 300 or 1000 mg/kg bw per day by gavage; $n = 25$ dams per group) did not influence the fetal sex distribution (Hardy et al., 2002).

Pregnant Wistar rats ($n = 9$ –12) were dosed by gavage from GD 10 to GD 16 with BDE-47 (20 mg/kg bw per day) or 6-OH BDE-47 (5 mg/kg bw per day) and offspring assessed for various developmental landmarks. No effects were seen in terms of sex ratio, anogenital distance, age at vaginal opening or age at preputial separation (Buitenhuis et al., 2004).

Following maternal exposure of Long-Evans rats ($n = 7$ –9 litters per group) to BDE-99 from GD 10 to GD 18 (1 or 10 mg/kg bw per day subcutaneously), dose-dependent delays of vaginal opening and a 20% increase in ovary weights were detected at the highest dose. In male offspring, acceleration of preputial separation was observed, together with reduced weights of the epididymis at both dose levels (by about 12% at the higher dose), increased weight of the ventral prostate at the lower dose (by about 25%) and a dose-dependent increase in dorsal prostate weight, measuring 20% at the higher dose (Ceccatelli, 2004). The decreased epididymis weight seen in this study is likely due to gestational exposure, since it was not observed in the study by Stoker et al. (2004a) using postnatal exposure to a technical PBDE mixture (DE-71), which contains mainly pentabrominated congeners. Gene expressions of AR, ER α and ER β and of insulin-like growth factor I (IGF-I) were studied in several reproductive organs. In the ventral prostate, marked decreases in AR mRNA were detected at both doses (less than 20% of control values), as well as dose-dependent reductions in ER α

mRNA (to zero level at the higher dose), ER β mRNA (to 5% of control values at the higher dose) and IGF-I mRNA (down to about 50%). In the dorsal prostate, IGF-I mRNA was unchanged, AR mRNA showed marked dose-dependent increases (3-fold at the higher dose), ER α mRNA was increased at the higher dose (about 2-fold) and ER β mRNA was reduced at both doses (by about 50%). These results demonstrate that different lobes of the prostate respond differentially to PBDE exposure. In the uterus, mRNA levels of the progesterone receptor (PR) were down-regulated in a dose-related manner (by about 50% at the higher dose). ER α was not altered, but ER β mRNA was up-regulated at the low dose (2-fold) and reduced at the high dose (down to 40% of control level). After injection of estradiol in gonadectomized rats, induction of IGF-I mRNA was reduced in the ventral prostate of low-dose rats (by about 60%) and dose-dependently elevated in the uterus (3- to 4-fold at the higher dose), thus demonstrating exposure-related influences on regulation of this gene (Ceccatelli, 2004). In the uterus, the IGF-I receptor is assumed to mediate actions of estradiol (Richards et al., 1996). Estradiol did not change ER α mRNA in uteri of exposed females, but ER β mRNA was strongly induced at the higher dose, while it was nearly zero in controls and in the lower PBDE dose group (Ceccatelli, 2004). In addition, AR mRNA was decreased in uteri of low-dose females (down to about 15% of control levels). There were also PBDE-related effects on gene expression in the brain (see section 2.2.2).

Using the same perinatal exposure protocol with BDE-99, marked decreases in circulating estradiol and testosterone were observed in weanling male offspring, which became more pronounced in adulthood (estradiol down to 20% of control values, testosterone approximately 40% of controls; $n = 8$ per group). Anogenital distance was marginally decreased in male offspring at the higher exposure level. Vaginal opening was delayed at 10 mg/kg bw per day in female rats, while the lower dose resulted in a slight acceleration of preputial separation in males ($n = 22$ – 25 litters per group). These findings were seen together with elevated sweet preference (by about 35% at the higher dose), which is a sexually dimorphic behaviour, thus indicating behavioural feminization in male rats ($n = 9$ – 12 per group). In addition, dose-dependent reductions in serum concentrations of the steroid hormone 1,25-dihydroxyvitamin D $_3$ (by about 50% at the higher dose) were detected in female offspring at weaning ($n = 8$ per group). Male offspring exhibited PBDE-induced alterations in conditioned taste aversion (40% change at higher dose; $n = 10$ – 11 per group) using 1,25-dihydroxyvitamin D $_3$ as the aversive stimulus (Lilienthal et al., 2004).

(iii) Summary of steroid effects

With the exception of one study on corticosterone (Fowles et al., 1994), all *in vivo* data so far have been obtained in rats. From the data available, early developmental exposure seems to be more effective than exposure in pubertal animals (see Tables 88 and 89 in section 10 below). Influences on pubertal onset and weights of reproductive organs have been observed at lower exposure levels following maternal treatment (Ceccatelli, 2004). However, these effects were found using a single congener (BDE-99), whereas the other study used the

technical mixture DE-71 (Stoker et al., 2004a). Since DE-71 contains mainly pentaBDE, influences by exposure period seem to be more likely. These differences may also explain the accelerated pubertal onset (preputial separation) in male offspring detected by Ceccatelli (2004) and Lilienthal et al. (2004), in contrast to delayed pubertal onset described by Stoker et al. (2004a). Marked reduction in circulating estradiol was found after gestational exposure to BDE-99 (Lilienthal et al., 2004), and this treatment also resulted in marked decreases in AR mRNA in the ventral prostate of exposed offspring (Ceccatelli, 2004). Anti-androgenic effects on DHT-induced AR activation and inhibition of AR agonist binding in vitro have been described for BDE-47 and BDE-100, in particular (Stoker et al., 2004b). Estrogenic activities have been reported for hydroxylated PBDE metabolites in vitro (Meerts et al., 2001).

(c) Immunotoxicity

Following exposure to high dietary doses of DecaBDE for 103 weeks, an increased frequency of splenic lesions was observed (NTP, 1986). The lesions were splenic fibrosis (males, 2240 mg/kg bw per day) and splenic haematopoiesis (females, 1200 and 2550 mg/kg bw per day). Lymphoid hyperplasia was also increased in high-dose male rats (2240 mg/kg bw per day).

The effects of a PentaBDE mixture (DE-71) on sheep erythrocyte plaque-forming cell (PFC) response and natural killer (NK) cell activity were studied in female C57BL/6J mice ($n = 6$) upon gavage dosing (Fowles et al., 1994). Single doses of PentaBDE (0, 0.8, 4, 20 or 500 mg/kg bw) did not affect the PFC response in mice. However, repeated daily dosing by gavage (14 days) significantly reduced the PFC response at doses of 18, 36 and 72 mg/kg bw per day and also decreased thymus weight at 72 mg/kg bw per day. NK cell activity, studied only after repeated dosing, was not altered by exposure to up to the highest tested dose of the PentaBDE mixture.

The PentaBDE mixture Bromkal 70-5-DE was administered to C57BL mice ($n = 8$; control $n = 12$) and Sprague-Dawley rats ($n = 6$; control $n = 10$) (in both species, female animals of Charles River strains) (0, 18 or 36 mg/kg bw per day), and the PBDE congener BDE-47 was administered only to mice (18 mg/kg bw per day) by daily gavage doses for 14 days (Thuvander & Darnerud, 1999). Twenty-four hours after the last dose, animals were killed, organs excised and lymphocytes obtained from thymus and spleen. Subsequently, analysis was conducted for spleen and thymus weights, splenic and thymic lymphocyte subset numbers and in vitro immunoglobulin G (IgG) production in pokeweed mitogen-stimulated splenocytes. Certain effects of the PBDE exposure were seen in exposed mice but not in rats. Mouse splenocyte numbers (total numbers, as well as CD4+, CD8+ and CD45R+ thymic lymphocyte subsets) were markedly decreased after exposure to BDE-47. Also, a reduced in vitro production of IgG antibodies from pokeweed-stimulated splenocyte cultures was observed in mice after exposure to Bromkal 70-5-DE at 36 mg/kg bw per day.

(d) *Neurotoxicity*

(i) *In vitro*

Mixtures

The administration of the PBDE mixture DE-71 (3–50 µg/ml medium) to primary cultures of Long-Evans rat cerebellar granule cells resulted in a stimulation of the release of [³H]arachidonic acid (ARA) by a phospholipase A₂ (PLA₂) dependent mechanism (Kodavanti & Derr-Yellin, 2002). The release was time-dependent and could be blocked by the PLA₂ inhibitor methyl arachidonylfluorophosphonate. Removal of external calcium caused a significant, but modest, reduction of the PBDE-stimulated ARA release. In contrast to the pentaBDE mixture DE-71, the octaBDE mixture DE-79 was not effective in this model. The potency of DE-71 to stimulate ARA release was similar to the potency of the PCB mixtures Aroclor 1016 and Aroclor 1254 when expressed in molar terms (>10 µmol/l). Since ARA and PLA₂ have been implicated in synaptic plasticity, the authors suggested that these findings indicate that alteration of neuronal ARA may be involved with the PBDE-induced effects on learning and memory in animals (Kodavanti & Derr-Yellin, 2002).

The pentaBDE mixture DE-71 inhibited the uptake of dopamine into synaptic vesicle preparations from adult rat brains, with an IC₅₀ value of 8 µmol/l. Only minor effects of pentaBDE were detected for uptake of dopamine and glutamate into synaptosomes (Mariussen & Fonnum, 2003). The observed effect was described by the authors as possibly related to changes in membrane potential. OctaBDE (DE-79) and decaBDE (DE-83R) were not effective in this system, in contrast to other brominated flame retardants, such as hexabromocyclododecane and tetrabromobisphenol-A.

Single congeners and metabolites

The cellular accumulation of BDE-47 was examined in primary cultures of neocortical cells, neurons and glia cells prepared from newborn Long-Evans rats. Incubation of cultures with BDE-47 at 0.01–3.0 µmol/l in serum-free medium for 60 min led to a concentration-dependent uptake in cells. There was a 100-fold accumulation in cells compared with medium; thus, 1 µmol/l in the medium resulted in a cellular concentration of about 100 µmol/l. The proportion of BDE-47 accumulated in cells was on average 15%, with 55% remaining in the medium and 30% associated with the plastic dish. Saturation was observed after 120 min. Cellular accumulation of BDE-47 decreased markedly when serum proteins were added to the incubation medium. These results show that the use of media concentrations underestimates cellular concentrations by about 2 orders of magnitude (Mundy et al., 2004).

BDE-99 caused an increase in apoptotic cell death in an astroglia cell line (human 132-1N1 astrocytoma cells) at concentrations of 50 µmol/l or higher (24-h exposure) and an inhibition of the mitochondrial reduction capacity (MTT assay), with an IC₅₀ of 26.5 µmol/l. Cytotoxicity, as assessed by trypan blue dye exclusion

and cellular LDH release, was not affected by BDE-99 concentrations up to 100 $\mu\text{mol/l}$. Translocation of three protein kinase C (PKC) isozymes was not diminished by preincubation with a PKC inhibitor (GF109203X) or down-regulation of PKC by the phorbol ester, phorbol myristate acetate. The results indicate that PKC activation is not critically involved in cytotoxic effects of BDE-99 in these cells. In addition, no effects on cytotoxicity were observed after application of the calcium chelator BAPTA-AM, the tyrosine kinase inhibitor genistein and a mitogen-activated protein kinase (MEK) inhibitor (PD98059). However, cytotoxicity was enhanced by a phosphatidylinositol 3 kinase inhibitor (LY290042) that is involved in cellular apoptotic processes (Madina et al., 2004).

(ii) *In vivo*

Mixtures: Rat

Following perinatal exposure of rat dams (strain not given) to the pentaBDE mixture DE-71 (0, 5, 30 or 100 mg/kg bw by gavage) from GD 6 to PND 21, impairments of cue-conditioned fear (up to a 5-fold change in the first minute) were observed in adult offspring (age not given). There were no observed effects on spatial learning, as assessed by the Morris water maze and context-conditioned fear (Taylor et al., 2003). The highest dose caused elevated baseline population spikes (nearly 50%; $n = 11$ per group) in the dentate gyrus of the hippocampus after high-frequency stimulation of the perforant path. Measurements of long-term potentiation (LTP), an electrophysiological model of synaptic plasticity, revealed impaired LTP at this dose level. These effects were thought to be related to the reduced serum T4 observed in offspring at PND 6 and PND 14 (Crofton et al., 2003; Gilbert et al., 2004).

Single congeners and metabolites: Mouse

Male NMRI mice were exposed by gavage to equimolar doses of BDE-47 (0, 0.7 or 10.5 mg/kg bw) or BDE-99 (0, 0.8 or 12 mg/kg bw) on PND 10. Animals were then tested for locomotor activity over a 60-min period at the ages of 2 and 4 months ($n = 8$ per group). Indices of spontaneous behaviour, as determined by locomotion, rearing and total activity, were significantly decreased by both PBDEs in a dose-related manner (down to 40% and 60% of control values at the higher doses of BDE-47 and BDE-99, respectively) during the first 20 min of the measuring period. During the last 20-min testing period, activity was usually increased compared with controls, mainly in the high-dose BDE-47 group and both dose groups of BDE-99. The ability of the mice in the same dose groups to habituate to a novel environment (habituation capability) appeared to become worse with age, as the indicated effects were more pronounced in mice at 4 months of age in comparison with 2 months. In addition, mice exposed to the higher dose level of BDE-99 exhibited signs of impaired reversal learning (learning and memory function) ($n = 16$ –18 per group) in a water maze when tested at 5 months of age (Eriksson et al., 2001).

In a subsequent study of similar design, three different time points (PND 3, 10 and 19) were used for the administration of a single gavage dose of BDE-99

(8 mg/kg bw or 14 μ mol/kg bw; $n = 10$ per group). The same pattern of impairment of motor behaviour as in the first study was seen in 4-month-old male NMRI mice treated on PND 3 or PND 10 (50% decrease in activity in the early testing phase and 8- to 10-fold increase in locomotion in the late phase of testing), while exposure on PND 19 was without effect. The application of 14 C-labelled BDE-99 revealed no differences between the three ages in terms of the amount of radioactivity in the brain (3.7–5.1% of administered dose) 24 h after treatment. Lower (1.3–2.8% of dose), but comparable, amounts of labelled BDE-99 were found in brains from mice of all groups 7 days after the administration, thus showing that the neurobehavioural effects are due not to differences in internal exposure in the brain, but to the time of exposure. This indicates a critical period for PBDE exposure to induce alterations in motor activity (Eriksson et al., 2002).

In a follow-up of this experiment, it was shown that the behavioural alterations induced by BDE-99 (0 or 8 mg/kg bw orally on PND 10; $n = 12$ per group) may be mediated by the cholinergic system. In control mice, nicotine (80 μ g/kg bw subcutaneously) increases motor activity about 3-fold in the early phase of the testing period, while in the mice exposed to BDE-99, the opposite effect (hypoactivity) was observed following nicotine dosing (Viberg et al., 2002). Subsequent investigations with BDE-99 (12 mg/kg bw by gavage on PND 10) demonstrated decreases by about 30% in densities of nicotinic cholinergic receptors in the hippocampus ($n = 10$ per group). One week prior to neurochemical measurements, these mice had been tested for locomotor behaviour. The known changes in spontaneous behaviour were detected at a BDE-99 dose of 12 mg/kg bw, whereas lower doses of 0.2 or 0.4 mg/kg bw were not effective (Viberg et al., 2004a). In addition, in a replicate experiment, but with a different strain of mice, it was shown that behavioural effects of BDE-99 can be detected in both sexes and two strains of mice (C57BL and NMRI). Exposure of C57BL mice ($n = 8$ per group per sex) to a single gavage dose of BDE-99 on PND 10 (0, 0.4, 0.8, 4.0, 8.0 or 16.0 mg/kg bw) resulted in the same pattern of dose-dependent influences on spontaneous behaviour when tested at 2, 5 and 8 months of age (Viberg et al., 2004b). At doses of ≥ 0.4 mg/kg bw, significant alterations in behaviour were noted in both sexes, which became more pronounced with age.

Dose-related effects on activity ($n = 10$ per group), learning and memory ($n = 19$ –24 per group) and nicotinic cholinergic receptors ($n = 10$ per group), similar to those described for BDE-99, were also found in male NMRI mice after exposure to BDE-153 at doses of 0, 0.45, 0.9 or 9.0 mg/kg bw by gavage on PND 10 (Viberg et al., 2003a). Significant alterations in spontaneous behaviour and decreased performance in the Morris water maze (spatial learning) were observed in mice from the middle and high dose groups.

Using the same experimental design, this group also examined effects of decaBDE (BDE-209) in neonatal NMRI mice. Three age points for dosing (PND 3, 10 and 19) were selected and compared, and three dose levels were used (PND 3 and 19: 0, 2.22 or 20.1 mg/kg bw; PND 10: 0, 1.34, 13.4 or 20.1 mg/kg bw by gavage; $n = 10$ per group). Tests of activity revealed the familiar pattern of effects as seen with BDE-99 and BDE-153 in NMRI mice treated with the highest dose on PND 3, with decreases by 40% in locomotion in the early phase and very marked

elevations in spontaneous behaviour during the late phase of testing. The alterations became more pronounced with aging from 2 to 4 and 6 months. Administration of BDE-209 on PND 10 and PND 19 was not effective at causing any behavioural alterations. The application of [^{14}C]decaBDE revealed that labelled decaBDE can be found throughout the body, including the brain, and that radioactivity increases in the brain from 24 h to 7 days after treatment when applied on PND 3 or 10. In contrast, only very low levels could be detected after administration on PND 19 (Viberg et al., 2003b).

These findings were extended in a subsequent study in which males of the same strain of mice were exposed by gavage to equimolar doses of BDE-183, BDE-203 or BDE-206 (21 $\mu\text{mol/kg}$ bw or 15.2, 16.8 or 18.5 mg/kg bw on PND 3 and PND 10, respectively). At 2 months of age, effects on locomotion were detected in mice exposed to BDE-203 at PND 3 or PND 10 and after exposure to BDE-206 on PND 10. In contrast, BDE-183 caused only minor effects. Alterations of spatial learning and memory (Morris water maze) were observed in mice at 3 months of age after exposure to both BDE-203 and BDE-206 on PND 10. Taken together, the octabrominated BDE-203 was described as being the most effective congener (Eriksson et al., 2004).

Maternal exposure of CD-1 Swiss mice to BDE-99 (0, 0.6, 6 or 30 mg/kg bw per day by gavage) from GD 6 to PND 21 caused a delay (approximately 2 days) in maturation of the screen climbing response as a measure of sensorimotor development in pups of the highest dose group around PND 14 ($n = 12\text{--}16$). Testing locomotor activity at different ages ($n = 6\text{--}8$ per group) revealed higher activity levels in mice from the low and middle dose groups at PND 34 and PND 60 (3- to 4-fold), while at PND 120, these animals showed reduced activity in comparison with controls (by 50–60%). The high dose did not differ from controls. Ultrasonic vocalizations and the homing test did not show exposure-related differences (Branchi et al., 2002).

When BDE-99 was administered by a non-stressful feeding method (dissolved in oil and given in drinking tubes) to CD-1 Swiss mice from PND 1 to PND 21 (0, 18 or 36 mg/kg bw per day), slight reductions in the activity of choline acetyltransferase were observed in the hippocampus, but not in striatum and cortex, in offspring on PND 26 (sex and response magnitude not given; Wiegand et al., 2003).

Single congeners and metabolites: Rat

Wistar rat dams were injected subcutaneously with BDE-99 at 0 or 30 mg/kg bw per day from GD 2 to GD 9 or from GD 11 to GD 19 and their offspring tested for locomotor activity around PND 70. Female, but not male, offspring exposed from GD 11 to GD 19 exhibited higher values in locomotion and rearing during the late phase of the measurement period (2- to 3-fold). No effects were found after exposure from GD 2 to GD 9 (Wiegand et al., 2003).

Following behavioural testing, the brains were examined for contents of proteins associated with the glutamate–nitric oxide–cGMP pathway, a major signal transduction system. Concentrations of calmodulin and guanylate cyclase were

raised by about 100% and 30%, respectively, in the cerebellum from rats exposed from GD 11 to GD 19. In the hippocampus, calmodulin was also increased by about 100%, while guanylate cyclase was unchanged. No alterations of these proteins were found in the cortex, and content of neural nitric oxide synthase (nNOS) was not changed in all three regions. Exposure from GD 2 to GD 9 led to significant changes in nNOS in the cerebellum (10% decrease) and in the hippocampus (about 15% increase), while no change was detected in the cortex. Calmodulin was reduced by about 20% in the hippocampus and the cortex, and guanylate cyclase was elevated by about 15% and 20% in the cerebellum and hippocampus, respectively. No influence of either exposure period was found on the contents of MAP kinase 2 (MAP-2). This protein was reduced by about 30% in the hippocampus, but not in the cerebellum and cortex, irrespective of early or late gestational exposure. Microdialysis studies showed that stimulation with the glutamate agonist *N*-methyl-D-aspartate (NMDA) resulted in an enhanced increase of extracellular cGMP, which was more pronounced after BDE-99 exposure from GD 2 to GD 9 (about 2-fold and 3-fold increase in peak levels after early and late exposure, respectively). These findings are supported by corresponding measurements *in vitro* and *ex vivo* (Wiegand et al., 2003).

Recently, findings of altered activity in mice were extended to male Sprague-Dawley rats exposed to a single BDE-99 dose of 0, 0.8, 8 or 16 mg/kg bw on PND 10. Adult rats exhibited dose-related effects on spontaneous behaviour similar to those seen in mice when tested at 2 months of age, with the effects being significant at doses of 8 and 16 mg/kg bw (Viberg, 2004; Viberg et al., 2004c).

Maternal exposure of Wistar rats to BDE-99 by gavage on GD 6 (0, 60 or 300 µg/kg bw; *n* = 10 per group) caused elevated basal locomotor activity in offspring in the higher dose group at weaning (about 25% increase) and in both dose groups at puberty (about 10% at both exposure levels). Similar changes were observed at PND 36 in rats after developmental treatment with propylthiouracil (0.05% in drinking-water from GD 7 to GD 21), which was used as a positive control for thyroid-mediated effects. However, by puberty, activity levels had normalized in this group compared with controls (Kuriyama et al., 2004a).

The same exposure protocol, but with a single dose of BDE-47 on GD 6 (0, 140 or 700 µg/kg bw by gavage; *n* = 17–22 dams per group), resulted in similar effects on different parameters of basal locomotor activity as in BDE-99-exposed rat offspring on PND 35 and PND 70. Effects on PND 70 were significant only in females, while on PND 35, males of the higher dose group were also affected. Increases of approximately 10–25% were found in this behaviour. In addition, activity in the open field and behaviour in the elevated plus maze were examined on PND 80 and PND 150, respectively. Both sexes of the high dose group exhibited raised activity in the open field (20% and 50% increase in females and males, respectively). Also, males of the high dose group spent more time than controls in open and closed arms of the elevated plus maze, suggesting activity-related differences. The number of entries in open arms of the maze was increased (by approximately 50%) in males at the high dose, but decreased (by about 25%) in females at the low dose (Kuriyama et al., 2004c).

A different protocol, using exposure of Long-Evans rats to BDE-99 (0, 1 or 10 mg/kg bw per day, subcutaneous injection from GD 10 to GD 18), resulted in elevated sweet preference in adult male offspring, indicating behavioural feminization (see above). This effect was found together with decreased circulating concentrations of sex steroids. In addition, male offspring exhibited dose-dependent elevations in conditioned taste aversion induced by 1,25-dihydroxyvitamin D₃ at 6–7 months of age. Decreased serum concentrations of this steroid hormone had been detected in female offspring at weaning (see above). The onset of catalepsy induced by haloperidol was more rapid in PBDE-exposed adult offspring at both dose levels (about 50% increase in latencies; $n = 10$ –11). No effects were seen in context- versus cue-conditioned fear ($n = 10$ per group per condition) when rats were tested on the test day after conditioning; however, exposed males exhibited significantly less activity after aversive stimulation on the conditioning day (about 50–60% decrease in comparison with controls), indicating enhanced reactivity (Lilienthal et al., 2004). Brain slices from littermates of these rats were examined for LTP *ex vivo* ($n = 6$ –8 per group). Recordings from cortical slices of immature rats demonstrated reduced LTP and paired pulse facilitation at the higher dose level (depression by 10–20%). In hippocampal slices, only effects on LTP were detected (decreased by about 20%). These effects persisted in aged rats (>1 year), despite the decline of internal exposure levels in adipose tissue to control values (Wiegand et al., 2003, 2004).

After exposure to the same dosing protocol, the sexual dimorphism in PR mRNA expression in the ventromedial hypothalamic nucleus (VMH) was abolished in female offspring by BDE-99 at both doses due to a decrease in PR mRNA. No changes were observed in the medial preoptic area (MPO), but there were exposure-related elevations of ER α mRNA in both brain regions. Also, mRNA of preproenkephalin, the precursor of the neuropeptide enkephalin, was found to be increased in the VMH in both sexes and decreased in the medial preoptic area in male offspring, but only at the lower dose (Lichtensteiger et al., 2003, 2004). Adult male offspring from the high dose group that were gonadectomized and treated with estradiol ($n = 6$ –9 per group) exhibited a 30% increase in PR mRNA in the VMH compared with ectomized and estradiol-treated controls and a decrease by approximately 25% at the low dose. In contrast, in females, a dose-related decrease to about 70% of control values was found at the high dose. In the MPO, there were decreases in both sexes and at both dose levels. In intact female offspring, there was a marked reduction (down to about 10% of control values) in mating behaviour in the high dose group.

(iii) Summary of neurotoxic effects

The majority of investigations examining neurotoxicity *in vivo* used exposure to single congeners in mice and rats. In mice, single exposure of neonates on 1 postnatal day was chosen in almost all experiments. Decreased locomotor activity in the early phase of measurement and impaired habituation of activity in the late phase were observed in these studies (see Table 90 in section 10). Progressively, more pronounced effects were detected with ageing of mice, with the early postnatal period of exposure being more sensitive (Eriksson et al., 2002; Viberg et

al., 2003b). BDE-99 and BDE-153 appear to be more potent than BDE-47 in altering these measures (Eriksson et al., 2001; Viberg et al., 2004a, 2004b). BDE-209 was also reported to induce neurobehavioural changes, but at higher doses compared with the lower brominated congeners (Viberg et al., 2003b). For BDE-99, a BMD has also been calculated based on results from Viberg et al. (2004b) (Sand et al., 2004; see Table 85 in section 10). Effects on locomotor activity were also seen in the other experiments with mice using perinatal exposure to BDE-99 (Branchi et al., 2002); exposed young mice tended to be hyperactive compared with controls between PND 34 and 60 but hypoactive by PND 120. In rats, exposure on 1 day postnatally resulted in the same effects on activity and habituation found in mice (Viberg et al., 2004c). Studies from one group reported altered activity levels after exposure to a very low dose of BDE-47 and BDE-99 on GD 6 (Kuriyama et al., 2004a, 2004c; see Table 91 in section 10). Perinatal exposure to the technical mixture DE-71 did not influence spatial learning and context-conditioned fear (Gilbert et al., 2004), but impaired cue-conditioned fear (Taylor et al., 2003). On the same behavioural task, altered reactivity to aversive stimulation was also found after gestational treatment with BDE-99 (Lilienthal et al., 2004). In this study, effects on haloperidol-induced catalepsy were also found, together with alterations in steroid-dependent behaviour. In addition, BDE-99 abolished the sexual dimorphism in PR mRNA expression in the VMH of female offspring and markedly reduced mating behaviour (Lichtensteiger et al., 2003, 2004). Perinatal exposure to DE-71 impaired LTP in the hippocampus (Gilbert et al., 2004), whereas BDE-99 caused decreases of LTP in the cortex after gestational treatment. This difference was also found in aged offspring when adipose tissue concentrations of BDE had declined to control values (Wiegand et al., 2003, 2004). LTP is the electrophysiological correlate of synaptic plasticity.

(e) Effects on development of reproductive organs

Post-weanling Wistar rats ($n = 15$) were treated by gavage to DE-71 (0, 3, 30 or 60 mg/kg bw per day) for either 20 (PND 22–41, females) or 31 consecutive days (PND 23–53, males) and then assessed for pubertal development and reproductive end-points. The commercial lot of DE-71 was reported to be composed of 58.1% pentaBDE and 24.6% tetraBDE. There was a significant delay (1.8 days) in vaginal opening in the high-dose females, while preputial separation was significantly delayed (1.7–2.1 days) in males from the two highest dose groups. Ventral prostate and seminal vesicle weights were also significantly decreased (15–19%) in males from the high dose group compared with controls (Stoker et al., 2004a). No significant differences were noted with respect to body weight gain in any treatment group.

Effects of the PBDE congener BDE-99 were presented in a short study in Wistar rats (Talsness et al., 2003). Pregnant rats (number not given) were given BDE-99 at 0, 60 or 300 $\mu\text{g/kg}$ bw by gavage as a single dose on GD 6. The female offspring were killed in estrus at about PND 90, and the ovaries were excised and studied using electron microscopy. At the 60 $\mu\text{g/kg}$ bw dose, destruction of luminal surfaces of the serosal epithelial cell was apparent, and organelles seemed to be in a process of dissolution. At 300 $\mu\text{g/kg}$ bw, the degenerative changes were more

pronounced, and the authors suggested a dissolution of the endoplasmic reticulum and tubular mitochondria. In sections from control animals, the cell structures seemed intact and of normal appearance. The number of animals in each group subjected to microscopy was not reported.

Pregnant Wistar rats were treated with a single oral dose of BDE-99 (0, 60 or 300 µg/kg bw; $n = 16-20$) on GD 6, with male offspring ($n = 12-20$) undergoing reproductive assessment between PND 140 and PND 160. There was a dose-related decrease in relative testis weight (approximately 10% at the highest dose group) and a minor decrease for both dose groups in relative epididymis weight. Spermatid, sperm number and daily sperm production were decreased by both PBDE doses, with maximum declines reaching approximately 34% of control values (Kuriyama et al., 2004a). No effects were observed with respect to serum LH or testosterone levels. While no effects were observed with respect to male sexual performance, only 39% and 21% of the 60 and 300 µg/kg bw dose groups, respectively, were able to achieve a second ejaculation during a 20-min mating period, compared with 53% of controls (statistically significant at the high dose).

Exposure of pregnant Wistar rats to daily oral doses (gavage) of either BDE-47 (20 mg/kg bw per day) or 6-OH BDE-47 (5 mg/kg bw per day) between GD 10 and GD 16 had no effect on offspring sex ratio, growth, timing of various developmental landmarks (vaginal opening, preputial separation) or estrous cycle length between PND 210 and PND 230 (Buitenhuis et al., 2004).

2.3 Observations in humans

2.3.1 Biomarkers of effect

In vitro immunotoxic response was studied in human lymphocytes after exposure to PBDE congeners at doses from 10^{-5} to 10^{-9} mol/l (Fernlof et al., 1997). No effects on pokeweed mitogen-stimulated DNA proliferation or IgG synthesis were found after exposure to BDE-47 or BDE-85.

2.3.2 Clinical observations

DecaBDE is the only PBDE for which limited human data are available. Skin sensitization potential of "decaBDE" (Dow Chemical, USA; containing 77.4% decaBDE, 21.8% nonaBDE and 0.8% octaBDE) was studied in 50 volunteers (Norris et al., 1975b). A 5% suspension of decaBDE in petroleum was applied to the skin 3 times per week for 3 weeks, followed by a challenge treatment 2 weeks after the last induction application. No skin sensitization responses were observed during the study. Another skin sensitization study was carried out in 80 male and 120 female volunteers, who were exposed to two batches of decaBDE (purity not stated) (described in IPCS, 1994). The volunteers were treated with nine induction patches at 2-day intervals, and the test substance was kept in contact with skin for 24 h. The induction regimen was followed by a period of 12 days without treatment, after which a new skin site was used for a 24-h challenge patch. Skin reactions were observed at 24 and 48 h after removal of the challenge patch. The study revealed no evidence of skin sensitization.

2.3.3 Epidemiological studies

Workers exposed to polybrominated biphenyls (PBBs) and PBDEs, including decaBDE, during manufacture were reported to have a higher than normal prevalence of primary hypothyroidism and a significant reduction of sensory and motor neuron conductance velocities, but no other neurological or dermatological changes (Bahn et al., 1980). It was not possible to conclude whether these changes were attributed to PBB or PBDE exposure; however, no decaBDE could be detected in serum of the exposed workers.

Four epidemiological studies have been conducted on workers of facilities where flame retardant polymers have been extruded (not retrievable, but reviewed in IPCS, 1994). The workers were potentially exposed to brominated flame retardants, including PBDEs, and in some cases also to polybrominated dibenzo-*p*-dioxins (PBDDs) and dibenzofurans (PBDFs). According to the International Programme on Chemical Safety (IPCS) review (IPCS, 1994), these studies did not find any adverse effects attributable to the exposure to these chemicals.

In an epidemiological study from Sweden, an association was reported between the risk of non-Hodgkin lymphoma (NHL) and adipose tissue levels of BDE-47 (Hardell et al., 1998). In the study, BDE-47 levels from 19 patients with NHL, 23 with malignant melanoma and 8 other cancer patients were compared with concentrations in 27 selected controls with no cancer diagnosis. The authors stated that a "nonsignificant elevated risk" was found when cases and controls were compared with the two highest concentration groups with the lowest group (<2.05 ng/g lipid), with an odds ratio of 1.9 (confidence interval [CI] 0.3–1.4) or 3.8 (CI 0.7–26), respectively.

In a follow-up study, Hardell and co-workers (2001) found higher BDE-47 levels in adipose/blood samples collected from Swedish patients diagnosed with NHL compared with matched controls. In samples from 80 patients, the mean BDE-47 level was 8.2 ng/g (0.1–134 ng/g), and in control samples (83 individuals), 2.4 ng/g (0.05–28 ng/g). The results from the patients were further grouped according to Epstein-Barr early antigen (EA) titres; patients with high BDE-47 levels and high EA titres had a higher odds ratio than samples showing lower EA titres. The odds ratios for these two groups were 21 (CI = 1.9–24) and 13, respectively.

In a study on Swedish and Latvian fish consumers, the levels of BDE-47 and several hormones were determined in plasma of 110 men with various consumption (0–32 meals per month) of Baltic Sea fish (Hagmar et al., 2001). The study showed a weak negative correlation between BDE-47 and TSH, after adjustment for age. However, BDE-47 could explain only approximately 10% of the variance in TSH ($P < 0.001$), and the authors concluded that such a significant correlation could result from pure chance.

3. ANALYTICAL METHODS

3.1 Commercial PBDE production

PBDE products are typically produced in three different degrees of bromination, mostly identified with an average bromine content of pentaBDE, octaBDE and decaBDE. These products contain diphenyl ethers with mainly 4–6, 6–10 and 10 bromine atoms, respectively. The number of PBDE congeners present in each of the commercial products is surprisingly small, i.e. 2 major and 10 minor components are present at levels exceeding 1% in the technical PentaBDE preparation Bromkal 70-5-DE (Sjödin, 2000). In commercial PCB mixtures, the number of PCB congeners is considerably larger. This smaller number of congeners is reflected in biological samples as well. The first report of the presence of PBDE congeners containing 4–6 bromines in fish from a Swedish river was given by Andersson & Blomkvist (1981).

3.2 Description of analytical methods

3.2.1 Introduction

The analytical procedure from collection of samples to the final identification and quantification of the analytes consists of a number of well defined steps. Each of these steps is equally important to the final results. Depending on the type of sample, the kind of sampling or sampling procedure that will be seen as the best way to receive relevant and representative data for the samples must be considered. Storage of samples collected is also an important aspect for saving samples over longer periods of time. The procedure used for preparation of the samples must be designed in a way that the analytes remain intact while the remaining sample matrix is removed and/or degraded. For instrumental analysis, the technique applied must be sensitive and specific enough to identify and quantify the analytes of interest. In the following, techniques and methods used in various laboratories are reviewed.

3.2.2 Congeners analysed

The total number of PBDE congeners is 209. For reasons of occurrence in samples and analytical capability, only a limited number of congeners have been measured in the last few years. This number ranges between three and nine congeners (given in **bold** in Table 8). Due to increasing analytical power and availability of standards, the number of congeners measured in experienced laboratories is in the mid-30s.

3.2.3 Screening tests

The knowledge on screening tests for PBDEs is quite limited. Behnisch et al. (2003) did development work in this area. They measured for up to 13 individual PBDE congener DR-CALUX-REP and Micro-EROD-REP values. The most sensitive component was found to be BDE-126.

Table 8. Thirty-two PBDE congeners measured in biological samples^a

Bromines per molecule	BDE congener number
MonoBDEs	1, 2, 3
DiBDEs	7, 10, 13, 15
TriBDEs	47 , 49, 66 , 75, 77
PentaBDEs	85, 99 , 100 , 116, 126
HexaBDEs	138, 153 , 154 , 155, 166
HeptaBDEs	181, 183
OctaBDEs	197, 203
NonaBDEs	207
DecaBDE	209

^a The numbers given in bold should be "standard" in laboratories involved.

3.2.4 Quantitative methods

Due to the analytical method applied for the detection of PBDEs, there are important restrictions in the procedure. The application of the isotope dilution method by using ¹³C-labelled standards is strongly recommended. The electron capture detection (ECD) and electron capture negative ionization–low-resolution mass spectrometry (ECNI-LRMS) techniques measure only halogens or bromine-containing substances. In Table 9, the advantages and disadvantages of different detection techniques for PBDEs are given. However, the distinction between advantages and disadvantages of a method is dependent on the particular application and on personal preferences.

An important part of the analytical procedure is the determination of the adequate detection limit. Table 10 gives an overview of typical sample amounts and resulting detection limits. The detection limits are estimations and relevant for measurements performed by use of modern high-resolution mass spectrometric (HRMS) instrumentation.

(a) Extraction, cleanup methods

Several methods for extraction of biological samples have been proposed in the literature. For extraction of solid material, the Soxhlet procedure is used in many laboratories. This method is, however, a time-consuming technique that, in addition, requires large quantities of organic solvent. Other techniques include supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) and solid-phase extraction (SPE). A schematic representation of analytical methods used for extraction and cleanup of solid and lipid biological samples is given in Figure 3.

Table 9. Advantages and disadvantages of different detection techniques for PBDEs

Detection	Advantages	Disadvantages
ECD	Low-cost analysis, maintenance cost low, relatively easy to use	Fair sensitivity for PBDEs, instability of linear range, poor selectivity, no isotope dilution method possible
EI-LRMS	Facilitates the use of ^{13}C -labelled standards, good selectivity	Relatively low sensitivity
ECNI-LRMS	Good sensitivity, good selectivity for brominated compounds	Interference with other brominated components possible, frequent source maintenance required, no isotope dilution method possible
EI-HRMS	Good sensitivity, very good selectivity, use of ^{13}C -labelled standards, "gold standard" in PBDE analysis	Purchase cost, maintenance cost, needs highly trained personnel

ECD, electron capture detection; ECNI, electron capture negative ionization; EI, electron impact; HRMS, high-resolution mass spectrometry; LRMS, low-resolution mass spectrometry

Table 10. Required sample amounts and typical detection limits

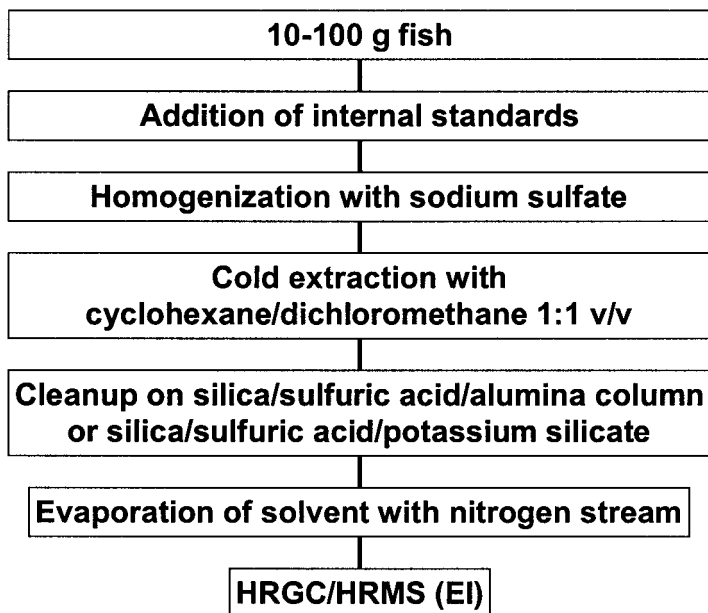
Sample type	Typical sample amount	Limit of detection (LOD) ^a lipid based (ng/g)
Human milk, 3% lipid	10–25 ml	0.005
Cows' milk, 3% lipid	10–100 ml	0.005
Human blood, 0.3% lipid	10–25 ml	0.05
Human serum, 0.5% lipid	5–15 ml	0.05
Fish, 1–10% lipid	10–200 g	0.01
Meat, 2–20% lipid	5–100 g	0.01

^a LOD for tetra- and pentaBDE congeners.

An overview of various typical treatments of biological samples is presented in Table 11 for food and in Table 12 for human samples. The tables present information on pretreatment of samples, extraction type, cleanup procedure, type of detection and typical recovery rates.

As can be seen from both tables, there is a general difference in pretreatment of liquid and solid samples. Solid samples are normally mixed with sodium sulfate and then eluted by an adequate solvent, whereas liquid samples are extracted by a liquid–liquid extraction procedure.

Figure 3. Example of analytical treatment of fish samples for the analysis of PBDEs



Adapted from Pöpke et al. (2004)

(b) Instrumental analysis

Brominated substances are often analysed under chemical ionization conditions, monitoring the negative ions formed by electron capture reactions (ECNI). The predominant ions formed from organobromine substances under such conditions are the bromine isotopes m/z 79 and 81. This technique is more sensitive and less costly than other alternatives, such as HRMS. However, the latter technique has a higher selectivity than MS (ECNI) detection of bromine ions, since the accurate mass of the molecular ion or fragment ion is recorded. The higher specificity of HRMS to reduce the risk of misinterpretation of interfering substances resulted in the selection of this method as the “gold standard” in determination of PBDEs.

The response of PBDE congeners studied — especially under ECNI — decreases with increasing numbers of bromine atoms in the molecule. Hence, the implementation of a congener-specific analysis using authentic reference standards is of importance. In the case of applying HRMS, this issue is easily resolved by using the ^{13}C -labelled internal standards. The use of this type of internal standard offers the exact observation of the whole analytical procedure inclusively and the knowledge of the recoveries of the surrogates used.

Table 11. PBDE analysis (extraction/cleanup/detection) for biota samples (mussels, fish, marine mammals, birds, eggs, meat)

Sample	Pre-treatment	Extraction	Cleanup	Detection	Recovery (%)	References
Fish	Mixing with Na ₂ SO ₄	Soxhlet (16 h), DCM/Hex = 1:1	(1) Florisil, elution with Hex/DE = 94:6 (2) Treatment with conc. H ₂ SO ₄	AED	>80	Johnson & Olson (2001)
Fish, marine mammals	Mixing with Na ₂ SO ₄	Soxhlet (6 h), Hex/Acet = 3:1	(1) GPC for lipid removal (2) Silica, elution with Hex (3) Treatment with concentrated H ₂ SO ₄	ECNI-MS	not reported	de Boer et al. (2001)
Fish	Mixing with Na ₂ SO ₄	Soxhlet (24 h), Hex/Acet = 1:1	(1) GPC for lipid removal (2) Silica, elution with DCM	EI or ECNI-MS	70–120	Dodder et al. (2002)
Fish	Mixing with Na ₂ SO ₄	Column extraction with 300 ml DCM	(1) GPC on Bio-beads SX3, eluent DCM/Hex (2) Silica, elution with DCM	EI-HRMS	65–120	Alaee et al. (2001)
Salmon	Mixing with Na ₂ SO ₄	SLE with Hex/Acet and Hex/DE	(1) GPC for lipid removal (2) KOH/EtOH partitioning neutral compounds (3) Silica, elution with Hex (4) Treatment with concentrated H ₂ SO ₄	ECNI-MS	80–90	Asplund et al. (1999)
Fish, fish feed	Mixing with Na ₂ SO ₄	Soxhlet (2 h), Hex/Acet = 3:1	Acidified silica, elution with Hex	EI-MS, ECNI-MS	>80	Jacobs et al. (2002)
Fish oil	Dilution in Hex	–	Acidified silica, elution with Hex	ECNI-MS	>80	Jacobs et al. (2002)
Seal, herring	Mixing with Na ₂ SO ₄	Column extraction with 300 ml Hex/Acet = 7:3 and 300 ml Hex/DE = 9:1	(1) GPC on PL-gel, eluent Hex/DCM = 1:1 (2) Florisil, elution with Hex/DCM = 1:1	EI-MS	50–115	Haglund et al. (1997)
Fish, bird eggs	Homogenization with Ultra-Turrax	SLE, Hex/Acet (1:2.5) and Hex/DE (9:1)	(1) Treatment with conc. H ₂ SO ₄ (2) GPC, eluent DCM/Hex = 1:1 (3) Silica, elution with Hex and Hex/DE = 4:1	ECNI-MS	39–65	Jansson et al. (1991)

Table 11. (contd)

Sample	Pre-treatment	Extraction	Cleanup	Detection	Recovery (%)	References
Bird eggs	Mixing with Na ₂ SO ₄	ASE (3 × 15 min, with Hex/Acet = 1:1)	(1) GPC (2) Silica, elution with Hex/Tol = 6:4	EI-LRMS	not reported	Herzke et al. (2001)
Bird eggs	Mixing with Na ₂ SO ₄	Soxhlet with D/H = 1:1	(1) GPC (2) Florisil, elution with Hex	EI-HRMS	not reported	Norstrom et al. (2002)
Fish, meat, vegetables	Freezing-drying	(1) Saponification with KOH/EtOH (2 h) (2) LLE with Hex	(1) Multicolumn with AgNO ₃ /silica – acid silica – silica – basic silica, elution with DCM/Hex = 5:95 (2) Active carbon, elution with DCM/Hex = 1:3	EI-HRMS	>80	Ohta et al. (2002)
Fish	Mixing with Na ₂ SO ₄	Cold extraction with DCM and CHex	Multicolumn with acid silica – silica – basic silica, alumina	EI-HRMS	>60	Päpke et al. (2004)
Milk	Adding of K-oxalate and EtOH	Extraction with DE and ether	Multicolumn with acid silica – silica – basic silica, alumina	EI-HRMS	>60	Päpke et al. (2004)
Fish	Mixing with Na ₂ SO ₄	Soxhlet with D/H = 1:1	(1) GPC (2) Florisil, silica, alumina	EI-HRMS	not reported	Hites et al. (2004)

Solvents: Acet, acetonitrile; CHex, cyclohexane; DCM, dichloromethane; DE, diethyl ether; EtOH, ethanol; Hex, hexane; Tol, toluene

Methods: AED, atomic emission detection; ASE, accelerated solvent extraction; D/H, deuterium/hydrogen ratio; ECNI, electron capture negative ionization; EI, electron impact; GPC, gel permeation chromatography; HRMS, high-resolution mass spectrometry; LLE, liquid–liquid extraction; LRMS, low-resolution mass spectrometry; MS, mass spectrometry; SLE, supported liquid extraction

Table 12. PBDE analysis (extraction/cleanup/detection) of human tissues and fluids (adipose tissue, serum, milk)

Sample	Sample pretreatment	Extraction	Cleanup	Detection	Recovery (%)	References
Adipose tissue	Drying with Na ₂ SO ₄	Soxhlet (2 h), Hex/DCM/Acet = 3:1:1	(1) Acid silica (2) Acid silica/alumina, elution Hex	EI-MS	81–102	Covaci et al. (2002a, 2002b)
Adipose tissue	Drying with Na ₂ SO ₄ , mixing with alumina	SFE with CO ₂ , 40 °C, 30.4 MPa, trapping on PX21/C ₁₈ , elution with Hex/DCM	–	EI-MS, TOF-MS	not reported	van Bavel et al. (1999)
Adipose tissue/liver	Homogenization	Ultra Turrax extraction with i-PrOH/Hex = 2:3 and Hex	(1) Lipide x 5000 partitioning (after mixing with i-PrOH, formic acid) (2) Column elution with MeOH in H ₂ O and Acet (3) Alumina and silica columns, elution with Hex (4) GPC on Bio-beads SX-3	EI-HRMS	57–84	Meironyté Guvenius et al. (2001)
Adipose tissue	Homogenization	Soxhlet (24 h), Tol	(1) Silica gel (2) Activated carbon (Carbopack C) (3) Activated alumina	EI-HRMS	42–104	Strandman et al. (1999)
Human breast adipose tissue	Homogenization	Hex/DCM	(1) GPC (2) Florisil, elution with Hex	ECNI-MS	not reported	She et al. (2000); Petreas et al. (2002)
Serum	HCl and i-PrOH addition	Hex/MTBE = 1:1	(1) Washing with KCl solution (2) KOH/EtOH partitioning (3) Treatment with concentrated H ₂ SO ₄ (4) Acid silica, elution with Hex	ECNI-MS	69–95	Sjödin et al. (1999)

Table 12. (contd)

Sample	Sample pretreatment	Extraction	Cleanup	Detection	Recovery (%)	References
Serum	(1) Formic acid/ i-PrOH (4:1) (2) Ultrasonication (3) Dilution with H ₂ O	SPE on Isololute ENV+ (200 mg, 6 ml)	(1) Lipid decomposition with conc. H ₂ SO ₄ (2) Wash with H ₂ O, acetate buffer and H ₂ O/MeOH (3) Elution with DCM/MeOH = 1:1	ECNI-MS	56–111	Thomsen et al. (2001)
Milk	Homogenization, mixing with formic acid and Lipide x 5000	(1) Wash with MeOH/H ₂ O (2) Elute with Acet	Alumina and silica columns, elution with Hex GPC on Bio-beads SX-3, elution with Hex/DCM	EI-HRMS	57–84	Meironyté Guvenius et al. (2001)
Milk	(1) Formic acid/ i-PrOH (4:1) (2) Ultrasonication (3) Dilution with H ₂ O	SPE on Osais HLB (500 mg, 6 ml)	(1) Lipid decomposition with conc. H ₂ SO ₄ (2) Wash with H ₂ O, acetate buffer and H ₂ O/MeOH (3) Elution with DCM/MeOH = 1:1	ECNI-MS	49–83	Thomsen et al. (2003)
Milk	Saponification with ethanolic KOH	LLE with Hex	(1) Multilayer column acid silica – silica – base silica, elution with Hex	EI-HRMS	>80	Ohta et al. (2002)
Milk	–	LLE with Hex/Acet	(1) Conc. H ₂ SO ₄ (2) GPC (3) Florisil, elution with H	EI-HRMS	85–10	Ryan & Patry (2000)
Milk	Adding of K-oxalate and EtOH	Extraction with DE and ether	Multicolumn with acid silica – silica – basic silica, alumina	EI-HRMS	>60	Päpke et al. (2001)
Milk	Adding of K-oxalate and EtOH	Extraction with DE and ether	GPC, multicolumn with acid silica – silica	EI-HRMS	not reported	Fürst (2001)

Table 12. (contd)

Sample	Sample pretreatment	Extraction	Cleanup	Detection	Recovery (%)	References
Whole blood	Adding of EtOH and water	LLE with Hex/ISOP	Multicolumn with acid silica – silica – basic silica, alumina	EI-HRMS	>60	Päpke et al. (2004)

Solvents: Acet, acetonitrile; DCM, dichloromethane; DE, diethyl ether; EtOH, ethanol; Hex, hexane; i-PrOH, 2-propanol; ISOP, isopropanol; MeOH, methanol; MTBE = methyl *tertiary*-butyl ether; Tol, toluene

Methods: ECNI, electron capture negative ionization; EI, electron impact; GPC, gel permeation chromatography; HLB, hydrophilic–lipophilic balance; HRMS, high-resolution mass spectrometry; LLE, liquid–liquid extraction; MS, mass spectrometry; SFE, supercritical fluid extraction; SPE, solid-phase extraction; TOF, time of flight

Following EPA method 1614 (US EPA, 2003), recoveries of ^{13}C standards of PBDEs should be expected within the ranges shown in Table 13.

Table 13. Recoveries of internal standards

PBDE group	BDE congener number	Recovery (%)
TriBDEs	28	50–150
TetraBDEs	47	50–150
PentaBDEs	99, 100	50–150
HexaBDEs	153, 154	50–150
HeptaBDEs	183	50–150
DecaBDE	209	25–200

(c) Selection of GC columns

The physical and chemical properties of BDE-209 put great demands on the analytical method, including sampling, extraction and cleanup, as well as final chromatographic separation. The problems encountered during the analysis of high-molecular-mass BDE congeners are associated with thermal instability, rather than their high boiling points. The degradation of, particularly, BDE-209 is increased with temperatures, time spent at elevated temperatures and presence of catalytic sites. For best yield of the decabrominated congener, these parameters should be kept as low as possible. The GC separation of PBDEs is often performed on two separate columns, a 30- to 60-m-long column for analysis of the low-molecular-mass BDE congeners and a shorter column for the analysis of the high-molecular-mass BDE-209.

Björklund et al. (2003) demonstrated that columns with supposedly similar stationary phases may result in a large difference in the yield of PBDEs. Furthermore, losses of high-molecular-mass BDE congeners do occur in the GC column and are correlated with the column length and the stationary film thickness. The time, temperature and catalytic sites all contribute to reduce the yield of the high-molecular-mass congeners. To obtain a high yield of these components, especially for BDE-209, short inert columns with a thin stationary phase are preferred. In Table 14, possible columns for PBDE analysis are given.

Table 15 shows the increasing relative response for BDE-209, depending on column type and length.

(d) GC injection techniques

The injection of PBDEs into the GC system is a critical and important part of the chromatographic analysis. Thus, careful selection and optimization of the injection techniques have to be performed in order to reduce the discrimination of these compounds. Splitless is the most commonly used injection technique for GC

separation of PBDEs. However, both the septum-equipped temperature programmable injector, the programmable temperature vaporizing injector as well as on-column injectors have been successfully used. Large-volume injections using either programmable temperature vaporizing in solvent elimination mode or the loop-type interface have also been used (Tollbäck et al., 2003).

Table 14. Columns for possible use in PBDE analysis

Column	Length (m)	Inner diameter (mm)	Phase thickness (μm)	Comments
J & W DB-5	15	0.25	0.10	
J & W DB-5	15	0.25	0.25	
J & W DB-1 MS	5, 15, 30	0.25	0.10	
Agilent HP-1	15	0.25	0.10	
Agilent XLB	15, 30	0.25	0.10	Extremely low bleed
Varian factorFOUR™	5, 15, 30	0.25	0.10	Ultra low bleed

From Björklund et al. (2003)

Table 15. Relative response for BDE-209 vs BDE-99 and estimated LOD for BDE-209 on different columns

Column	Estimated LOD (30 m) (picograms injected)	Relative response, BDE-209 vs BDE-99		
		30 m	15 m	5 m
FactorFour	1.3	0.06	0.36	0.46
XLB	250	0	0.008	— ^a
DB-5MS	0.9	0.05	0.33	0.50

From Björklund et al. (2003)

LOD, limit of detection

^a Not investigated.

In Table 16, the yield for the high-boiling decaBDE using different injection techniques is presented.

Table 16. Yield of BDE-209 obtained using different injection techniques

Injection type	Peak area BDE-209 (instrument response)
Mean splitless	20 000
Optimized splitless	50 000
On-column	165 000
Direct injection	165 000

From Tollbäck et al. (2003)

(e) *Photolytic decomposition of PBDEs*

Various studies have shown that PBDEs (especially decaBDE) and other brominated organic compounds in solvents undergo rapid photolytic debromination in the presence of ultraviolet light under laboratory conditions (Sellström et al., 1998; Herrmann et al., 2003). Due to this fact, it is strongly recommended that all analytical treatments be undertaken in brown glass or in glassware covered with aluminium foil.

(f) *Analysis of method blanks*

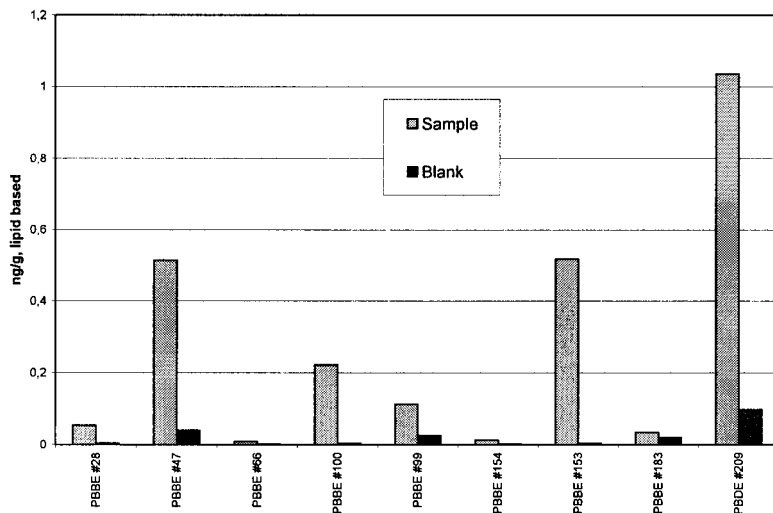
The analysis of blank samples has a special importance for the determination of PBDEs in various biological tissues. Due to marginal contamination of solvents and adsorbents used during PBDE analysis, all material has to be tested in advance. In parallel to method development, the reduction of a potential blank contamination needs special attention.

Along with each batch of samples, blank samples should be analysed as well. Values for samples should be reported only if the sample level is at least twice the blank level.

In Figure 4, a fish sample contaminated with low levels of PBDEs is compared with a blank sample. The figure indicates the relatively low influence of the blank on the sample values. It should be mentioned that blanks — depending on sample type, sample amount, applied cleanup procedure and finally laboratory equipment — may be quite different. Therefore, it is strongly recommended that a separate block of blanks be available at least for each type of sample.

In the case of an unsatisfying influence of blanks on samples, a significant reduction of the blank values could be achieved by the following procedures:

- Rotary evaporators should not be used in order to reduce the risk of contamination. Volume reduction can be reached by moderate heating or by a gentle stream of cleaned nitrogen/air.
- All glassware should be rinsed by analytical-grade solvents prior to use.
- Solvents and reagents should be tested before the laboratory procedures.
- Silica gel and sodium sulfate should be pre-washed.
- No plastic equipment should be used.
- Due to potential contamination of solvents and chemicals via air, containers/bottles should be closed as soon as possible after usage.
- The methodology should be miniaturized by reducing solvent volumes, if possible.

Figure 4. Comparison of PBDE levels in a blank and a fish sample

Reprinted from *Talanta*, Vol. 63, Pöpke, O., Fürst, P. & Herrmann, T., Determination of polybrominated diphenyl ethers (PBDEs) in biological tissues with special emphasis on QC/QA measures, pp. 1203–1211, 2004, with permission from Elsevier.

(g) Example of a GC/HRMS run

In Figure 5, a typical GC/HRMS run of a medium-contaminated fish sample is presented. In this figure, only one trace of each isomeric pattern is shown.

(h) Quality control and quality assurance

Quality control (QC) and quality assurance (QA) represent an important tool of the total analytical concept. In total, more than 30% of the whole analytical effort is covered by QC/QA measures.

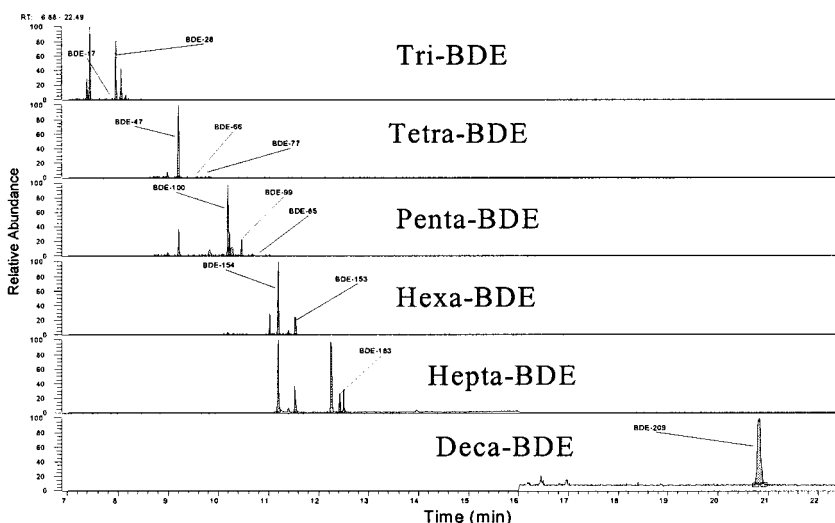
(i) Internal measures

Internal measures performed on a routine basis include:

- regular chemical and glassware checks (blanks), once a block of 4, 6 or 10
- regular checks of so-called instrument blanks (GC/MS)
- regular checks of QC samples (e.g. blood pools) (GC/MS)
- daily calibration verification tests
- regular GC performance tests (separation, retention time windows)

- identification based on definite abundance ratio and retention time criteria, with the use of internal and external standards
- quantification based on the isotope dilution method with the use of internal and external standards
- establishing a five- to seven-point calibration curve and regular repetition of single concentration points
- regular method performance checks by analysing control samples of known PBDE concentrations
- daily MS performance checks to control the resolution and sensitivity

Figure 5. Mass fragmentograms of PBDEs in a fish sample



Reprinted from *Talanta*, Vol. 63, Pöpke, O., Fürst, P. & Herrmann, T., Determination of polybrominated diphenyl ethers (PBDEs) in biological tissues with special emphasis on QC/QA measures, pp. 1203–1211, 2004, with permission from Elsevier.

(ii) External measures

External measures should include:

- regular participation in interlaboratory QC studies and proficiency tests covering those matrices that are regularly analysed
- exchange of samples and control measurements of standards and samples with other qualified laboratories

More detailed information with respect to QA/QC measures is given by Pöpke et al. (2004).

4. SAMPLING PROTOCOLS

There are no specific guidelines for sampling protocols for food samples to be analysed for their PBDE content. Therefore, basic rules for sampling for organic contaminants or pesticides should be followed. The primary requirement is a representative, homogeneous laboratory sample with no secondary contamination.

4.1 Personnel

A qualified, authorized person should perform sampling.

4.2 Representative sample

Samples must be representative of the lots or sublots from which they are taken. Compliance with maximum levels or action levels should be established on the basis of the concentrations determined in the laboratory sample.

- Lots are identifiable quantities of food delivered at one time and determined by the official to have common characteristics, such as origin, variety, type of packaging, packer, consigner or markings. In the case of fish, they should be of comparable size.
- Sublots are designated parts of a large lot to which the sampling method is applied. Each subplot must be physically separate and identifiable.
- An incremental sample is a quantity of material taken from a single place in a lot or subplot. As far as possible, incremental samples should be taken at various places distributed throughout the lot or subplot.
- An aggregate sample is the combined total of all the incremental samples taken from the lot or subplot. It should be at least 1 kg, unless impractical.
- A laboratory sample for the purposes of enforcement, trade and refereeing should be taken from the homogenized aggregate sample, unless this conflicts with Member States' regulations on sampling. The sample used to ensure enforcement should be large enough to allow at least duplicate analysis.

4.3 Packaging, transport and storage of aggregate and laboratory samples

Each aggregate and laboratory sample should be placed in a clean, inert container offering adequate protection from contamination, loss of analytes by adsorption to the internal wall of the container or damage in transit. Glassware offers good protection from contamination and can be cleaned easily. Polyethylene and polypropylene containers also provide protection against damage during transit. Containers made from halogenated substances (such as polyvinyl chloride) are not considered suitable for this purpose. Although PBDEs are

chemically stable, samples must be stored and transported in such a way that the food sample does not deteriorate. In particular, the fat content should not be changed (e.g. by microbiological or enzymatic processes), as the content of the compounds in food of animal origin is generally calculated on a fat basis.

4.4 Human milk samples

No guidelines are set for storage and transport of human milk samples. However, the guidelines recommended by WHO in the case of dioxins can be used. Specifically, WHO recommended the addition of potassium dichromate tablets to human milk samples during collection of portions and for transport in the third round of studies of exposure, if freezing of the portions cannot be guaranteed. This helps to avoid microbiological deterioration of the samples. If the portions can be frozen immediately after collection and the collected portions can be shipped in a frozen state, addition of potassium dichromate tablets is unnecessary.

4.5 Sealing and labelling

Each sample taken for official use should be sealed at the place of sampling and identified following Member States' regulations. A record must be kept of each sampling, permitting each lot to be identified unambiguously and giving the date and place of sampling, together with any additional information likely to be of assistance to the analyst.

4.6 Edible parts

For determination of PBDEs in food, only the edible parts are analysed. Vegetables should be washed with water to separate them from adhering soil.

5. EFFECTS OF PROCESSING

No data were available on the effects of processing on PBDE levels in foods. However, as PBDEs are chemically stable, lipophilic substances, little changes in PBDE content with processing would be expected.

Results of studies on the effect of processing on the dioxin content of foods are assumed to apply in the absence of studies on PBDEs. Smoking of meat or fish samples has been shown to increase the dioxin and furan content, depending on the smoking conditions (Körner & Hagenmaier, 1990; Mayer, 1998; Mayer & Jahr, 1998). Broiling of hamburger samples resulted in an approximately 50% decrease in the total toxic equivalents (wet weight) per hamburger, but the decrease appeared to be due solely to the decrease in wet weight associated with loss of water and loss of PCDDs/PCDFs with the fat (Schechter et al., 1996). In further studies, it was shown that the total toxic equivalents (PCDDs/PCDFs and PCBs) in hamburger, bacon and catfish decreased by an average of 50% as a result of broiling. However, the concentration remained the same in hamburger,

increased by 84% in bacon and decreased by 34% in catfish (Schechter et al., 1997). On average, the total measured concentration (pg/kg whole weight) increased by 14% in hamburger and by 29% in bacon and decreased by 33% in catfish (Schechter et al., 1998). In a study of the effect of pan-frying beef patties, the PCDD/PCDF concentration was reduced by 40–50% by cooking. Most of the reduction was accounted for by the amount in fat liberated from the patties during cooking (Petroske et al., 1997, 1998). No *de novo* synthesis of dioxins was observed after deep frying of scallops of pork covered with egg and crumbs either with or without salt and pepper. The frying temperature was high (180 °C).

Based on these results and the assumption that processing will have the same effect on PBDE levels as it does on dioxin levels, it is unlikely that PBDEs are formed or lost during usual cooking processes. Changes in PBDE content can be expected to be seen on a fresh weight basis owing to changes in fat and water content. As a whole, the PBDE content on a mass balance basis is expected to be constant in meat, fat and juices. However, since PBDEs concentrate in the fat portions of the food, such as the fatty tissue of the fish, removing the skin and visible fat and using cooking methods that allow fat to drip off can reduce levels in foods as consumed and the associated exposures.

6. LEVELS AND PATTERNS OF CONTAMINATION OF FOOD COMMODITIES

6.1 Surveillance data

Data were submitted by two countries, Canada and Germany, and published studies summarizing PBDE concentrations for various types of food samples were submitted by the Netherlands, the United Kingdom and the United States. Published data were also available for Finland, Japan, Spain and Sweden.

6.1.1 Canada

The PBDE data available for Canada were from two total diet studies (TDS) conducted in Whitehorse in 1998 (Health Canada, 2004a) and Vancouver in 2002 (Health Canada, 2004b), as well as a specific survey on fish and seafood conducted in 2002 in Vancouver, Toronto and Halifax (Health Canada, 2004c).

Each TDS consists of the purchase of foods at retail outlets, preparation and cooking of individual foods where applicable, combining some food samples into composites and laboratory analyses. The foods are typically purchased from three or four supermarkets and processed as for consumption 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). The processed foods are then mixed according to each category to make composites for analysis. For PBDEs, about 50 food composites consisting of foods known to contain these compounds, such as those of animal origin and relatively high fat content, along with a few other individual food samples, have been analysed. The data available from these two TDS are provided in Table 17.

Table 17. PBDE concentrations in food samples collected in Canada (TDS)

Code	Food	Concentration (ppt), wet based		Code	Food	Concentration (ppt), wet based	
		Whitehorse	Vancouver			Whitehorse	Vancouver
A01	Whole milk	0	3.39	C03	Liver paté	n/a	244.8
A02	2% milk	0	0.11	D01	Marine fish	101.23	1164.9
A03	1% milk	0	0.15	D02	Freshwater fish	374.88	1461.9
A04	Skim milk	0	0.01	D03	Canned fish	n/a	36.3
A05	Evaporated milk, canned	24.44	0.02	D04	Shellfish	101.64	58
A06	Cream	24.19	20.81	E01	Meat soups	21.6	5.5
A07	Ice cream	60.35	18.35	E03	Soup broth	n/a	0.2
A08	Yoghurt	5.66	8.47	E04	Soups, dehydrated	1.51	1.3
A09	Cheddar cheese	67.21	94.9	I01	Cooking fat	121.44	121.4
A10	Cottage cheese	1.61	0.5	I02	Margarine	6.59	4.4
A11	Processed cheese	62.35	81.4	I04	Mayonnaise	n/a	96.7
A12	Butter	55.5	264.5	J01	Chocolate	n/a	189.7
B01	Beef steak	150.97	46.2	L03	Baby dinner	n/a	45.2
B02	Beef roast	48.07	25.3	L04	Baby dinner	43.36	16
B03	Ground beef	227.55	120.8	L05	Formula, milk	14.48	0.3
B04	Fresh pork	143.53	40.8	L06	Formula, soya	n/a	1.1
B05	Pork cured	72.76	169.2	L08	Baby dinner, meat	152.68	57.6

Table 17. (contd)

Code	Food	Concentration (ppt), wet based		Code	Food	Concentration (ppt), wet based	
		Whitehorse	Vancouver			Whitehorse	Vancouver
B06	Veal	n/a	205.7	M02	Frozen entrée	n/a	47.1
B07	Lamb	50.84	39.6	M04	Frozen entrée	210.05	n/a
B08	Cold cuts	195.13	217.4	M06	Frozen dinner	13.35	n/a
B09	Luncheon meat, canned	99.13	248.4	N01	Pizza	71.05	274.9
B10	Organ meats	31.38	19.2	N02	French fries	104.22	35.6
B11	Wieners	1188.74	163.2	N03	Hamburger	21.76	58.5
C01	Eggs	333.43	79.6	N04	Fish burger	14.03	n/a
C02	Poultry	79.41	37.7				

From Health Canada (2004a, 2004b)

n/a, not applicable; ppt, part per trillion (equivalent to pg/g or ng/kg)

The survey on fish and seafood sampled farmed and wild-caught fish and seafood products sold at the retail level. Samples of farmed and wild-caught char, oysters, salmon, shrimp and tilapia were analysed for PBDEs. At the time of sampling, there was only limited availability of wild-caught oysters, salmon, shrimp and tilapia in retail outlets, and edible portions only (skin and bone removed) were analysed for the various targeted contaminants. The data are shown in Table 18.

6.1.2 Finland

Kiviranta et al. (2004) measured concentrations of PBDEs in 10 market basket surveys consisting of almost 4000 individual food samples representing 228 different food items collected between April 1997 and June 1999 from super-markets, farmers' markets and food producers and wholesalers in Finland. Five PBDE congeners were analysed (BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154). The concentrations of the sum of PBDEs ranged from 0.82 to 850 pg/g fresh weight, and the fish basket had the highest concentrations of PBDEs. Table 19 summarizes the detected concentrations.

6.1.3 Germany

Values for various food samples were submitted from Germany. Time of collection was between May 2001 and September 2003. All reported values are summarized in Tables 20 to 22. The concentrations are given for total PBDEs (sum of BDE-28, BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154) in ng/g lipid. Due to the relatively high limit of quantification (LOQ) of 1 ng/g lipid, only values for positive samples are reported in the summary tables.

Additional data on contamination levels in foods in Germany were available from several publications. Concentrations in 13 fish samples from the German market analysed by Pöpke et al. (2004) are reported in Table 23.

6.1.4 Japan

Ashizuka et al. (2004) analysed foods collected by a market basket food study. The authors measured the levels of PBDEs in various fish samples and the TDS food groups. The PBDE values found in the fish samples are relatively high, between 79 and 747 pg/g wet weight (see Figure 6). Of the 13 food group samples in the TDS, PBDEs were found in Groups IV (Oil), X (Fish), XI (Meat and eggs) and XII (Milk and milk products) at concentrations of 122, 1259, 65 and 8.6 pg/g, respectively. In the other groups, PBDEs were below the detection limit.

Ohta et al. (2002) measured the concentrations of PBDEs in fish, shellfish, meat and vegetables sold in two food markets in the city of Hirakata, Osaka prefecture. Table 24 summarizes the measured concentrations.

Table 18. Contaminants in retail fish and seafood products: Summary statistics for total PBDE levels

Species	Source	n	PBDE concentration (ppb)				
			Mean	Standard error of the mean	Standard deviation	Minimum	Maximum
Char	Farmed	5	1	0.4	1	0.4	2.7
	Wild	5	0.6	0.1	0.3	0.3	1.1
Oysters	Farmed	11	0.7	0.1	0.5	0.006	1.4
	Wild	4	0.4	0.08	0.2	0.3	0.6
Salmon	Farmed	19	2.2	0.3	1.4	0.4	5.5
	Wild	3	0.6	0.2	0.3	0.1	1.3
Shrimp	Farmed	13	0.2	0.06	0.2	<0.001	0.7
	Wild	4	0.1	0.05	0.09	0.009	0.2
Tilapia	Farmed	12	0.6	0.4	1.4	0.04	5
	Wild	3	0.1	0.09	0.2	0.01	0.3

From Health Canada (2004c)

ppb, part per billion (equivalent to µg/kg or ng/g)

Table 19. Concentrations of PBDEs and fat percentages of 10 market baskets and total diet basket

Food basket	Fat content (%)	Sum of PBDEs (pg/g fresh weight)	
		NQ = 0	NQ = LOQ
(1) Liquid milk products	2	0.82	2
(2) Solid milk products	21	34	40
(3) Fish	6.4	850	850
(4) Meat and eggs	11	13	15
(5) Fats	79	180	220
(6) Cereal products	2.1	15	15
(7) Potato products	0.34	1.3	1.4
(8) Vegetables	0.9	17	17
(9) Fruits and berries	1.3	3.8	4.2
(10) Beverages, spices, sweets		5.4	5.5
Total diet basket	3.5	43	43

From Kiviranta et al. (2004)

LOQ, limit of quantification; NQ, not quantified

Table 20. PBDEs in milk, milk products and eggs and poultry^a

Sample type	Total number of			% positive samples	Positive samples (values in ng/g lipid)	
	Samples	ND samples	Positive samples		Mean	Range
Farm collection milk/raw milk	96	91	5	5	2	1–4
Cheese	32	31	1	3	2	
Goat cheese	4	4	0	0		
Butter	38	34	4	11	1.3	1–2
Eggs	106	76	30	28	1.7	1–5
Chicken	38	24	14	37	3.6	1–12
Duck, goose	22	11	11	50	2	
Turkey	10	3	7	70	3.7	2–7

ND, not detected

^a LOQ = 1 ng/g.

Table 21. PBDEs in fish samples from the German market^a

Sample type	Total number of			% positive samples	Positive samples (values in ng/g lipid)		Lipid content (%)
	Samples	ND ^b samples	Positive samples		Mean	Range	
Butterfish	6	6	0	0	ND	–	19.7
Herring shark	4	0	4	100	43	5–131	0.4
Plaice	29	0	29	100	28.3	5–84	1.4
Trout	16	0	16	100	33.4	8–100	3.1
Renke	6	0	6	100	34.8	9–55	5.8
Pike	3	0	3	100	150	122–172	0.2
Roach	5	0	5	100	38.4	16–68	0.9
Bream	2	0	2	100	20.5	14–27	2.9
Tench	2	0	2	100	26.5	26–27	3.2
Carp	2	0	2	100	17.5	17–18	6.7
Eel	5	0	5	100	145	8–383	19.5
Perch	12	0	12	100	205	56–422	0.3
Cod	11	1	11	92	43	ND–109	0.3
Ocean perch	44	1	44	98	16.7	ND–56	2.7
Nile perch	2	1	1	50	1 ^a	ND–1	1.4
Victoria Lake perch	1	1	0	0	–	–	0.1

ND, not detected

^a LOQ = 1 ng/g.^b Only from positives.

Table 22. PBDEs in meat samples from the German market^a

Sample type	Total number of			% positive samples	Positive samples (values in ng/g lipid)		Lipid content (%)
	Samples	ND samples	Positive samples		Mean	Range	
Rabbit	14	6	8	57	11	1–59	3.2
Lamb	1	1	0	0	–		2.7
Sheep	2	2	0	0	–		–
Fallow deer (Damwild)	1	0	1	100	1		–
Calf	2	2	0	0	–		–
Cow (Kuh/Rind)	36	34	2	6	3	1–4	6.5 ^b
Bull	2	2	0	0	–		–
Horse	1	0	1	100	2		–
Pig	48	38	10	21	4.6	1–16	–
Game	2	0	2	100	2	2	1.1
Wild boar	2	0	2	100	1	1	2.3

ND, not detected.

^a LOQ = 1 ng/g.^b Only from $n = 4$.

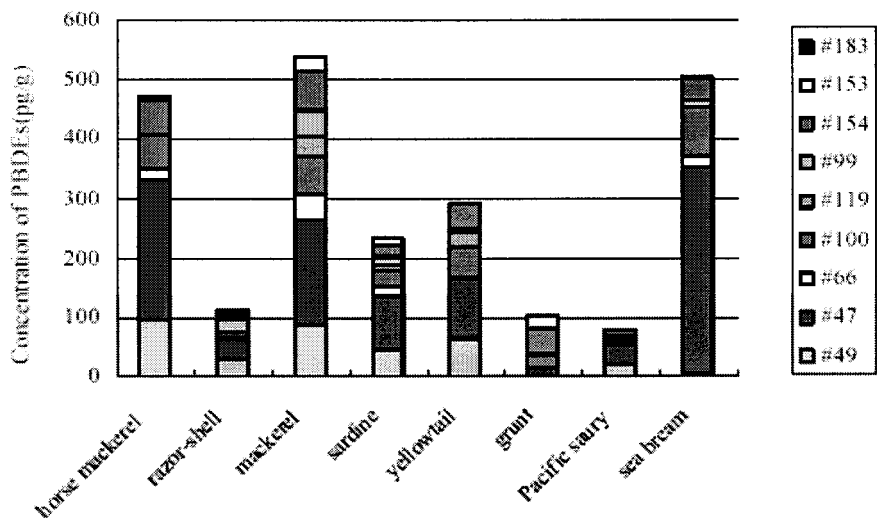
Table 23. Concentrations of PBDE congeners in fish samples from the German market

Sample	Lipid content (%)	Concentrations (ng/g lipid) of BDE congener number:											
		17	28	47	66	77	99	100	153	154	183	209	Total
Herring	20.7	0.13	0.45	7.43	0.56	0.03	2.61	2.15	0.12	0.40	ND	ND	13.9
Salmon	12.7	0.01	ND	1.01	0.06	ND	0.37	0.20	0.02	0.09	ND	ND	1.76
Plaice	2.3	0.15	0.28	3.57	0.32	ND	1.23	0.57	0.19	0.35	0.04	ND	6.65
Trout	9.6	0.05	0.27	5.61	0.33	ND	1.98	0.93	0.14	0.42	ND	ND	9.74
Ocean perch	3.6	0.02	1.57	34.7	0.40	0.01	1.09	4.77	0.95	3.76	0.04	0.36	47.6
Ocean perch	3.2	ND	0.39	11.2	0.18	ND	0.28	1.9	0.29	2.1	ND	0.04	16.4
Halibut	13.6	ND	ND	0.34	0.02	ND	0.05	ND	ND	0.02	ND	ND	0.42
Halibut	11.1	ND	0.19	3.0	0.13	ND	0.28	0.34	0.07	0.34	ND	ND	4.35
Halibut	11.2	0.05	0.47	6.0	0.35	ND	0.73	1.12	0.23	0.91	ND	ND	9.86
Coalfish	0.51	0.02	ND	1.54	0.02	ND	0.46	0.13	0.03	0.03	NA	0.42	2.66
Pike-perch	0.56	0.08	0.23	20.5	0.64	0.10	4.17	4.22	0.85	3.85	0.11	2.79	37.6
Victoria perch	1.8	ND	ND	0.67	0.01	ND	0.15	0.10	0.10	0.25	0.21	1.04	2.53
Catfish	3.1	ND	0.21	3.77	0.27	ND	1.45	0.73	0.16	0.38	0.12	1.18	8.27

From Pöpke et al. (2004)

ND, not detected; NA, not analysed

Figure 6. Levels and congener profiles of PBDEs in fish samples



Taken from Ashizuka, Y., Nakagawa, R., Hori, T., Tobiishi, K. & Iida, T. (2004) Levels of polybrominated diphenyl ethers and polybrominated dioxins in fish, total diet study food groups and Japanese meals. *Organohalogen Compd.*, **66**, 2553–2559.

Table 24. PBDE concentrations in food samples collected in the city of Hirakata, Japan

Food	PBDE (pg/g fresh weight)	Food	PBDE (pg/g fresh weight)
Young yellowtail	1650	Salmon	593
Young yellowtail	1580	Yellow tuna	18.5
Young yellowtail	1720	Yellow tuna	17.7
Young yellowtail	1620	Yellow tuna	26.2
Mackerel	1550	Short-necked clam	61.3
Mackerel	1400	Short-necked clam	43.5
Mackerel	1540	Short-necked clam	52.4
Mackerel	1280	Spinach	134
Yellowtail	1320	Potato	47.6
Yellowtail	985	Carrot	38.4
Salmon	1040	Pork	63.6
Salmon	897	Beef	16.2
Salmon	813	Chicken	6.25

From Ohta et al. (2002)

6.1.5 The Netherlands

The Netherlands Institute of Fisheries Research (RIVO) investigated background concentrations of PBDEs in 91 samples of food products consumed by the Dutch population. Table 25 lists the number of samples analysed per food group, and Table 26 summarizes the concentrations in these foods.

Table 25. Food products analysed by RIVO

Category	Product	Number of (pooled) samples
Dairy and dairy products	Blue-veined cheese	11
	Hard cheese	2
	Milk	6
	Whipping cream	2
	Coffee creamer	1
Eggs	Egg	9
Meat and poultry	Beef	7
	Pork	5
	Poultry	9
Animal fats	Fat of cattle	4
	Fat of pigs	3
Fish	Plaice	1
	Salmon	1
	Mackerel	2
	Herring	2
	Eel	8 ^a
	Mussel	2
	Shrimp	1
Vegetable oil	Oil	2
	Sunflower olive oil	6

From de Winter-Sorkina et al. (2003)

^a Smoked eel ($n = 1$), IJsselmeer eel ($n = 2$), hatched eel ($n = 3$) and imported eel ($n = 2$).

Table 26. Mean concentrations per food category, according to scenario 1 ($<LOD = 0.5 \times LOD$) and 2 ($<LOD = 0$)

Category	$n > LOD$	Mean concentration (ng/g)	
		Scenario 1	Scenario 2
Cheese	2	0.3	0.04
Beef	4	0.7	0.08
Pork	2	2.1	0.08
Poultry	5	0.3	0.04
Herring	2	12.9	2.14
Salmon	1	3.4	0.57

From de Winter-Sorkina et al. (2003)

6.1.6 *Spain*

Food samples — from local markets, big supermarkets and grocery stores from seven cities (Barcelona, Tarragona, Lleida, Girona, L'Hospitalet de Llobregat, Badalona and Terrassa) of Catalonia — were collected between June and August 2000. For collection of samples, two groups were made up. The first group included meat of beef (steak, hamburger), pork (loin, sausage), chicken (breast) and lamb (steak); fish (hake, sardine) and shellfish (mussel); vegetables and tubers (lettuce, tomato, potato, green beans, cauliflower); fresh fruits (apple, orange, pear); and eggs. The second group included cow milk (whole, semi-skimmed) and dairy products (yoghurt, cheese); cereals (bread, pasta, rice); pulses (lentils, beans); fats (margarine) and oils (olive, sunflower); tinned fish (tuna, sardine); and meat products (ham, hot dogs, salami). Two composite samples were analysed for each food item in group 1. Each composite was made up of 10 individual samples, which were collected in five different places. For foods in group 2, only one composite sample was analysed for each food item. This composite was made up of eight individual samples of similar weights, which were collected in four different places of the same city. The sums of the tetra- to octabrominated congeners were determined for each sample. In total, 54 samples were analysed. The detection limits varied from 5 to 40 ng/kg dry weight, depending on the specific food and the respective congeners. Tables 27 and 28 summarize the measured concentrations. The highest concentrations of total PBDEs were found in oils and fats, followed by fish and shellfish, meats and meat products and eggs (Bocio et al., 2003).

6.1.7 *United Kingdom*

A survey was conducted in late 2001 to determine the concentrations of PBDEs in brown trout and eels from locations upstream and downstream from a site known to have manufactured both penta- and octaBDE until the late 1990s. Table 46 in section 7.3.1 summarizes the detected concentrations (UK COT, 2004). In addition, a survey of the concentrations of PBDE congeners in food samples from the 2001 TDS was conducted. None of the congeners measured was detectable in this analysis.

6.1.8 *United States*

A market basket survey has been performed by Schechter et al. (2004a). Thirty-two food samples purchased from three Dallas, Texas, supermarket chains in 2003 were analysed for 13 individual PBDE congeners (Table 29).

Further data on meat samples and dairy products from Schechter et al. (2004c) are presented in Tables 30 and 31.

In addition, data on PBDE concentrations in meat and meat products were reported by Huwe (2004) (Table 32). Individual values below the limit of detection (LOD = 3 times the standard deviation of the blanks) were considered non-

Table 27. PBDE concentrations (ng/kg wet weight) in food samples collected in Catalonia, Spain^a

BDE	Vegetables (n = 8) ^b	Tubers (n = 2)	Pulses (n = 2)	Cereals (n = 4)	Fruits (n = 6)	Fish and shellfish (n = 8)	Meat and meat products (n = 15)	Eggs (n = 2)	Milk (n = 2)	Dairy products (n = 2)	Fats and oils (n = 3)
Tetra	4.0 (3.9)	0.5 (0)	2.3 (2.0)	2.2 (0)	0.4 (0)	158.3 (158.2)	23.5 (23.3)	17.3 (17.3)	8.0 (8.0)	10.7 (10.7)	169.7 (169.7)
Penta	1.4 (1.3)	0.5 (0)	0.6 80	2.2 (0)	0.4 (0)	115.9 (115.8)	24.9 (24.7)	25.8 (25.8)	5.2 (5.2)	23.4 (23.4)	157.7 (157.7)
Hexa	0.4 (0)	0.9 (0)	1.1 (0)	4.5 (0)	0.7 (0)	47.4 (47.0)	13.5 (12.8)	11.9 (11.9)	0.5 (0)	2.0 (0)	139.7 (138.0)
Hepta	0.7 (0)	1.8 (0)	2.2 (0)	8.9 (0)	1.4 (0)	5.4 (3.0)	23.9 (22.5)	4.4 (3.3)	1.1 (0)	4.0 (0)	77.0 (73.7)
Octa	1.4 (0)	3.7 (0)	17.9 (0)	17.9 (0)	2.9 (0)	6.8 (1.4)	23.4 (19.1)	4.7 (0)	2.1 (0)	7.9 (0)	43.7 (30.3)
Sum	7.9 (5.2)	7.4 (0)	10.7 (2.0)	35.7 (0)	5.8 (0)	33.9 (325.3)	109.2 (102.4)	64.5 (58.3)	16.9 (13.2)	47.9 (34.1)	587.7 (669.3)

From Bocio et al. (2003)

^a For each food group, two values are given. The first and second (in parentheses) values were calculated assuming that when a congener was below the LOD, the concentration was equal to one-half of the respective LOD (first value) or zero (second value).

^b n = number of composite samples analysed.

detects (ND) and set to either LOD/2 or zero (in parentheses) before the averages were calculated.

Table 28. PBDE concentrations in food samples collected in Catalonia, Spain

Food	PBDE concentration ^a	
	ng/kg, lipid weight	ng/kg, wet weight
Vegetables		8 (5)
Tubers		7 (0)
Pulses		11 (2)
Cereals		36 (0)
Fruits		6 (0)
Whitefish	2359 (2052)	88 (37)
Shellfish	3140 (2961)	88 (83)
Tinned fish	2117 (1997)	260 (246)
Bluefish	10 839 (10 804)	1019 (1016)
Pork and pork products	597 (565)	172 (166)
Chicken	247 (0)	10 (0)
Beef and beef products	290 (248)	42 (36)
Lamb	261 (182)	31 (21)
Eggs	530 (482)	64 (58)
Dairy products	677 (557)	48 (34)
Whole milk	630 (525)	24 (20)
Unskimmed milk	618 (402)	10 (6)
Vegetable oils and fats	805 (795)	804 (794)
Margarine	188 (145)	155 (120)

From Bocio et al. (2003)

^a Data were calculated assuming that when a congener was below the LOD, the concentration was equal to one-half of the respective LOD. Values in parentheses were calculated assuming that ND = 0.

Further data for meat samples collected in three northern California markets were published by Luksemburg et al. (2004) and are summarized in Table 33.

6.1.9 Other data

Hites et al. (2004) is another important source of data on PBDE contamination of fish. Hites et al. (2004) analysed farmed and wild salmon produced and bought worldwide. The highest PBDE concentrations were found in farmed salmon.

Figure 7 and Table 34 summarize the PBDE concentrations detected in fish from Europe and the United States. The total BDE mean values from fish samples collected in the United States were 10 times higher than those detected in fish samples collected in Europe. This difference may be due to the fact that the samples from the United States were mainly from rivers and lakes, while the fish samples from Europe were mainly marine fish.

Table 29. PBDE concentrations in 32 food samples purchased in Dallas, Texas

Food	PBDE concentration (pg/g wet weight)	Food	PBDE concentration (pg/g wet weight)
Margarine	0.9	Salmon fillet 2	3078
Evaporated milk 1	28.2	Catfish	2450
Evaporated milk 2	29.6	Bacon 1	0
Milk formula	30.3	Pork	41
Low-fat yoghurt	31.5	Ground beef	78.3
Ice cream	149	Bacon 2	104
Evaporated goat milk	290	Chicken breast	283
Butter	412	Ground turkey	713
Cheese	679	Duck	1282
Tilapia fillets	8.5	Wieners	1333
Texas shrimps	106	Pork sausage	1373
Rainbow trout	536	Non-fat milk	0
Catfish fillet	1547	Soya formula	16.9
Salmon	1752	Chicken eggs	73.7
Shark	1920	Calf liver	115
Salmon fillet 1	1994	Chicken liver	2835

Adapted from graphs in Schecter et al. (2004c)

Another collection of PBDE data in fish samples of different origins is given in Table 35. Mean concentrations for samples collected from different countries range between 14 and 2200 ng/g lipid.

Tritscher et al. (2003) reported on PBDE values for different types of fish, one egg sample and five cow milk samples. The concentration of total PBDEs in fish ranged between 1.1 and 5.4 ng/g lipid. The lowest value of all six samples was found in Whiting, Peru, the only sample originating from the Southern Hemisphere (Figure 8). The highest concentration in the egg yolk sample was found for BDE-99 and BDE-47, at 1.1 and 0.5 ng/g lipid, respectively; the total BDE concentration was reported to be 2.2 ng/g lipid. The total PBDE values for the five milk samples

Table 30. PBDE concentration in meat and meat products

BDE congener number	PBDE concentration ^a (ng/g lipid)							
	Sliced bacon	Sliced bacon	Butcher block pork	Ground pork	Beef tenderloin filet	Extra lean ground beef	Chicken breast	Fresh ground chicken
	(35% fat)	(43% fat)	(8.9% fat)	(21.5% fat)	(13.7% fat)	(14% fat)	(4.9% fat)	(7.25% fat)
17	0.0020	ND (0.01)	0.0016	ND (0.01)	ND (0.01)	0.0018	ND (0.01)	ND (0.01)
28	ND (0.01)	ND (0.01)	ND (0.01)	ND (0.02)	ND (0.01)	ND (0.01)	0.0093	ND (0.02)
47	0.085	ND (0.05)	0.078	0.25	0.26	0.17	1.2	0.15
66	ND	ND (0.01)	NA	ND (0.01)	ND (0.01)	0.0040	NA	ND (0.01)
85	0.0039	NA	NA	0.014	0.013	NA	NA	ND (0.01)
99	0.048	ND (0.04)	0.18	0.35	0.29	0.24	2.6	0.26
100	0.014	ND (0.01)	0.020	0.060	0.050	0.033	0.35	0.064
138	ND (0.01)	ND (0.01)	0.0018	0.020	ND (0.01)	0.0030	0.046	ND (0.01)
153	0.013	ND (0.01)	0.011	0.087	0.036	0.035	0.25	0.056
154	0.0080	ND (0.01)	0.014	0.070	0.027	0.018	0.22	0.035

Table 30. (contd)

BDE congener number	PBDE concentration ^a (ng/g lipid)							
	Sliced bacon	Sliced bacon	Butcher block pork	Ground pork	Beef tenderloin filet	Extra lean ground beef	Chicken breast	Fresh ground chicken
	(35% fat)	(43% fat)	(8.9% fat)	(21.5% fat)	(13.7% fat)	(14% fat)	(4.9% fat)	(7.25% fat)
183	0.040	ND (0.01)	0.015	0.092	0.028	ND	0.066	0.080
209	0.080	ND (0.08)	0.13	ND (0.01)	ND (0.08)	0.072	1.00	1.1
Total ^b	0.29	ND	0.46	0.94	0.70	0.58	5.8	1.8

Table 30. PBDE concentration in meat and meat products (contd)

BDE congener number	PBDE concentration ^a (ng/g lipid)						
	Young duck with giblets	Fresh ground turkey	Calf liver sliced	Chicken liver	Wieners	Chinese style sausage	Pork sausage
	(75% fat)	(11% fat)	(6.4% fat)	(13% fat)	(33% fat)	(26.2% fat)	(24% fat)
17	ND (0.01)	0.0018	0.0019	0.0023	ND (0.01)	ND (0.01)	ND (0.01)
28	ND (0.01)	ND (0.01)	0.0070	0.0080	ND (0.01)	ND (0.01)	ND (0.03)
47	0.38	0.88	0.14	5.2	1.2	ND (0.1)	1.6
66	0.0036	0.0076	NA	0.041	0.0043	ND (0.01)	ND (0.01)
85	0.020	NA	0.0075	0.21	0.034	0.012	0.071

Table 30. (contd)

BDE congener number	PBDE concentration ^a (ng/g lipid)						
	Young duck with giblets	Fresh ground turkey	Calf liver sliced	Chicken liver	Wieners	Chinese style sausage	Pork sausage
	(75% fat)	(11% fat)	(6.4% fat)	(13% fat)	(33% fat)	(26.2% fat)	(24% fat)
99	0.81	2.0	0.16	9.6	2.1	0.15	2.9
100	0.16	0.49	0.025	2.0	0.16	0.024	0.31
138	0.0097	0.035	ND (0.01)	0.14	0.022	ND (0.01)	0.024
153	0.070	0.30	0.045	1.1	0.32	0.023	0.34
154	0.057	0.22	0.028	0.99	0.15	0.019	0.23
183	0.042	0.33	0.098	0.088	0.043	0.026	0.062
209	0.15	2.2	1.3	2.2	ND (0.09)	ND (0.2)	0.21
Total ^b	1.7	6.4	1.8	22	4.1	0.53	5.8

From Schechter et al. (2004c)

NA, not analysed; ND, not detected

^a For PBDEs that were not detected, the LOD is provided in parentheses, where available.

^b In calculating the totals, ND was assumed to be equal to 0.

Table 31. PBDE concentration in milk and milk products

BDE congener number	PBDE concentration ^a (ng/g lipid)										
	Evap- orated milk	Evap- orated milk	Evap- orated goat milk	Milk- based instant formula	Low-fat yoghurt	Sweet cream salted, butter	Original cream cheese	Pas- teurized process cheese	Natural cheese gouda	Pasteur- ized prepared cheese	Cottage cheese
	(6.6% fat)	(6.3% fat)	(6.7% fat)	(3.4% fat)	(1.3% fat)	(78% fat)	(39% fat)	(19% fat)	(26.2% fat)	(11.6% fat)	(4.72% fat)
17	ND (0.01)	ND (0.01)	0.0030	ND (0.01)	0.016	ND (0.01)	0.00095	ND (0.01)	ND (0.01)	ND (0.01)	ND (0.01)
28	ND (0.01)	ND (0.01)	0.038	ND (0.01)	0.074	ND (0.01)	ND (0.01)	ND (0.01)	ND (0.01)	ND (0.02)	ND (0.03)
47	0.24	0.19	1.6	ND (0.01)	0.72	0.21	0.25	0.24	0.29	0.24	0.29
66	NA	NA	0.027	ND (0.09)	0.019	ND (0.01)	0.0040	ND (0.01)	ND (0.01)	0.0069	ND (0.01)
85	NA	NA	NA	NA	NA	NA	NA	ND (0.01)	0.021	ND (0.01)	ND (0.01)
99	0.13	0.20	1.5	0.36	0.62	0.22	0.20	0.18	0.22	0.20	0.30
100	0.029	0.036	0.41	0.032	0.11	0.052	0.031	0.031	0.047	0.036	0.056
138	ND (0.01)	0.0031	ND	0.0079	0.0042	ND (0.01)	NA	ND (0.01)	ND (0.01)	ND (0.01)	ND (0.01)
153	0.020	0.030	0.44	0.042	0.077	0.021	0.015	0.019	0.032	0.020	0.029
154	0.0067	0.013	0.12	0.032	0.032	0.016	0.0073	0.014	0.018	0.014	0.021

Table 31. (contd)

BDE congener number	PBDE concentration ^a (ng/g lipid)										
	Evap- orated milk	Evap- orated milk	Evap- orated goat milk	Milk- based instant formula	Low-fat yoghurt	Sweet cream salted, butter	Original cream cheese	Pas- teurized process cheese	Natural cheese gouda	Pasteur- ized prepared cheese	Cottage cheese
	(6.6% fat)	(6.3% fat)	(6.7% fat)	(3.4% fat)	(1.3% fat)	(78% fat)	(39% fat)	(19% fat)	(26.2% fat)	(11.6% fat)	(4.72% fat)
183	0.0033	ND (0.01)	0.18	0.0059	0.11	0.0067	ND (0.01)	0.011	0.0060	ND (0.01)	ND (0.02)
209	ND (0.03)	ND (0.03)	0.085	0.41	0.72	ND (0.2)	1.2	0.077	ND (0.09)	0.15	ND (0.3)
Total ^b	0.43	0.47	4.4	0.89	2.5	0.53	1.7	0.58	0.63	0.67	0.69

From Schecter et al. (2004a)

NA, not analysed; ND, not detected

^a For PBDEs that were not detected, the LOD is provided in parentheses, where available.^b In calculating the totals, ND was assumed to be equal to 0.

Table 32. Concentrations of major PBDEs in blanks, bacon and meat trimmings^a

BDE	PBDE concentration (pg/sample)									
	Blanks, <i>n</i> = 7		Bacon, <i>n</i> = 11		Chicken fat, <i>n</i> = 17		Pork fat, <i>n</i> = 11		Beef fat, <i>n</i> = 9	
	Average	LOD (3 × SD)	Average	Range	Average	Range	Average	Range	Average	Range
28/33	1.5	2.6	1.7 (0.7)	ND–4.6	1.7 (0.5)	ND–6	3.3 (2.1)	ND–23	1.3 (0)	ND
47	31.8	56.9	83.0 (62.3)	ND–454	424.0 (419)	ND–2764	518.0 (513)	ND–3897	50.4 (28)	ND–178
85	1.7	4.2	3.3 (1.4)	ND–15	22.9 (22.4)	ND–182	13.2 (12.2)	ND–66	2.5 (0.6)	ND–5.3
99	31.2	52.5	104.9 (88.2)	ND–624	742.7 (742)	60–4447	510 (508)	ND–2972	70.6 (53)	ND–279
100	4.4	5.9	14.0 (12.7)	ND–85	151.8 (151)	10–859	85.7 (85.7)	9–559	11.4 (9.8)	ND–48
153	2.6	7.3	27.4 (26.4)	ND–140	126.1 (126)	17–576	61.6 (61.6)	8–178	13.9 (13)	ND–42
154	2.4	5.6	14.8 (13.5)	ND–85	38.9 (37.9)	ND–126	43.2 (43.0)	ND–137	7.5 (6.3)	ND–24
183	3.1	14.7	46.9 (42.9)	ND–135	84.9 (82.7)	ND–469	46.8 (43.5)	ND–134	7.3 (0)	ND
209	914	3385	1693 (0)	ND	1845 (251)	ND–4275	1913 (374)	ND–4120	1693 (0)	ND
Σtri to hepta BDEs			296 (248)	ND–7831	1593 (1582)	86–8965	1282 (1269)	17–7831	165 (111)	ND–586
Σtri to hepta BDEs, lipid weight			818 (634)	ND–4642	2508 (2412)	124– 15 139	2603 (2488)	35–16 329	251 (178)	ND–835
% lipid			39.1	30.7–46.3	65.6	56.0–71.6	51.0	37.2–71.6	62.3	45.2– 73.4

From Huwe (2004)

LOD, limit of detection; ND, not detected; SD, standard deviation

^a Sample data are blank-subtracted.

Table 33. Concentration of PBDEs in beef and fowl meat purchased in three food markets in northern California, USA

	PBDE concentration (pg/g wet weight)								
	Meat products			Fowl products					
	Ground beef (grain fed)	Ground beef (free range)	Ground deer	Chicken thighs	Chicken thighs (free range)	Duck	Goose	Ground turkey	Pheasant
BDE-7	ND	ND	ND	ND	ND	ND	ND	ND	ND
BDE-13	ND	ND	ND	ND	ND	ND	ND	ND	ND
BDE-15	ND	ND	ND	ND	ND	0.60	ND	ND	ND
Total diBDE	ND	ND	ND	ND	ND	0.60	ND	ND	ND
BDE-17	ND	ND	ND	ND	ND	1.3	ND	ND	ND
BDE-25	ND	ND	ND	ND	ND	ND	ND	ND	ND
BDE-28	ND	0.36	ND	ND	ND	4.2	0.40	0.36	0.55
BDE-35	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total triBDE	ND	0.36	ND	ND	ND	5.4	0.40	0.36	0.55
BDE-47	45	10	120	37	12	187	20	77	26
BDE-49	ND	0.61	ND	ND	ND	7.3	ND	1.7	0.75
BDE-65	ND	ND	ND	ND	ND	2.8	ND	ND	ND
BDE-75	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total tetraBDE	45	11	120	37	12	195	20	79	26
BDE-85	ND	ND	6.5	1.3	ND	ND	1.3	2.9	1.2
BDE-99	75	17	179	53	21	209	29	165	34

Table 33. (contd)

	PBDE concentration (pg/g wet weight)								
	Meat products			Fowl products					
	Ground beef (grain fed)	Ground beef (free range)	Ground deer	Chicken thighs	Chicken thighs (free range)	Duck	Goose	Ground turkey	Pheasant
BDE-100	ND	3.4	33	13	5.2	144	5.4	47	7.3
BDE-126	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total pentaBDE	95	20	218	68	26	370	36	216	42
BDE-138	ND	ND	ND	ND	ND	44	ND	2.3	ND
BDE-153	ND	2.8	27	33	3.4	1220	5.3	38	5.9
BDE-154	ND	1.2	14	7.8	1.9	170	1.9	21	2.9
BDE-155	ND	ND	ND	ND	ND	ND	ND	1.3	ND
Total hexaBDE	ND	4.8	41	41	5.3	1440	7.2	66	8.8
BDE-181	ND	ND	ND	ND	ND	11	ND	ND	ND
BDE-183	14	5.1	ND	112	5.3	146	2.5	71	3.5
Total heptaBDE	24	5.1	ND	112	5.3	174	2.5	71	3.5
BDE-197	ND	5.5	ND	35	ND	46	1.6	26	5.8
BDE-203	ND	1.5	ND	ND	ND	17	ND	9.0	ND
Total octaBDE	ND	7.0	ND	46	ND	110	1.6	45	5.8
BDE-207	ND	11	ND	30	16	23	5.9	26	8.7
Total nonaBDE	ND	15	ND	30	21	33	5.9	32	14

Table 33. (contd)

	PBDE concentration (pg/g wet weight)								
	Meat products			Fowl products					
	Ground beef (grain fed)	Ground beef (free range)	Ground deer	Chicken thighs	Chicken thighs (free range)	Duck	Goose	Ground turkey	Pheasant
BDE-209	ND	113	ND	284	417	188	123	147	106
Total BDEs ^a	164	177	379	618	486	2516	196	656	207

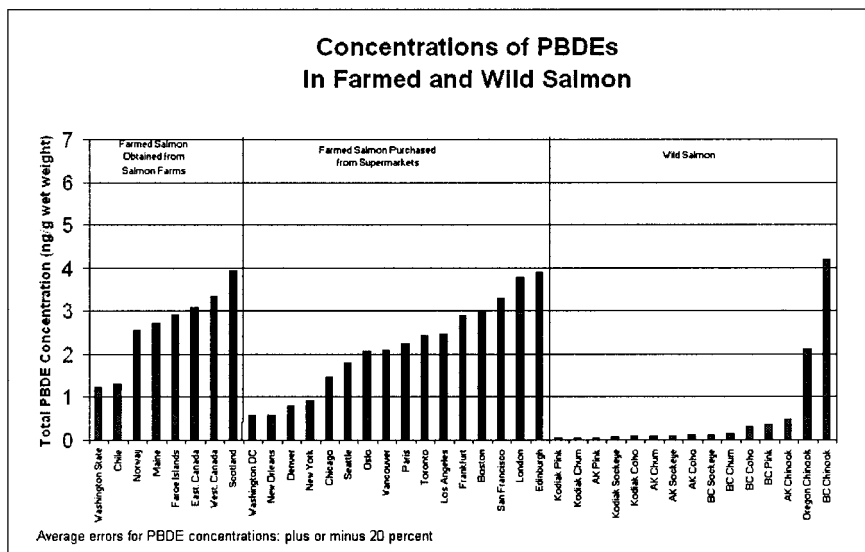
From Luksemburg et al. (2004)

ND, not detected

^a In calculating the totals, ND is assumed to be equal to the LOQ (where LOQ varies from 10 to 250 pg/g for all congeners).

from northern France ranged between 0.12 and 0.55 ng/g lipid. In all cases, the highest concentration was found for BDE-47.

Figure 7. Concentrations of PBDEs in farmed and wild salmon



Taken from Hites, R., Foran, J., Schwager, S., Knuth, B., Hamilton, C. & Carpenter, D. (2004) Global assessment of polybrominated diphenyl ethers in farmed and wild salmon. *Environ. Sci. Technol.*, **38** (19), 4945–4949.

6.2 Distribution curves

There were large differences in the amount, detail and quality of the data from the various countries. In particular, the data available differed with respect to the number of congeners analysed, whether concentrations were reported on a lipid or a wet weight basis and the sensitivity of the method used. Some data were available only as the sum of the congeners, and in some instances it was not clear which congeners were included in these sums. Further, in some cases, an upper-bound approach was used, i.e. substituting the LOQ (or LOD) for those congeners that were not quantified, in calculating total PBDEs. In other cases, the concentration of the contaminants may have been underestimated, when a lower-bound approach, with a zero value used for those congeners that were not quantified, was used. In some cases, the reported summaries did not clarify which approach was used. When data were available on a per lipid basis, they were converted to a per wet weight basis if the associated percent lipid information was available in the database or publication. Several studies summarizing concentrations expressed concentrations on a per fat basis and did not provide an estimate of the fat content of the food samples. For these studies, an average estimate based on the fat content of similar foods from the other studies was used. It was not always possible to determine which congeners were analysed; however, when that information was

Table 34. PBDE concentrations and congener distributions in fish

Location	Type	Date	Repetitions	PBDE concentration (ng/g lipid)						Reference
				47	99	100	153	154	ΣPBDE	
Germany	Eel, river	2001	5	4.50	0.14	0.98	0.21	0.48	6.31	Lepom et al. (2002)
Netherlands	Mackerel	1997	1	5.40	1.90	1.80			9.10	de Boer & Denneman (1998)
Baltic Sea	Herring	1985	4	10.3	1.70	1.57			13.6	Haglund et al. (1997)
Baltic Sea	Three species	1998	22	10.8	1.47	1.60	0.95	0.48	15.3	Burreau et al. (1999)
Switzerland	Rainbow trout	2002	4	11.5	2.27	1.70	0.27	0.36	16.1	Zennegg et al. (2003)
Baltic Sea	Herring	1998	3	12.4	4.14		0.75		17.3	Strandman et al. (1999)
Greenland	Three species	2000	36	15.6	0.69	1.28			17.6	Christensen et al. (2002)
Scotland and Belgium	Salmon	2001	13	10.9	2.87	3.56	1.01	0.81	19.2	Jacobs et al. (2002)
Sweden	Whitefish	1986	35	15.0	7.20	3.90			26.1	Sellström et al. (1993)
Sweden	Herring	1987	50	24.1	9.33	4.01			37.4	Sellström et al. (1993)
Baltic Sea	Sprat	1998	9	49.4	6.34		1.03		56.7	Strandman et al. (1999)
Switzerland	Whitefish	2002	8	44.3	24.0	4.63	1.21	1.52	75.6	Zennegg et al. (2003)
North Sea	Several species	1999	28	47.6	11.2	13.5	1.17	3.36	76.8	Boon et al. (2002)
Sweden	Herring	1987	260	130	23.0	13.0			166	Sellström et al. (1993)
Germany	Bream, river	2001	22	127	0.49	31.8	4.80	18.2	182	Lepom et al. (2002)
Baltic Sea	Salmon	1995	8	132	35.0	37.0	3.20	6.00	213	Asplund et al. (1999)
Baltic Sea	Salmon fillet	1991	1	167	52.0	44.0	4.20	11.0	278	Haglund et al. (1997)

Table 34. (contd)

Location	Type	Date	Repetitions	PBDE concentration (ng/g lipid)						Reference
				47	99	100	153	154	ΣPBDE	
Sweden	Several species	1987	12	269	41.8	34.5			345	Sellström (1996)
Sweden	Pike, rivers	1995	14	226	128	53.9			408	Sellström et al. (1998)
Sweden	Arctic char	1987	15	400	64.0	51.0			515	Sellström et al. (1993)
European means				81.8	19.9	16.0	1.58	4.27	119	
SD				23.4	6.77	4.38	0.46	1.89	32.9	
Geometric means				33.6	5.54	6.23	0.97	1.71	49.1	
Slocan River, USA	Whitefish	1996	3	4.20	4.70	1.50	0.93	0.76	12.1	Rayne et al. (2003)
British Columbia, Canada	Sole	1992	26	14.7	7.36	4.45	6.16	1.51	34.2	Ikonomou et al. (2002)
Columbia River, USA	Whitefish	1992	4	16.8	22.8	5.20	3.00	2.00	49.8	Rayne et al. (2003)
Columbia River, USA	Whitefish	1992	2	20.0	27.7	8.20	4.10	2.90	62.9	Rayne et al. (2003)
Michigan & Illinois, USA	Two species	1999	36	34.0	7.28	6.83	8.96	11.4	68.4	Rice et al. (2002)
British Columbia, Canada	Sole	2000	60	48.5	16.8	15.4	6.21	4.93	91.8	Ikonomou et al. (2002)

Table 34. (contd)

Location	Type	Date	Repetitions	PBDE concentration (ng/g lipid)						Reference
				47	99	100	153	154	ΣPBDE	
Columbia River, USA	Whitefish	2000	9	63.4	83.4	22.3	12.9	7.40	189	Rayne et al. (2003)
Great Lakes, Canada and USA	Lake trout	2000	40	151	37.0	19.9	9.96		217	Luross et al. (2002)
Kootenay Lake, USA	Whitefish	1998	5	125	135	38.2	17.0	13.8	330	Rayne et al. (2003)
Great Lakes	Several species	1999	20	208	59.0	45.5	14.7	40.4	368	Dodder et al. (2002)
Columbia River, USA	Whitefish	1996	1	132	184	43.5	23.8	14.8	398	Rayne et al. (2003)
Columbia River, USA	Whitefish	2000	12	179	227	68.8	32.9	20.0	527	Rayne et al. (2003)
Columbia River, USA	Whitefish	1994	1	185	263	71.6	40.6	30.2	590	Rayne et al. (2003)
Columbia River, USA	Whitefish	1995	4	325	479	148	63.7	44.0	1060	Rayne et al. (2003)
Lake Michigan	Salmonids	1996	21	1340	239	249	30.3	116	1970	Manchester-Neesvig et al. (2001)
Kootenay River, USA	Suckers	2000	6	2110	6.60	461	24.4	168	2770	Rayne et al. (2003)
Lake Michigan, Canada and USA	Trout	1996	6	1700	600	360	110	200	2970	Asplund et al. (1999)

Table 34. (contd)

Location	Type	Date	Repetitions	PBDE concentration (ng/g lipid)						Reference
				47	99	100	153	154	ΣPBDE	
Virginia, eastern	Three species	1998	25	4540	783	1410	235	235	7200	Hale et al. (2001)
North American means				622	177	165	35.8	53.7	1050	
SD				275	53.7	79.5	13.3	18.5	423	
Geometric means				136	63.1	38.9	16.0	16.6	308	

From Hites et al. (2004)

SD, standard deviation

available, the BDE-28, BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154 congeners were the ones most reported analysed. Therefore, the following analyses assume that, at a minimum, these six congeners were analysed in all studies. For the studies in which additional congeners were analysed and concentrations of the individual congeners were available, the assessment restricted the analyses to the six congeners listed above.

Table 35. PBDEs in fish samples, various studies

Area	Reference	Year of collection	Sample	<i>n</i>	Mean values (range) (ng/g lipid weight)
Norway (13 lakes)	Schlabach et al. (2001)	1999	Trout	1/lake	43.2 (7.9–124) ^a
Baltic Sea (7 sites)	Nylund et al. (2001)	1999	Herring	12–20/site	17.0 (12–30.7) ^b
German market	CVUA (2001)	2001	Plaice	44	30 (151) ^c
			Rosefish	64	14 (196) ^c
Scotland	Jacobs et al. (2002)	1999	Salmon	8	53.6 (1.1–85.2) ^a
Belgian market		2001		5	19.6 (3.1–52.1) ^a
San Francisco Bay area	Holden et al. (2003)	2002	Perch	6	696
			Halibut	4	2235
			Bass	4	1925
			Shark	1	489
River Elbe	Lepom et al. (2002)	2001	Bream	22	198 (26–728) ^a
			Eel	5	6.3 (3.6–21.4) ^a

^a Range of individual data.

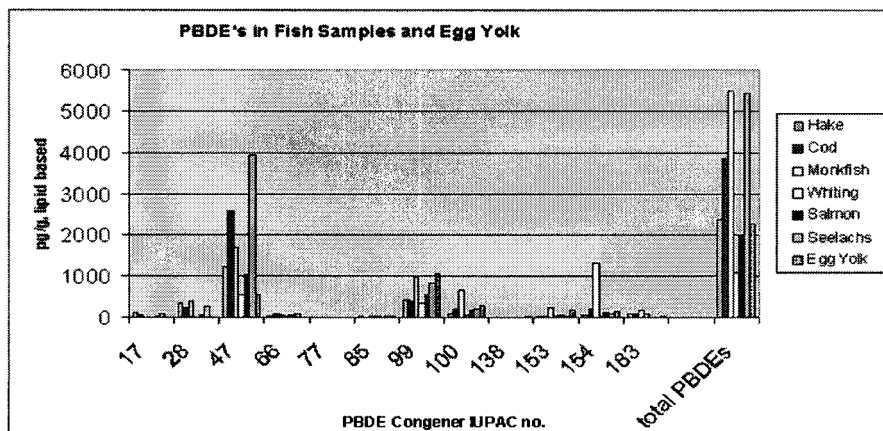
^b Range of means.

^c Maximum.

Concentration data available on fish samples collected in the United Kingdom were clearly identified as targeted samples and were therefore excluded from the analyses. It was impossible to identify analytical results for targeted samples for the other countries; all the remaining data were therefore considered representative of total contamination of foods.

Foods for which PBDE contamination data were available were grouped in six food groups: Meats and products (including beef, pork, poultry and game), Dairy and dairy products, Fish and shellfish, Fats and oils, Eggs and Fruits and vegetables.

Figure 8. PBDEs in fish samples and egg yolk



Taken from Tritscher, A., Stadler, R., Scanlan, F., Collingro, C. & Pöpke O. (2003) Determination of polychlorinated diphenylethers in samples of raw cow's milk, fish and egg. *Organohalogen Compd.*, **61**, 131–134.

Data were generally available in an aggregated form; although individual data were available from some studies, there were not enough samples per study to allow the generation of a full curve for the distribution of concentrations. As agreed at the FAO/WHO workshop on exposure assessment of contaminants (WHO, 2000), the data from the various studies were grouped by geographical region, and a lognormal distribution was assumed for each food group and each region. To estimate the mean of the distribution, the aggregated data were weighted as a function of the number of initial samples. Each result was multiplied by the number of individual samples in the original survey, and the sum of the products was then divided by the total number of individual samples to obtain a weighted mean of the contamination of foods by PBDEs. In a second step, the geometric standard deviation (GSD) of the distribution was derived by calculating the weighted standard deviation of the log-transformed means available for each country and food group. An average GSD of 2 was used to represent variability between countries within a given geographical region.

In addition, a GSD was estimated from the studies for which data on individual samples were available. An average GSD of 5.6 was used for fish and shellfish, whereas an average standard deviation of 2.6 was used for the other food groups. The within-food component of the variation represents variation in concentrations in different portions of one food group bought in one area. This component is not used in estimating long-term intakes, because the long averaging time for PBDE intake renders "meal-to-meal" variation irrelevant to the consideration of long-term risk. In the case of long-term intakes, it is assumed that consumers choose food randomly with respect to the distribution of concentrations of contaminants and will therefore have an intake over time that is an approximation of the

true mean of that distribution. This component, however, would be used, in conjunction with the between-countries variation, in the calculation of short-term intakes if acute exposures to PBDEs are considered of interest.

National data were aggregated by region when sufficient data were available: Western Europe, North America and Far East. There were no data for countries in other regions. Two sets of distributions were generated and used in the regional intake assessments: "lower-bound" distributions, where estimates were derived from studies setting non-detects at zero; and "upper-bound" distributions, where estimates were derived from studies setting non-detects at the LOD. The percentiles of these distribution curves were determined, and the median values and 90th percentiles are presented in Tables 36 and 37. In addition, Figures 9 to 12 illustrate these distributions for two food groups and two regions.

7. DIETARY INTAKE ASSESSMENT

7.1 Introduction and background

PBDEs have been detected at low levels in food, human milk, outdoor air, indoor air, water, household dust, human blood and human fat tissue. The main sources of exposure of humans to PBDEs are through food, human breast milk and dust (Health Canada, 2004f). In 1979 and 1980, the first record of the occurrence of PBDEs in fish samples was published by Andersson & Blomkvist (1981). Concentrations between 950 and 27 000 ng/g lipid were found in fish sampled along the Swedish river Viskan, where numerous textile industries are located. These industries have used various brominated flame retardants in the production of textiles. Due to the importance of fish in the diet worldwide, a number of fish studies have been undertaken. PBDEs accumulate in fatty tissues and fluids such as milk due to their physicochemical properties.

7.2 Methods

7.2.1 Definitions

The following definitions were adopted:

- *Dietary intake*: The dietary intake of PBDEs is defined as the amount of these contaminants that is ingested in food per unit time. Dietary intake is expressed in one of two ways: intake per unit of time, or intake per kilogram body weight per unit of time. The latter measure requires that data on body weight be available. In the current assessment, intake is expressed as picograms of total PBDEs per capita per day.
- *Region*: When applied to concentrations and diets, region is an area composed of individual nations or other geopolitical units that are likely to have separate food sources and markets but common dietary characteristics. The five Global Environment Monitoring System Food Contamination Monitoring and Assessment Programme (GEMS/Food) regional diets fit this definition.

Table 36. Weighted mean and derived median and 90th percentile of the simulated concentration distributions of PBDEs used in the long-term intake assessment

Region	Parameter	PBDE concentrations (ng/g of fresh weight)					
		Dairy & dairy products	Eggs	Fish & shellfish	Fruits & vegetables	Meat & poultry	Fats & oils
Derived using summaries with ND = 0							
Europe	Weighted mean	0.030	0.048	1.782	0.007	0.078	0.267
	GSD	2	2	2	2	2	2
	Median	0.023	0.037	1.364	0.005	0.060	0.204
	90th percentile	0.055	0.090	3.316	0.013	0.146	0.496
North America	Weighted mean	0.100	0.207	0.918	0.007 ^a	0.351	0.925
	GSD	2	2	2	2	2	2
	Median	0.077	0.158	0.703	0.005	0.269	0.708
	90th percentile	0.187	0.384	1.708	0.013	0.654	1.722
Derived using summaries with ND = LOD							
Europe	Weighted mean	0.249	0.128	1.872	0.010	0.232	0.944
	GSD	2	2	2	2	2	2
	Median	0.190	0.098	1.433	0.007	0.178	0.723
	90th percentile	0.463	0.239	3.484	0.018	0.433	1.757

Table 36. (contd)

Region	Parameter	PBDE concentrations (ng/g of fresh weight)					
		Dairy & dairy products	Eggs	Fish & shellfish	Fruits & vegetables	Meat & poultry	Fats & oils
North America	Weighted mean	0.249 ^a	0.128 ^a	1.872 ^a	0.010 ^a	0.246	1.083
	GSD	2	2	2	2	2	2
	Median	0.190	0.098	1.433	0.007	0.188	0.829
	90th percentile	0.463	0.239	3.484	0.018	0.457	2.016
Far East	Weighted mean	0.249 ^b	0.128 ^b	0.910	0.073	0.029	0.944 ^b
	GSD	2	2	2	2	2	2
	Median	0.190	0.098	0.697	0.056	0.022	0.723
	90th percentile	0.463	0.239	1.694	0.136	0.053	1.757

GSD, geometric standard deviation; LOD, limit of detection; ND, not detected

^a No data available for the North American region; data from the Western European region used instead.

^b No data available for the Far Eastern region; data from the Western European region used instead.

Table 37. Weighted mean and derived median and 90th percentile of the simulated concentration distributions of PBDEs used in the acute intake assessment

Region	Parameter	PBDE concentrations (ng/g of fresh weight)					
		Dairy & dairy products	Eggs	Fish & shellfish	Fruits & vegetables	Meat & poultry	Fats & oils
Derived using summaries with ND = 0							
Europe	Weighted mean	0.030	0.048	1.782	0.007	0.078	0.267
	GSD	3.26	3.26	6.40	3.26	3.26	3.26
	Median	0.015	0.024	0.314	0.003	0.039	0.132
	90th percentile	0.066	0.108	3.393	0.015	0.176	0.598
North America	Weighted mean	0.100	0.207	0.918	0.007 ^a	0.351	0.925
	GSD	3.26	3.26	6.40	3.26	3.26	3.26
	Median	0.050	0.102	0.162	0.003	0.173	0.457
	90th percentile	0.225	0.463	1.748	0.015	0.787	2.074
Derived using summaries with ND = LOD							
Europe	Weighted mean	0.249	0.128	1.872	0.010	0.232	0.944
	GSD	3.26	3.26	6.40	3.26	3.26	3.26
	Median	0.123	0.063	0.330	0.005	0.115	0.466
	90th percentile	0.557	0.288	3.565	0.022	0.521	2.116

Table 37. (contd)

Region	Parameter	PBDE concentrations (ng/g of fresh weight)					
		Dairy & dairy products	Eggs	Fish & shellfish	Fruits & vegetables	Meat & poultry	Fats & oils
North America	Weighted mean	0.249 ^a	0.128 ^a	1.872 ^a	0.010 ^a	0.246	1.083
	GSD	3.26	3.26	6.40	3.26	3.26	3.26
	Median	0.123	0.063	0.330	0.005	0.121	0.535
	90th percentile	0.557	0.288	3.565	0.022	0.551	2.428
Far East	Weighted mean	0.249 ^b	0.128 ^b	0.910	0.073	0.029	0.944 ^b
	GSD	3.26	3.26	6.40	3.26	3.26	3.26
	Median	0.123	0.063	0.161	0.036	0.014	0.466
	90th percentile	0.557	0.288	1.734	0.164	0.064	2.116

GSD, geometric standard deviation; LOD, limit of detection; ND, not detected

^a No data available for the North American region; data from the Western European region used instead.

^b No data available for the Far Eastern region; data from the Western European region used instead.

Figure 9. Simulated distributions of PBDEs in dairy products for use in the long-term intake assessment for the Western European region

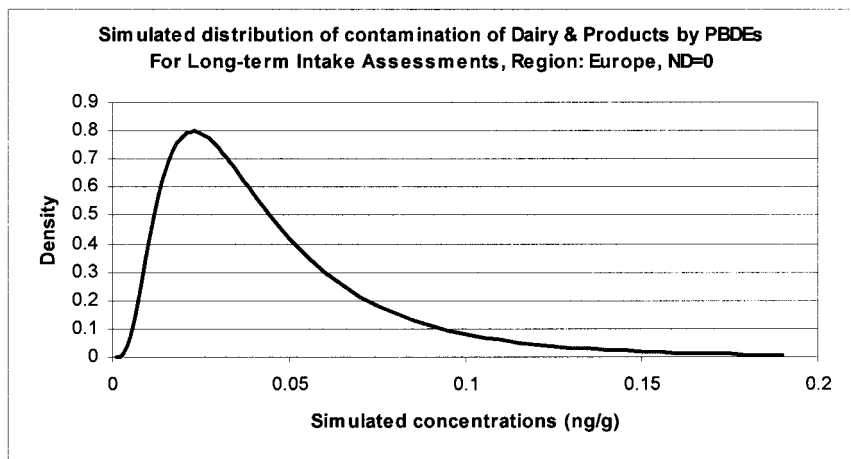
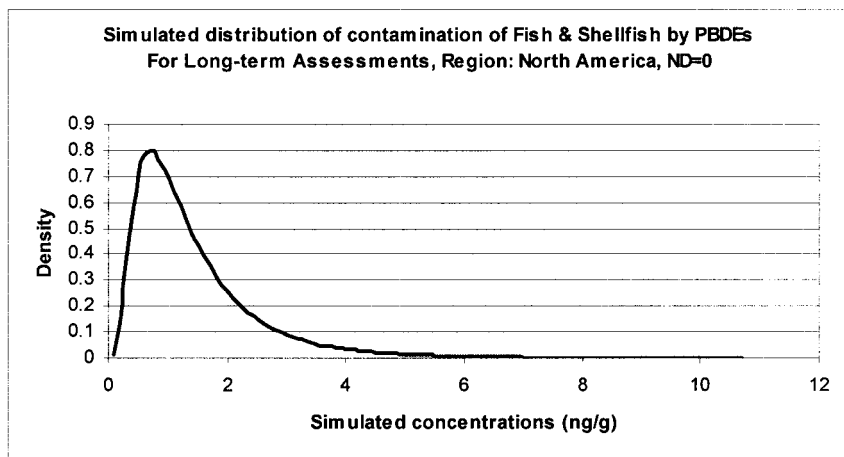


Figure 10. Simulated distributions of PBDEs in fish and shellfish for use in the long-term intake assessment for the Northern American region



- *Between-person variation:* When applied to intake and food consumption, between-person variation is defined as variation between individuals in a population within a nation or other geopolitical unit that is likely to have common food sources and markets.
- *Between-country variation:* When applied to concentrations, between-country variation is defined as variation between long-term mean concentrations in

Figure 11. Simulated distributions of PBDEs in dairy products for use in the acute intake assessment for the Western European region

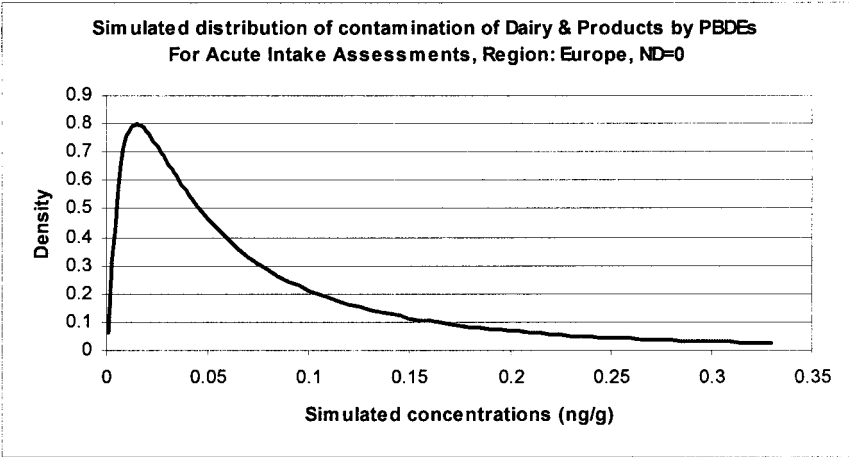
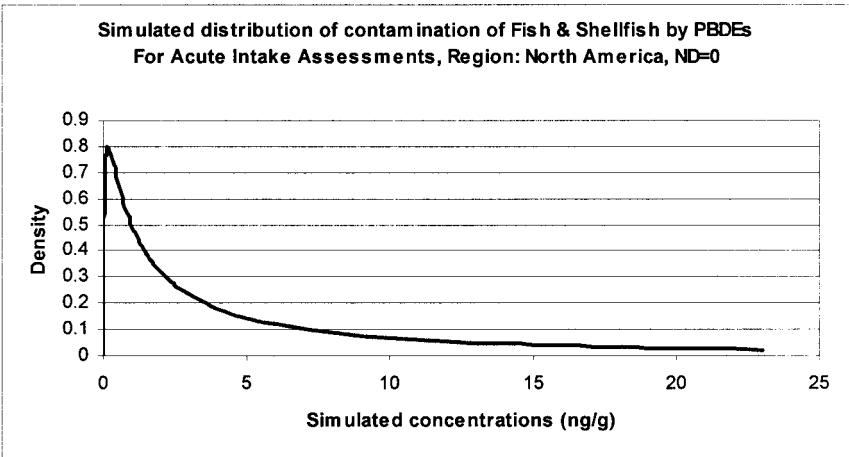


Figure 12. Simulated distributions of PBDEs in fish and shellfish for use in the acute intake assessment for the Western European region



specific food groups in areas that predominantly do not share food sources or markets. National boundaries are assumed to define these populations within an acceptable degree of error for this analysis.

- *Within-food variation:* When applied to concentrations, within-food variation is defined as variation between consumed portions of a given food group during the period considered in the analysis. For example, the within-food variation in

PBDE concentration for the group "Fish" would comprise the variation in PBDE concentration from one meal to the next during the period of exposure (lifetime or other) of that individual. This variation is composed of variation due to differences between species and variation related to differences between fish of the same species. The within-food variation in PBDE concentration is assumed to be equivalent to the between-sample variation for the samples considered for each food group in this analysis.

7.2.2 Intake calculations

Mean intake per person can be calculated from mean food consumption, the composition of the diet and the mean concentrations of PBDE in food from a local market, as follows:

Equation 1:

$$\bar{J}_T = \bar{I}_f \sum_{i=1}^N \bar{C}_i f_i$$

where \bar{J}_T is the long-term mean personal intake of a contaminant, \bar{C}_i is the mean concentration (in different portions) of the contaminant in food group i , \bar{I}_f is the mean food consumption (g/day), N is the number of food groups considered and f_i is the fraction of food group i that contributes to total food consumption.

The contribution of a food group i to the total intake of PBDEs is obtained from the partial intake, J_i for group i , as follows:

Equation 2:

$$\bar{J}_i = \bar{I}_f \bar{C}_i f_i$$

If the mean food consumption per person and the mean concentration are considered random variables, it becomes possible to evaluate the distribution of the dietary intake by a certain population. The approach used corresponds to the method for assessing intake of contaminants and toxins in food recommended by an FAO/WHO workshop on the topic (WHO, 2000). In short, the following procedure was followed:

1. Concentration distributions indicating between-country variation in the mean were constructed for various regions and food groups from the data on occurrence submitted by the countries. These distributions were assumed to be lognormal (see section 6.2).
2. Data on food consumption were used to estimate mean consumption and the between-person variation in food consumption in different diets. Lognormal food consumption distributions were constructed for each diet. Additionally,

the contribution of the recognized food groups to total food consumption in these diets was derived from data on food consumption.

3. The dietary intake of a particular population was assessed by combining the concentration and the food consumption distributions for that population by Monte Carlo simulations (with 10 000 trials for each simulation). In each Monte Carlo trial, dietary intake was estimated by multiplying random realizations of food consumption and concentrations in different food groups sampled from their distributions (Eq. 1). The concentrations were weighted according to the fraction that each food group contributes to total food intake (Eqs. 1 and 2). The collective intake estimates obtained by the Monte Carlo approach thus form a distribution of dietary intake for each population studied. The distributions are characterized by the median intake and two high percentiles (80th and 90th). Note that the 90th percentile is a realistic estimate corresponding to the mean intake that is exceeded by 10% of the population considered.

7.2.3 *Compounds*

Dietary intake was calculated for the sum of the following PBDEs: BDE-28, BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154.

7.2.4 *Distributions of concentrations*

Data on occurrence were available for various countries (see section 6.1), and these data were used to compile regional distributions of concentrations, each distribution characterized by two parameters, the median (see Table 38) and a GSD. Two sets of distributions were derived: the first was derived assuming non-detects are set at zero, while the second was derived assuming non-detects are set at the LOD. Since the data available from the various countries were not always derived using the same sets of assumptions, different countries were included in the two sets of distributions. In order to construct complete data series for each region, missing data are typically replaced by data for the closest region, e.g. Western European data for North America. However, since the fish concentration data were shown to be different between the two regions when non-detects were set at 0, it was not possible to use the Western European data as a surrogate for the missing North America data when non-detects were set at the LOD. Since Japan was the only country in the Far Eastern region for which data were available, data from Western Europe had to be used as a surrogate for the foods missing data in the Far Eastern region. Essentially, the variation in concentrations within each food group and region consists of a "between-country" and a "within-food" component.

(a) Between-country variation

In calculating long-term intakes, only the between-country component of the variation in concentration is relevant. Such variation implies that each region has areas with less and more contaminated areas. In other words, it is assumed that

persons living in a country where there is higher contamination will not "dilute" their daily intake of PBDEs by eating food from a country where there is less contamination, nor will persons living in a less contaminated area frequently consume foods from a more contaminated area. The between-country variation was estimated from the results available for this assessment, which consisted mainly of means of aggregated data, i.e. measurements in pooled samples or means of series of individual measurements (see section 6.2). The GSD of a data series of means thus refers to the variation between those means.

The GSD values ranged from 1.2 to 10.8 and clustered around 2. On the basis of these results, a universal between-country GSD of 2 was assumed.

(b) Within-food variation

The within-food component of the variation represents variation in concentrations in different portions of one food group bought in one area. This component is not used in long-term intake estimates, because the long averaging time for PBDE intake renders "meal-to-meal" variation irrelevant to the consideration of long-term risk. In the long-term intake assessment, it is assumed that consumers choose food randomly with respect to the distribution of concentrations of contaminants and will therefore have an intake over time that is an approximation of the true mean of that distribution.

An accurate estimate of the GSD for within-food variation requires measurement of concentrations in a set of individual products within one food group and one area. Some of the data available were on a per individual sample. The GSDs varied from 4.7 to 7.0 in the case of fish and shellfish and from 1.2 to 5.2 for the other foods. On the basis of these results, an average within-food GSD of 5.6 was assumed for fish and shellfish and of 2.6 for the other foods. The within-food variation should be used in estimation of acute (short-term) intakes.

7.2.5 National diets

National diet estimates were summarized by the various intake studies available. Some were based on TDS, others on national consumption surveys. Only mean intake estimates were provided.

7.2.6 International diets

Calculations were also performed for the GEMS/Food regional diets (WHO, 1998). These diets are derived not from data on food consumption but from food production and import and export balances, as summarized by the FAO in their Food Balance Sheets. Comparison with the detailed results of national food consumption surveys shows that this type of data on food consumption provides estimates that are more than 15% higher than actual mean food consumption (WHO, 1998). The same estimate of between-person variation in total food consumption that was assumed for dioxins (1.3) was assumed here.

7.3 Estimates of dietary intake

7.3.1 National estimates for adults

National intake estimates were submitted by Australia, Canada, The Netherlands and New Zealand. The Canadian estimate was based on the Canadian TDS. The Australian and New Zealand estimates were derived using concentration data from the Canadian TDS and consumption estimates from Australia and New Zealand. The Netherlands estimate was based on a report prepared by RIVO and consumption data from the Dutch National Food Consumption Survey. In addition, estimates for other countries were available in published reports and studies.

(a) Australia

No Australian or Codex maximum permitted levels or Australian analytical data exist for PBDE concentrations in foods at present. Australia submitted estimated exposures calculated using the concentration levels of PBDEs in Canadian foods, analysed in samples of the food groups reported in the Canadian 1998 Whitehorse and 2002 Vancouver TDS (Health Canada, 2004a, 2004b) and Australian food consumption data. Specifically, the exposure assessment was conducted using Australian food consumption data with Canadian mean and maximum concentration data derived from the two Canadian TDS.

Estimated dietary exposures to PBDEs based on Canadian concentration data and Australian food consumption data are presented in Table 38.

Table 38. Estimated dietary exposure to PBDEs using Canadian concentration data and Australian food consumption data

Exposure units	All respondents ^a		Consumers only ^b		
	(n = 13 858)		(n = 13 810)		
	Mean	Median	Mean	Median	95th percentile
<i>Using mean concentrations</i>					
pg/day	37.1	27.0	37.2	27.2	103.9
pg/kg bw per day	0.6	0.4	0.6	0.4	1.8
<i>Using maximum concentrations</i>					
pg/day	48.8	33.6	49.0	33.7	142.0
pg/kg bw per day	0.8	0.5	0.8	0.6	2.4

^a Respondents: This includes all members of the survey population whether or not they consumed a food that contains the contaminant.

^b Consumers only: This includes only the people who have consumed a food that contains the contaminant.

(b) Canada

Intake estimates for Canada are available from Ryan & Patry (2001) and from Health Canada (2004d, 2004e). Ryan & Patry (2001) estimated the PBDE intake of Canadian adults by sampling commercial foods from an ongoing total diet market basket study. This resulted in a daily intake of total PBDEs of about 44 ng. The main intake of PBDEs was observed to be correlated with the intake of meat. Health Canada estimated the total diet PBDE intake based on food composites collected from Whitehorse in the winter of 1998 and from Vancouver in the spring of 2002. The intake estimate is based on an average consumption rate for all ages and both sexes using a 60-kg person (Table 39). On inspection of the data, it appears that the intake of PBDEs by Canadians from consumption of commercial foods is about 30–40 ng total. Based on limited information, this value does not appear to have changed substantially from 1998 to 2002.

Table 39. Dietary intakes of total PBDEs in two Canadian cities in 1998 and 2002

Food group	PBDE intake (ng/day)	
	Vancouver 2002	Whitehorse 1998
Dairy	5.9	3
Meat	12.5	29.6
Fish	8.6	1.2
Other	3.4	4.5
All foods	30.4	38.2

(c) Finland

Kiviranta et al. (2004) measured the concentrations of PBDEs in 10 market baskets consisting of almost 4000 individual food samples representing 228 different food items, as well as in the total diet basket. The fish basket contributed most to the concentrations of PBDEs, in which the lower-bound range was from 0.82 to 850 pg/g. The associated average daily intake of these substances by the Finnish adult population was 44 ng/day (Table 40). Fish contributed most to the PBDE intake.

(d) Japan

For Japan, two independent intake studies have been reported by Ashizuka et al. (2004). They compared the results of their market basket food study and the duplicate study. The average composition of the total diet for Fukuoka residents is given in Table 41. The total dietary PBDE intakes per day and per person for the two estimating methods are calculated as follows:

Market basket study: 114 ng/day

Table 40. Average intakes of PBDEs in Finland based on 10 market baskets and total diet basket

Market baskets	NQ = 0		NQ = LOQ	
	PBDE intake (ng/day)	%	PBDE intake (ng/day)	%
(1) Liquid milk products	0.35	0.8	0.86	0.9
(2) Solid milk products	1.1	2.6	1.3	2.9
(3) Fish	23	55	23	52
(4) Meat and eggs	1.8	4.2	2	4.5
(5) Fats	6.5	15	7.9	18
(6) Cereal products	2.8	6.6	2.8	6.2
(7) Potato products	0.16	0.4	0.17	0.4
(8) Vegetables	1.9	4.5	1.9	4.3
(9) Fruits and berries	0.85	2	0.93	2.1
(10) Beverages, spices, sweets	3.9	9.2	4	8.8
Sum of baskets	43		45	
Total diet basket	44		44	

LOQ, limit of quantification; NQ, not quantified

Meal samples study (duplicate study): 68.2 ng/day (range: 10.8–212.7 ng/day)

(e) The Netherlands

Mean dietary intakes of PBDEs were estimated by de Winter-Sorkina et al. (2003) using data collected by RIVO (section 6.1) and the consumption data from the third Dutch National Food Consumption Survey. The calculated mean dietary intakes of the “middle” scenario (derived assuming $ND = LOD/2$) are 3.2–3.5 ng/kg bw per day. When the ND was set at zero (the “low” scenario), the estimated intake is 0.2 ng/kg bw per day (Table 42).

(f) New Zealand

No New Zealand or Codex maximum permitted levels or New Zealand analytical data exist for PBDE concentrations in foods at present. New Zealand submitted estimated exposures calculated using the concentration levels of PBDEs in Canadian foods, analysed in samples of the food groups reported in the Canadian 1998 Whitehorse and 2002 Vancouver TDS (Health Canada, 2004a, 2004b) and New Zealand food consumption data. Estimated dietary exposures to PBDE based on mean and maximum Canadian concentration data and New Zealand food consumption data are presented in Table 43.

Table 41. Average composition of total diet of average person in Fukuoka, Japan

Food group	Average weight (g/day)	% (by weight) of total diet
Rice and rice products	409.0	25.6
Grains, seeds and potatoes	192.8	12.1
Sugar and confectionaries	32.6	2.0
Oils	15.2	1.0
Legumes and legume products	73.2	4.6
Fruits	113.9	7.1
Carrots and green leafy vegetables	86.9	5.4
White leafy vegetables, mushrooms and seaweeds	184.6	11.5
Fish and fish products	172.2	10.8
Meat and eggs	82.1	5.1
Milk and milk products	107.9	6.8
Other processed foods	122.5	7.7
Water	5.6	0.4
Total	1598.5	100.0

From Ashizuka et al. (2004)

(g) Spain

The dietary intake of PBDEs for an adult male was 97.3 ng/day (ND = $0.5 \times$ LOD) or 81.9 ng/day (ND = 0), as given in Table 44. The greatest contribution to these values corresponds to fish and shellfish, with approximately one third of the total intake.

(h) Sweden

Darnerud et al. (1998b), using primarily Nordic data, estimated the exposure of the Swedish population to PBDEs from food in a report to the Nordic Council of Ministers. Their estimates were based on the upper range of total PBDE levels in herring caught in the Baltic Sea (528 ng/g lipid). The total PBDE intake was estimated by assuming a similar relative intake from different dietary sources as described earlier in a Swedish estimation for PCBs (Wicklund-Glynn et al., 1996). Consequently, according to this very approximate calculation, the total PBDE intake for the Nordic consumer would be 200–700 ng/day.

A more detailed Swedish intake estimate was published by Darnerud et al. (2000), on the basis of PBDE levels (BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154) in market basket samples collected in 1999. Analyses of food group homogenates were performed, and not detected values were recognized as $0.5 \times$

LOD. Total PBDE intake was obtained by adding intake from fish, meat, dairy products, eggs, fats/oils and pastry food groups. Using this method, total intake in Sweden was estimated to be 51 ng/day. About 50% of the intake originated from fish. All data given above are summarized by Darnerud et al. (2001).

Table 42. Total average dietary intake of PBDEs by the Dutch population, according to scenario 1 ($ND = 0.5 \times LOD$) and scenario 2 ($ND = 0$)

Compound	Mean intake (ng/kg bw per day) ^a	
	Scenario 1	Scenario 2
BDE-28	0.01	0.01
BDE-47	0.7	0.5
BDE-99	0.5	0.3
BDE-100	0.2	0.1
BDE-153	1.0	0.1
BDE-154	0.5	0.2
Sum PBDEs	3.2 (scenario A) ^b	0.2 (scenario A)
	3.5 (scenario B) ^c	0.2 (scenario B)

From de Winter-Sorkina et al. (2003)

^a The mean body weight of Dutch National Food Consumption Survey participants was 65.8 kg.

^b Scenario A: congener scenario.

^c Scenario B: food group scenario.

Table 43. Estimated dietary exposure to PBDE using Canadian concentration data and New Zealand food consumption data

Exposure units	All respondents ^a (<i>n</i> = 4636)		Consumers only ^b (<i>n</i> = 4624)		
	Mean	Median	Mean	Median	95th percentile
<i>Using mean concentrations</i>					
pg/day	48.7	33.1	48.8	33.2	140.8
pg/kg bw per day	0.7	0.5	0.7	0.5	1.9
<i>Using maximum concentrations</i>					
pg/day	65.4	41.8	65.6	41.9	199.4
pg/kg bw per day	0.9	0.6	0.9	0.6	2.7

^a Respondents: This includes all members of the survey population whether or not they consumed a food that contains the contaminant.

^b Consumers only: This includes only the people who have consumed a food that contains the contaminant.

Table 44. Estimated dietary intake of PBDEs by adult population of Catalonia, Spain^a

Food group	Daily consumption (g)	PBDE intake (ng/day)
Vegetable	226 (15.7)	1.8 (1.2)
Pulses	24 (1.7)	0.3 (0.05)
Cereals	206 (14.3)	7.4 (–)
Tubers	74 (5.1)	0.6 (–)
Fruits	239 (16.6)	1.4 (–)
Fish and shellfish	92 (6.4)	30.7 (29.9)
Meat and meat products	185 (12.8)	20.2 (18.9)
Eggs	34 (2.4)	2.2 (2.0)
Dairy products	106 (7.3)	5.1 (3.6)
Milk	217 (15.0)	3.7 (2.9)
Fats and oils	41 (2.8)	24.1 (23.3)
Total intake	1444 (100)	97.3 (81.9)
		1.4 (1.2) ^b

From Bocio et al. (2003)

^a Results are given for a male adult of 70 kg bw. In parentheses are percentages of total consumption. Data were calculated assuming that when a congener was below the LOD, the concentration was equal to one half of the respective LOD. Values in parentheses were calculated assuming that ND = 0.

^b Total intake expressed in ng/kg bw per day.

The latest intake study for Sweden was presented by Lind et al. (2002). The intake data of total PBDEs in female Swedish subjects are shown in Table 45. For females, a mean daily intake of 0.63 ng/kg bw (40.8 ng/day) was found. The intake data for male individuals are quite similar to the female data, at a mean of 0.58 ng/kg bw per day.

(i) *United Kingdom*

Table 46 summarizes the intake of PBDEs from brown trout and eels from locations upstream and downstream from a site known to have manufactured both penta- and octaBDE until the late 1990s.

A preliminary assessment of human exposure to PBDEs in the United Kingdom is given by Wijesekera et al. (2002). They reported data on a duplicate-diet study for 10 individuals. The daily dietary intake of total PBDEs (total of BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154) was calculated using data on the total content of the diet samples and food mass ingestion data for the individuals consuming each sample. The estimated median lower-bound value of 90.5 ng had a range between 37.2 and 235 ng/person per day.

Table 45. Intake of PBDEs from different food groups in Sweden, females age 17–74

Food group	n	PBDE intake (ng/day)			
		Mean	Median	Range	95th percentile
All fish	629	30.2	17.1	0.0–654	87.5
Fatty Baltic fish	629	9.37	2.02	0.0–551	43.5
Other fatty fish	629	18.8	9.90	0.0–598	64.1
Other fish	629	4.87	2.79	0.0–48.9	12.5
Meats, chicken	626	2.38	2.28	0.0–8.15	4.74
Dairy products	626	3.38	3.15	0.0–12.4	6.75
Vegetable fats	626	1.60	1.26	0.0–12.1	4.17
Other fats	626	2.76	2.51	0.0–11.6	6.02
Eggs	626	0.52	0.31	0.0–3.77	1.88
<i>Total intake</i>					
ng/day	621	40.8	28.1	1.28–666	96.4
ng/kg bw per day	621	0.63	0.43	0.02–11.7	1.42

From Lind et al. (2002)

(j) *United States*

Intake estimates for the United States are based on concentration data detected in the market basket survey conducted by Schechter et al. (2004a) (section 6.1.8). Figure 13 summarizes the estimated daily intake of PBDEs for 20- to 49-year-old males and females. The estimated intakes, 2.0 ng/kg bw and 1.4 ng kg/bw, are about 2–3 times higher than the daily intake in Europe.

(k) *Summary of national intake estimates*

A summary of the eight dietary PBDE intake studies performed worldwide to date is given in Table 47.

Table 46. Estimated average dietary intake of PBDEs following consumption of trout or eels from the Skerne-Tees river system

Sampling location	Species	Number of samples	Concentration range ^a (µg/kg fresh weight)	Maximum intake (µg/portion) ^b	Maximum average intake (µg/kg bw per day) ^c
Ricknall Grange ^d	Trout	5	12–14	1.6	0.003
Haughton Road ^e	Trout	7	59–197	24	0.056
Ricknall Grange ^d	Eels	1	53	3.7	0.0088
Oxenfield Bridge ^e	Eels	5	164–288	20.2	0.048

From UK COT (2004)

^a Reported as sum of BDE-28, BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154.

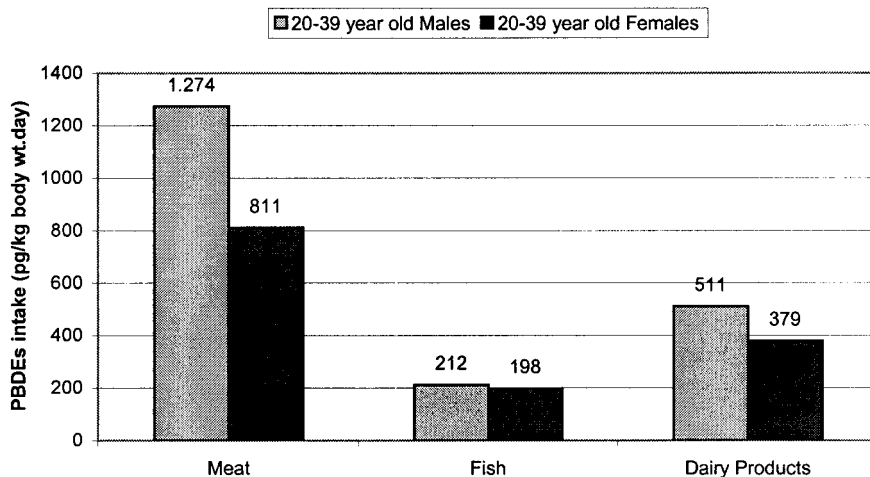
^b Portion sizes were assumed to be 120 g trout or 70 g eels, as cited in the United Kingdom Ministry of Agriculture, Fisheries and Food's Food Portion Sizes.

^c Average daily intake was calculated assuming consumption of one portion of trout or eels per week and an adult body weight of 60 kg.

^d Control site for Skerne River.

^e Test site showing the highest concentration of PBDEs in trout or eels.

Figure 13. Daily PBDE intake estimate for adults in the United States, 2003



Taken from Schecter, A., Päpke, O., Tung, K.C., Staskal, D. & Birnbaum, L. (2004a) Polybrominated diphenyl ethers contamination of United States food. *Environ. Sci. Technol.*, **38** (20), 5306–5311.

Table 47. Summary of data on human exposure to PBDEs through the diet

Country	Characteristics of the study	PBDE intake (ng/day)	Remarks	Reference
Australia	Canadian total diet study, Australian food consumption (per capita)	Mean intake: 37.1 pg/day Maximum intake: 48.8 pg/day	ND = 0	Food Standards Australia New Zealand (2004)
Canada	Food basket study, most food samples of animal origin	44		Ryan & Patry (2001)
	TDS conducted in Whitehorse in 1998 and Vancouver in 2002	38.2 (Whitehorse) 30.4 (Vancouver)	Calculations for intake were done assuming that ND = 0	Health Canada (2004a, 2004b)
Catalonia (Spain)	TDS	81.9 (lower)	ND = 0	Bocio et al. (2003)
	54 samples	97.3 (upper)	ND = LOD	
	11 food groups	sum of tetra- to octaBDEs		
Finland	10 market baskets and total diet basket	Market baskets		Kiviranta et al. (2004)
		43	ND = 0	
		45	ND = LOD	
		Total diet basket		
		44	ND = 0	
		44	ND = LOD	
Japan	a) Market basket study b) Duplicate-diet study	a) 114 b) 68.2 (10.8–212.7)		Ashizuka et al. (2004)
Netherlands	TDS	13 (low estimate)	ND = 0	de Winter-Sorkina et al. (2003)
	84 samples		ND = ½ LOD	
	6 food groups	213 (middle estimate)		
New Zealand	Canadian TDS, New Zealand food consumption	Mean intake: 48.7 pg/day Maximum intake: 65.4 pg/day	ND = 0	Food Standards Australia New Zealand (2004)

Table 47. (contd)

Country	Characteristics of the study	PBDE intake (ng/day)	Remarks	Reference
Sweden	Market basket samples: fish, meat, dairy products, eggs, fats/oils, pastry	51 sum of congeners 47, 99, 100, 153, 154	Calculations for intake were done assuming that ND = ½ LOD	Darnerud et al. (2001)
Sweden	Food of animal origin, National Swedish Diet Inventory	40.8 (mean) females (18–74 years) sum of congeners 47, 99, 100, 153, 154	Calculations for intake were done assuming that ND = 0	Lind et al. (2002)
United Kingdom	Duplicate-diet samples	90.5 (median) sum of congeners 47, 99, 100, 153, 154	Calculations for intake were done assuming that ND = 0	Wijesekera et al. (2002)
United States	Food basket study, 49 individual samples, most food samples of animal origin	Females: 1.4 ng/kg bw Males: 2.0 ng/kg bw	ND = 0	Schecter et al. (2004a)

LOD, limit of detection; ND, non-detects; TDS, total diet study

7.3.2 Regional estimates

The regional estimates for dietary intake presented use the concentration distributions derived by adjusting only for between-country variability and represent long-term intake estimates. The concentration distributions used in the assessment are those for Western Europe, the Far East (data from Japan) and North America (data from Canada and the United States).

Consumption data for the regional estimates were based on GEMS/Food regional diets. As these diets provide only point estimates of intakes, distributions of intake (representing interperson variability) assumed a lognormal distribution with a GSD of 1.3. Table 48 summarizes these distributions.

The estimated median long-term intakes from the GEMS/Food regional diets are listed in Table 49.

The estimated long-term intakes of PBDEs in the GEMS/Food regional diets (Tables 49 and 50) are considerably higher than those for the national diets summarized in section 7.3.1, particularly for the Western European region and the

Northern American region. This difference is driven by the fact that food consumption is overestimated in the GEMS/Food regional diets, as they are based on food production balances instead of actual food consumption.

Table 48. Statistical descriptors of the food consumption distributions used in the assessment (based on the GEMS/Food regional diets)

Region	Parameter	Per capita food consumption estimate (g/day)					
		Dairy & dairy products	Eggs	Fish & shellfish	Fruits, vegetables, roots & tubers	Meat & poultry	Fats & oils
Europe and North America	Mean	336.1	37.6	46.8	826.0	217.3	49.4
	GSD	1.3	1.3	1.3	1.3	1.3	1.3
	Median	303.7	34.0	42.3	746.6	196.4	44.6
	90th percentile	425.1	47.6	59.2	1045.0	274.8	62.5
Far East	Mean	32.8	13.1	31.5	372.8	47.0	16.0
	GSD	1.3	1.3	1.3	1.3	1.3	1.3
	Median	29.6	11.8	28.5	337.0	42.5	14.5
	90th percentile	41.5	16.6	39.9	471.6	59.5	20.3

GSD, geometric standard deviation

Table 49. Statistical descriptors (median, 80th and 90th percentiles) of estimated distributions of long-term PBDE intake in GEMS/Food regional diets

Source of concentration data	Diet	Total PBDE intake (ng/day per person), using distributions derived assuming ND = 0			Total PBDE intake (ng/day per person), using distributions derived assuming ND = LOD		
		Median	P80	P90	Median	P80	P90
Western Europe	European	109	166	210	250	350	420
North America	European	189	264	318	259	361	433
Far East	Far Eastern	NA	NA	NA	73	103	125

LOD, limit of detection; NA, not available; ND, not detected; P80, 80th percentile; P90, 90th percentile

A large difference is observed between the estimates using the lower-bound concentration distributions (generated with ND = 0) and those derived using the upper-bound concentration distributions (generated with ND = LOD), reflecting the large uncertainty in the concentration estimates due to the large number of non-detects in the data and to the fact that different studies were used in the

generation of these sets of distributions. When estimates using the lower-bound distributions are considered, the Western European intake estimates are driven by the fish and shellfish intakes, whereas meat, fat and oil and fish and shellfish intakes drive the North American intake estimates (Table 50). On the other hand, when estimates using the upper-bound distributions are considered, the Western European intake estimates are driven by the fish and shellfish and dairy intakes, whereas dairy intakes drive the North American intake estimates and fish and shellfish and fruit and vegetable intakes drive the Far Eastern intake estimates (Table 50). It should be noted that these estimates are based in some instances on a very small number of studies and are therefore largely uncertain.

Table 50. Contribution of the various food groups to the mean long-term intake in the GEMS/Food regional diets

Diet	Dairy & dairy products	Eggs	Fish & shellfish	Fruits & vegetables	Meat & poultry	Fats & oils	Total
Contributions to total diet at the mean level (ng/day) (ND = 0)							
Western Europe	10	2	81	6	17	13	128
North America	25	8	42	6	63	46	188
Far East	NA	NA	NA	NA	NA	NA	NA
Per cent contributions to total diet at the mean level (ND = 0)							
Western Europe	8%	1%	64%	4%	13%	10%	100%
North America	13%	4%	22%	3%	33%	24%	100%
Far East	NA	NA	NA	NA	NA	NA	NA
Contributions to total diet at the mean level (ng/day) (ND = LOD)							
Western Europe	81	5	85	8	49	45	274
North America	81	5	42	8	52	52	240
Far East	8	2	28	27	1	13	79
Per cent contributions to total diet at the mean level (ND = LOD)							
Western Europe	30%	2%	31%	3%	18%	17%	100%
North America	34%	2%	17%	3%	22%	22%	100%
Far East	10%	2%	36%	34%	2%	17%	100%

LOD, limit of detection; NA, not available; ND, non-detects

7.3.3 Dietary intake for infants

Nearly all PBDE intake studies are calculated for adults. In Table 51, preliminary estimates of the daily PBDE intake by nursing infants via human milk are presented. On the basis of this information, it can be concluded that the daily intake for breastfed infants is between 1 and 2 orders of magnitude higher than that for adults.

Table 51. Daily PBDE intake in nursing infants (calculation made under the assumption of consumption of 800 ml milk per day with a lipid content of 3%)

	Germany	United States	Viet Nam
PBDE concentration in human milk (ng/g lipid)	2.3 ^a	30 ^b	0.48 ^b
Total PBDE intake per day (ng)	48.3	630	10
Total PBDE intake per day (ng/kg bw)	9.7	126	2

^a Fürst (2001).

^b Schecter et al. (2004c).

7.4 Potential sources of intake other than food

The knowledge of pathways/sources other than food for the daily intake of PBDEs is quite limited. Potential additional pathways for intake are inhalation of air and the uptake of dust (especially household dust). Detailed information on the importance of these pathways is difficult to obtain. Wijesekera et al. (2002) analysed nine indoor air samples for PBDEs (presented in Table 52).

Table 52. PBDE concentrations in indoor air samples

Location	Sum PBDEs (ng/m ³) ^a	Location	Sum PBDEs (ng/m ³)
1W	0.77	3D	2.35
2W	15.9	7D	1.62
4W	17.9	9D	0.91
5W	1.43		
6W	5.73		
8W	9.1		
Median W	2.3	Median D	1.62

From Wijesekera et al. (2002)

D, domestic; W, workplace

^a Total of BDE-47, BDE-99, BDE-100, BDE-153, BDE-154.

The adult respiration rate was estimated as 20 m³/day. Due to the low level of outdoor air PBDE concentrations (low pg/m³ range; de Wit, 2002), no remarkable

influence was expected. Using the measured data, assuming a 100% absorption of intake and taking into account a 40-h weekly working time, a daily human exposure to PBDEs via inhalation was calculated at 32.9 ng/person. The intake via contaminated dust is more difficult to estimate. Various authors have described wide ranges of contamination of household dust. Data from Leonards et al. (2001), Knoth et al. (2002, 2003), Sjödin et al. (2004b), Stapelton et al. (2004), Schecter et al. (2005) and Wilford et al. (2005) are presented in Table 53.

Table 53. PBDE concentrations in household dust

Area	Author	Year of collection	<i>n</i>	Mean values (range) (ng/g)
EU	Leonards et al. (2001)	1995	7 ^a	195
Germany	Knoth et al. (2002, 2003)	2000–2001	40	63
United States	Stapelton et al. (2004)	2004	17	5563 (780–29 700)
United States	Schecter et al. (2005)	2004	9	12 136 (705–69 283)
Canada	Wilford et al. (2005)	2002–2003	68	5500 (170–170 000)
United States	Sjödin et al. (2004b)	2004	10	4240 (534–28 763)
Australia	Sjödin et al. (2004b)	2004	10	1166 (506–12 772)
United Kingdom	Sjödin et al. (2004b)	2004	10	10 292 (952–54 313)
Germany	Sjödin et al. (2004b)	2004	10	17 (74–552)

^a Includes The Netherlands, Finland, Denmark (*n* = 2), Sweden, Italy (*n* = 2).

8. PREVENTION AND CONTROL

As with other lipophilic contaminants, control of PBDE residues in animal feed is likely to have an impact on the concentrations of PBDEs found in meat, poultry, farmed fish and other animal-derived products. In addition, indoor air and dust are currently being investigated as possible significant sources of human exposure to PBDEs from consumer products (Wilford et al., 2004; Jones-Otazo et al., 2005).

9. LEVELS AND PATTERNS OF CONTAMINATION OF HUMANS

9.1 Comparison of analytical data in different tissues

With respect to comparability of PBDE data determined in different samples, it is important to have data for concentrations in various tissues. As will be seen, a comparison is possible only in the case of lipid-based data. Hirai et al. (2003)

analysed the distribution of PBDEs among bile, blood, liver and adipose tissue. Table 54 gives the relationship between PBDE concentrations in these tissues.

Table 54. Comparison of PBDE congener levels among different tissues in Japan: Bile, blood, liver and adipose tissue

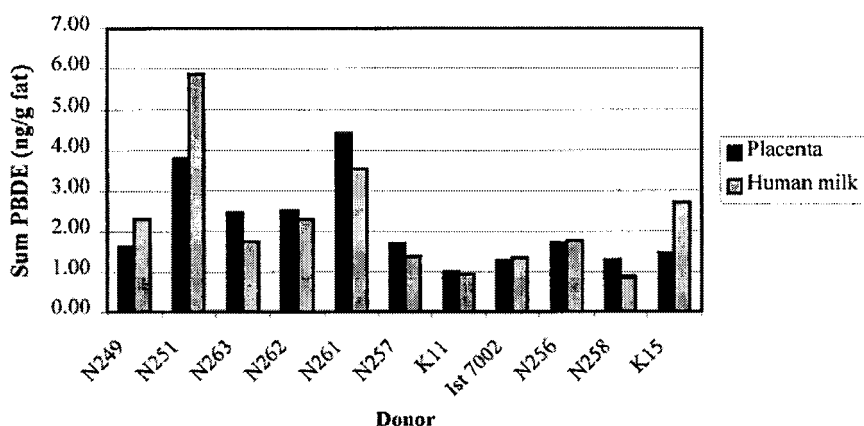
BDE	Mean \pm SD					
	Bile/ blood	Liver/ blood	Adipose tissue/ blood	Liver/ bile	Adipose tissue/ bile	Liver/ adipose tissue
71	1.1 \pm 0.6	1.5 \pm 0.6	1.4 \pm 0.8	2.7 \pm 3.1	2.3 \pm 1.8	1.2 \pm 0.7
47	1.6 \pm 1.5	1.2 \pm 0.4	1.6 \pm 0.7	3.3 \pm 3.6	4.3 \pm 5.9	1.0 \pm 1.2
100	1.6 \pm 1.5	1.1 \pm 0.4	1.7 \pm 0.7	2.3 \pm 2.3	3.9 \pm 3.8	0.7 \pm 0.5
99	2.2 \pm 2.6	1.3 \pm 0.6	1.6 \pm 0.7	3.4 \pm 3.0	4.6 \pm 4.6	0.9 \pm 0.9
153	1.5 \pm 1.3	1.1 \pm 0.5	1.8 \pm 1.1	2.3 \pm 2.2	4.2 \pm 5.9	0.7 \pm 0.4
Total (25 congeners)	1.3 \pm 1.0	1.2 \pm 0.4	1.7 \pm 0.9	2.4 \pm 2.4	3.8 \pm 5.1	0.8 \pm 0.6

From Hirai et al. (2003)

SD, standard deviation

A comparison of the PBDE distribution between human milk and placenta was undertaken for sum PBDEs (BDE-28, BDE-47, BDE-99 and BDE-153) by Strandman et al. (2000). As can be seen in Figure 14, the concentrations are similar in both tissues.

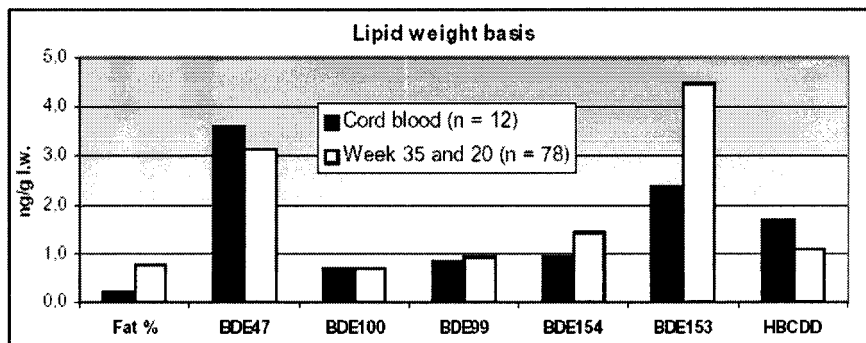
Figure 14. Sum of PBDEs in human milk and placenta from 11 donors



Taken from Strandman, T., Koistinen, J. & Vartiainen, T. (2000) Polybrominated diphenyl ethers (PBDEs) in placenta and human milk. *Organohalogen Compd.*, **47**, 61–64.

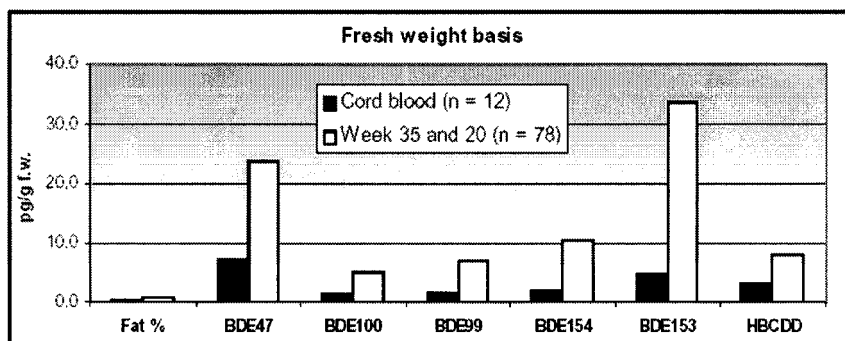
Weiss et al. (2004a, 2004b) compared PBDE concentrations in serum samples from Dutch mothers and from infants. As can be seen in Figures 15 and 16, the higher brominated congeners tend to have higher concentrations in the maternal samples when comparing the lipid-based values. Due to the low lipid content of cord blood, all values are significantly lower when reported on a fresh weight basis.

Figure 15. Mean PBDE concentrations on a lipid weight basis in Dutch mothers and infants



Taken from Weiss, J., Meijer, L., Sauer, P., Linderholm, L., Athanasiadis, I. & Bergman, A. (2004a) PBDE and HBCDD levels in blood from Dutch mothers and infants. In: *The Third International Workshop on Brominated Flame Retardants*. BFR 2004, Toronto, Ontario, 6–9 June 2004, pp. 71–74 (<http://www.bfr2004.com>).

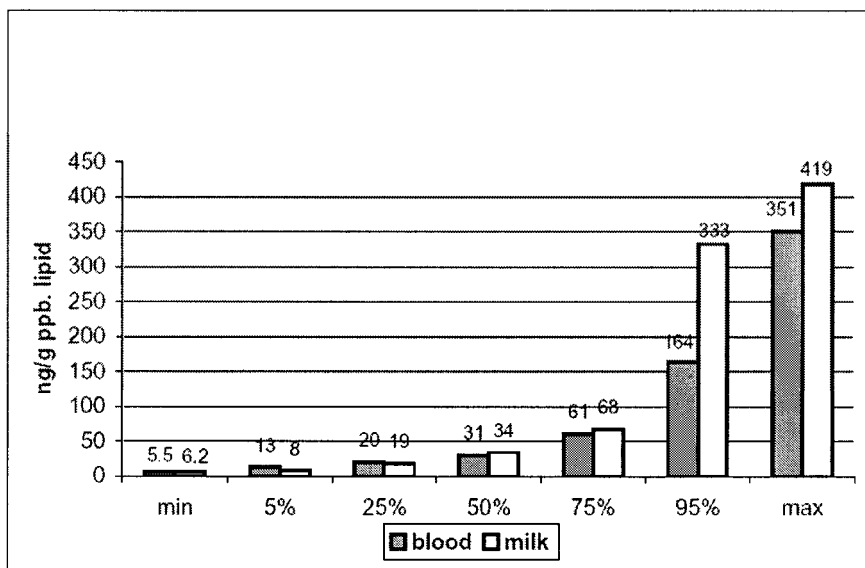
Figure 16. Mean PBDE concentrations on a wet weight basis in Dutch mothers and infants



Taken from Weiss, J., Meijer, L., Sauer, P., Linderholm, L., Athanasiadis, I. & Bergman, A. (2004a) PBDE and HBCDD levels in blood from Dutch mothers and infants. In: *The Third International Workshop on Brominated Flame Retardants*. BFR 2004, Toronto, Ontario, 6–9 June 2004, pp. 71–74 (<http://www.bfr2004.com>).

A comparison of PBDE concentrations in blood and human milk was reported by Schechter et al. (2004b). They analysed 52 milk samples and 29 blood samples collected in 2003. When looking at the total PBDEs, presented in Figure 17, a great similarity can be observed in both tissues. On the other hand, it is important to mention that the samples analysed are from different individuals.

Figure 17. PBDE levels in human milk (n = 52) and blood (n = 29) from the United States



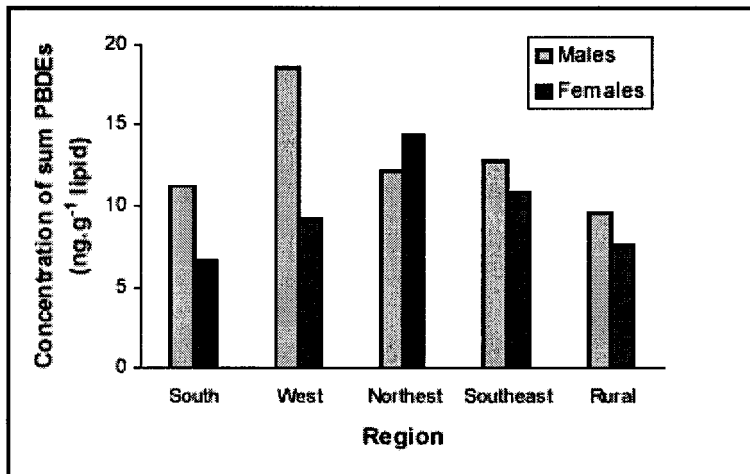
Taken from Schechter, A., Päpke, O., Tung, K.C., Joseph, J., Dahlgren, J. & Harris, T.R. (2005) Polybrominated diphenylether (PBDE) flame retardants in the US population: current levels, temporal trends, and comparison with dioxins, dibenzofurans and polychlorinated biphenyls. *J. Occup. Environ. Med.*, **47** (3), 199–211.

9.3 Different countries

9.3.1 Australia

The first data for PBDEs in the Australian population were provided by Harden et al. (2004). They analysed pooled samples collected in 2003 for different areas from Australia for males and females. Each pool consisted of about 100 individual samples. The age of the participants was between 31 and 45 years. Results of the study are given in Figure 18. The average total concentration (sum of 13 PBDEs) across all samples was 11.0 ± 3.9 ng/g lipid. Overall concentrations ranged from 6.7 ng/g lipid in a pooled sample collected from females from the South region to a maximum of 19 ng/g lipid in a pooled sample collected from males in the West region.

Figure 18. Concentration of PBDE congeners in blood sera from Australians aged 31–45 years



Taken from Harden, F., Toms, L.M., Ryan, J.J. & Müller, J. (2004) Determination of the levels of polybrominated diphenylethers (PBDEs) in pooled blood sera obtained from Australians aged 31–45 years. In: *The Third International Workshop on Brominated Flame Retardants*. BFR 2004, Toronto, Ontario, 6–9 June 2004, pp. 59–62 (<http://www.bfr2004.com>).

9.3.2 Belgium

Levels of selected PBDEs in Belgian human milk were published by Pirard et al. (2003). The samples, 14 from primiparous and multiparous women between the ages of 26 and 38 years, were collected between 2000 and 2001. The values are given in Table 55.

Table 55. BDE concentrations in investigated Belgian breast milk samples

BDE congener	Concentrations (ng/g lipid weight)	SD
BDE-28	0.09	0.17
BDE-47	1.69	1.90
BDE-100	0.17	0.209
BDE-99	0.35	0.319
BDE-154	0.12	0.082
BDE-153	0.43	0.388
Total BDEs	2.85	

From Pirard et al. (2003)

SD, standard deviation

9.3.3 Canada

For Canada, 10 individual human milk samples were obtained in 1992 as part of a countrywide survey of concentrations of organochlorines in Canadian women. The results of this study are shown in Table 56.

Table 56. Concentrations of PBDEs in individual human milk (*n* = 10) collected in 1992 from Ontario and Quebec

Sample no.	Concentration (ng/g, lipid basis)						
	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-183	Σ6 BDEs
1	0.10	1.64	1.11	0.25	0.30	0.15	3.55
2	0.12	2.09	1.26	0.36	0.27	0.08	4.18
3	ND (0.05)	0.31	0.10	0.07	0.21	0.10	0.79
4	0.07	0.91	0.24	0.10	0.14	ND (0.05)	1.46
5	0.13	0.70	0.30	0.16	0.34	ND (0.05)	1.63
6	0.15	2.46	0.87	0.42	0.23	0.10	4.23
7	0.26	4.57	1.70	0.65	1.31	0.71	9.20
8	1.22	18.72	5.63	2.14	0.76	ND (0.05)	28.47
9	ND (0.05)	0.65	0.22	0.12	0.35	0.39	1.73
10	0.10	1.85	0.43	0.16	0.14	ND (0.05)	2.68
Median	0.13	1.75	0.65	0.21	0.29	0.13	3.14
Average	0.22	3.39	1.19	0.44	0.41	0.15	5.79

From Ryan & Patry (2000)

ND, not detected

At the same time, pooled milk samples were also prepared by combining samples from 20 individuals from each of five geographic regions and from 100 and 200 individuals across Canada. The data are reported in Table 57.

Further information on levels of PBDEs in maternal human blood from ethnic groups living in different areas is presented by Ryan & van Oostdam (2004) (Table 58).

9.3.4 Czech Republic

Crhova et al. (2002) analysed adipose tissue samples collected postmortem between 2000 and 2001 from 24 individuals. The age of the donors ranged between 23 and 78 years. Concentrations are given in Table 59. In the groups studied, lower concentrations of PBDEs were observed in women than in men.

Table 57. Variation of PBDEs in composite samples of Canadian human milk samples

Region of origin	Time collected	No. of individual milk samples	BDE concentration (sum of six congeners) (ng/g milk lipid)
Maritimes	1992	20	19.08
Quebec	1992	20	18.75
Ontario	1992	20	2.57
Prairies	1992	20	5.70
Canada-wide	1992	100	16.24
Canada-wide	1981–1982	200	0.21

From Ryan & Patry (2000)

9.3.5 *Faroe Islands*

The aim of the study of Fängström et al. (2004b) was to determine the temporal trend for PBDEs in human milk samples from the Faroe Islands. It was demonstrated that an ongoing increase is taking place (Table 60).

9.3.6 *Finland*

The first measurements for the Finnish population were performed by Strandman et al. (1999), as given in Table 61. The tissue samples were randomly selected from an epidemiological population study. The range for the sum of three PBDE congeners was between 6.15 and 18.72 ng/g lipid.

Strandman et al. (2000) analysed pairs of placenta and human milk from 11 mothers in Finland, collected between 1994 and 1998. The data for the human milk samples are presented in Table 62.

9.3.7 *Germany*

The first PBDE data for Germany have been published by Schröter-Kermani et al. (2000). The study was conducted with blood samples archived by the German environmental specimen bank. Whole blood samples from 20 subjects (10 male and 10 female) participating in the monitoring programmes were chosen. The age of the participants ranged between 20 and 30 years. As can be seen in Table 63, a time-related increase was found. During the period 1985–1999, median PBDE concentrations in blood increased from 3.08 to 4.69 ng/g lipid.

Fürst (2001) compared PBDE data in human milk collected in 1992 and 2000. The values for most congeners were quite similar in the samples of either collection period. Slightly higher values were found only for BDE-153 and BDE-183 in the samples collected in 2000.

Table 58. PBDE congeners in maternal human blood plasma composites collected from northern populations

Code	Year of sampling	Ethnicity	Region	No. of individuals	Concentrations (ng/g, lipid basis)								
					#28	#47	#85	#99	#100	#153	#154	#183	ΣPBDE
M1	1998–1999	Caucasian	Inuvik	19	0.8	15.1	0.4	4.2	1.6	2.1	0.2	1.4	25.8
M2	1998–1999	Dene/Metis	Inuvik	40	1.2	12.6	0.8	4.9	4.1	6.7	0.7	1.1	31.9
M3	1994–1995	Inuit	Kitikmeot	13	0.2	4.8	0.8	7.6	2.1	2.5	0.9	0.8	19.6
M4	1994–1995	Inuit	Kitikmeot	34	0.3	5.5	0.9	9.5	1.7	1.9	0.7	0.5	21.0
M5	1994–1999	Caucasian	Mackenzie	24	0.5	6.7	0.2	3.0	0.9	0.8	ND (0.4)	0.9	13.1
M6	1994–1999	Caucasian	Mackenzie	29	1.4	23.5	1.1	11.5	4.1	3.3	0.9	0.8	46.5
M7	1994–1999	Caucasian	Mackenzie	29	1.1	7.2	0.2	2.8	1.1	1.5	0.3	1.1	15.3
M8	1994–1995	Dene/Metis	Mackenzie	21	0.9	7.9	ND (0.5)	3.7	1.0	0.8	ND (0.4)	0.6	14.9
M9	1994–1995	Dene/Metis	Mackenzie	10	0.8	10.3	0.2	3.7	1.1	1.0	0.3	0.5	18.0
M10	1994–1999	Inuit	Several	23	1.1	15.6	0.4	4.8	2.3	2.0	0.5	0.6	27.2
				Mean	0.8	10.9	0.5	5.6	2.0	2.3	0.5	0.8	23.3
				Range	0.2– 1.4	4.8– 23.5	ND– 1.1	2.8– 11.5	1.0– 4.1	0.8– 6.7	ND– 0.9	0.5– 1.4	13.1– 46.5

From Ryan & van Oostdam (2004)

ND, not detected (LOD given in parentheses, where available)

Table 59. Concentration of PBDE congeners in human adipose tissue

PBDE congener	Concentration (ng/g fat)					
	Male (n = 10)			Female (n = 14)		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
BDE-28	0.13	0.028	0.53	0.034	0.004	0.145
BDE-47	1.18	0.230	3.69	0.400	0.087	0.821
BDE-99	0.340	0.075	1.14	0.117	0.015	0.233
BDE-100	0.590	0.088	2.35	0.132	0.013	0.330
BDE-154	0.055	0.012	0.110	0.026	0.002	0.054
BDE-153	0.520	0.520	1.89	0.413	0.080	1.47
BDE-183	0.440	0.075	1.59	0.320	0.049	1.74
Total PBDEs	3.26	1.03	11.3	1.44	0.25	4.79

From Crhova et al. (2002)

Table 60. Concentrations of congeners identified in human milk from the Faroe Islands^a

	Concentrations (ng/g lipid weight)		
	1987	1994–1995	1998–1999
	(n = 10)	(n = 10)	(n = 10)
BDE-47	0.5	1.2	1.7
BDE-99	0.20	0.50	1.0
BDE-100	0.25	0.60	1.0
BDE-153	0.60	1.4	3.6
Sum PBDE	1.5	3.6	7.2

From Fångström et al. (2004b)

^a The samples were pooled with samples in each pool.

Very recent data for German mothers are reported by Vieth et al. (2004), with milk samples collected in 2001–2003. Statistical data from this investigation are presented in Table 64.

Significantly lower PBDE levels were observed in the group of mothers preferring a vegetarian or vegan diet and in the mothers nursing their second or third child. However, it should be pointed out that the sample size of mothers nursing their second or third child is higher in the group of vegetarians/vegans. BDE-209 was quantified in 40% of the human milk samples corresponding to background level.

Table 61. Concentration of three PBDE congeners in Finnish human adipose tissue

Age (years)	Concentration (ng/g fat)			
	BDE-47	BDE-99	BDE-153	Total BDEs (three congeners)
36	3.07	0.80	3.05	6.92
45	6.17	2.77	2.88	11.82
47	8.76	5.51	3.74	18.0
54	3.94	0.74	1.47	6.15
57	6.55	1.55	3.25	11.35
62	16.75	3.27	1.68	21.7
64	6.23	1.31	1.26	8.8
69	14.46	2.45	1.81	18.72
82	3.48	1.40	1.61	6.49
84	3.39	0.88	2.54	6.81
				11.7 (mean)

From Strandman et al. (1999)

Table 62. Concentrations of PBDEs in human milk (n = 11)

	Concentration (ng/g lipid)				
	BDE-28	BDE-47	BDE-99	BDE-153	Total BDEs (four congeners)
Mean	0.16	1.31	0.39	0.39	2.25
SD	0.15	1.15	0.23	0.20	1.73
Median	0.13	0.85	0.35	0.29	1.62
Minimum	0.04	0.30	0.14	0.19	0.67
Maximum	0.59	4.25	0.94	0.72	6.5

From Strandman et al. (2000)

SD, standard deviation

Table 63. Statistical data on Σ PBDE levels in human blood, time trend, Germany

Year	n	Concentrations (ng/g lipid)					
		Maximum	25th percentile	Median	Arithmetic mean	Geometric mean	75th percentile
1985	20	15.72	1.94	3.08	3.91	2.86	4.03
1990	20	12.35	1.73	3.88	4.89	3.57	6.82
1995	19	17.56	3.30	3.90	5.55	4.59	5.9
1999	20	12.61	3.98	4.69	5.57	4.87	7.27

From Schröter-Kermani et al. (2000)

Table 64. PBDE concentrations in human milk from Germany (ng/g fat) sampled between November 2001 and December 2003

BDE	Omnivores and vegetarians/vegans together					Omnivores		Vegetarians/vegans
	1st sampling period					2nd sampling period	1st sampling period	1st sampling period
	<i>n</i> = 62					<i>n</i> = 31	<i>n</i> = 37	<i>n</i> = 25
	Mean	Median	95th percentile	Maximum	<i>n</i> < LOQ ^a	Mean	Mean	Mean
BDE-28	0.04	0.04	0.12	0.17	9	0.04	0.05	0.04
BDE-47	0.82	0.54	3.52	4.50	1	0.78	0.99	0.58
BDE-66	0.01	0.01	0.03	0.06	14	0.01	0.02	0.01
BDE-99	0.25	0.17	0.92	1.30	2	0.25	0.30	0.16
BDE-100	0.21	0.17	0.67	1.10	0	0.19	0.23	0.18
BDE-153	0.63	0.53	1.54	1.90	0	0.50	0.66	0.57
BDE-154	0.02	0.02	0.06	0.07	0	0.02	0.03	0.02
BDE-183	0.09	0.04	0.34	0.63	12	0.06	0.10	0.07
BDE-209	0.17	0.10	0.59	1.00	37	0.11	0.17	0.17
Sum PBDEs	2.23	1.78	6.69	7.25		1.95	2.54	1.78

From Vieth et al. (2004)

^a Non-detects are set at one half the LOQ.

9.3.8 Italy

The first data of the PBDE concentrations in Italian nulliparous women of reproductive age were reported by De Felip et al. (2003). They analysed blood samples originating from three pools consisting of 10, 6 and 6 individuals, respectively. The mean concentration, as a sum of six congeners, was 2.0 ng/g lipid.

Ingelido et al. (2004) reported data on PBDE levels in human milk from mothers from the general population of Rome and Venice and its surroundings. The mean values, as presented in Table 65, range between 1.6 and 4.1 ng/g lipid. A positive correlation with the fish consumption was not observed.

Table 65. PBDE congener concentrations and distribution in pooled human milk samples from Venice and Rome

PBDE	Concentrations (ng/g lipid)			
	Venice (LC) ^a (n = 10)	Venice (MC) ^b (n = 13)	Venice (HC) ^c (n = 6)	Rome (n = 10)
BDE-17	0.004	0.004	<0.002	0.004
BDE-28	0.065	0.064	0.036	0.082
BDE-47	1.5	0.90	0.55	1.9
BDE-66	0.015	0.037	<0.006	0.019
BDE-85	0.035	0.045	0.018	0.074
BDE-99	0.41	0.51	0.14	0.97
BDE-100	0.28	0.19	0.15	0.48
BDE-138	<0.01	0.020	<0.01	0.013
BDE-153	0.41	0.47	0.60	0.47
BDE-154	0.025	0.047	0.020	0.070
BDE-183	0.061	0.19	0.050	0.092
ΣPBDEs	2.8	2.5	1.6	4.1

From Ingelido et al. (2004)

^a LC= low fish consumption.

^b MC = medium fish consumption.

^c HC = high fish consumption.

9.3.9 Japan

Human adipose tissue samples from around the Tokyo area in Japan were collected in 1970 (n = 10) and 2000 (n = 10) from 40- to 50-year-old female individuals (Choi et al., 2002). As can be seen in Table 66, a dramatic increase over time can be observed for all congeners.

Table 66. Concentrations of seven PBDE congeners and total PBDEs from Japanese human adipose tissue in 1970 and 2000

Compound	Concentrations (pg/g fat)			
	1970 (<i>n</i> = 10)		2000 (<i>n</i> = 10)	
	Median	Range	Median	Range
BDE-28	2.3	<1.0–7.6	76	47–487
BDE-47	17.0	4.4–60.4	459	109–979
BDE-100	2.1	<2.5–6.1	250	41–527
BDE-99	3.9	<2.5–13.9	118	42–362
BDE-154	<6.3	<6.3	60	14–104
BDE-153	<6.3	<6.3	382	122–631
Total PBDEs	29.2	6.8–78.4	1288	466–2753

From Choi et al. (2002)

Takasuga et al. (2002) published human PBDE residue data from a study for the development of halogenated components. Nine married couples, 37–48 years old, participated in the study. Concentrations for PBDEs are given in Table 67. The mean and median values are 3550 and 1902 pg/g lipid, respectively.

Table 67. PBDE concentrations in Japanese human blood for a 2-year study period

PBDE	Concentration (pg/g fat weight)			
	Average	Median	Minimum	Maximum
BDE-15	770	100	53	20 000
BDE-28	410	125	65	8 600
BDE-47	830	1200	100	14 000
BDE-99	210	670	130	63 000
BDE-100	260	140	57	1200
BDE-153	670	510	370	2100
BDE-183	160	140	56	520
Total PBDEs	3550	1902	951	37 250

From Takasuga et al. (2002)

9.3.10 Mexico

The first data on PBDE contamination of Mexican women were reported by Lopez et al. (2004). They analysed blood and milk samples originating from two different areas, 300 km apart. The authors stated that in some samples, BDE-209

was the dominant congener or was a significant contributor to the sum of PBDEs. As presented in Table 68, the concentrations are significantly higher in plasma than in milk.

Table 68. Concentrations of individual PBDEs and total PBDEs in plasma and milk samples from Mexico

Analyte	Concentration (ng/g lipid weight)			
	Plasma (San Luis Potosi City) (<i>n</i> = 5)		Milk (La Huasteca Potosina) (<i>n</i> = 7)	
	Mean	Range	Mean	Range
BDE-47	9.0	3.0–14.5	1.7	1.1–4.3
BDE-99	2.0	0.6–3.6	0.6	0.3–1.2
BDE-100	3.7	1.8–7.4	0.8	0.5–1.3
BDE-154	1.0	0.5–1.3	0.2	0.1–0.3
BDE-153	3.9	0.9–6.6	0.8	0.4–1.6
BDE-209	9.5	4.8–14.6	0.3	0.1–0.6
Sum PBDEs	29.7	21.5–37.5	2.1	0.8–5.4

From Lopez et al. (2004)

9.3.11 The Netherlands

In 1998, 108 breast milk samples were obtained from Dutch primiparous women and analysed for PBDEs (de Winter-Sorkina et al., 2003). The results of the study are given in Table 69.

Weiss et al. (2004a, 2004b) analysed a number of blood samples collected from Dutch mothers and infants. The data measured for the adults are given in Table 70.

9.3.12 Norway

For four different areas of Norway, Polder et al. (2004) analysed human milk samples collected in 2003 (Table 71). The median values found for Norway range between 1.66 and 2.52 ng/g lipid.

A time trend-related study on PBDEs in serum samples from the general population in Norway was performed by Thomsen et al. (2001). As with other countries, a time trend was observed in Norway as well (Figure 19).

Additional data from Norway on a congener-specific basis are presented in another study from Thomsen et al. (2004). The samples (*n* = 130) were obtained from individuals reporting a normal consumption of fish and game. The samples collected in 1999 are presented in Table 72.

Table 69. Statistical summary of PBDE congener concentrations measured in 103 Dutch breast milk samples taken in 1998

Congener	Number > LOD	Concentration (ng/g fat)				
		Minimum	Maximum	Median	Mean	Relative standard deviation
BDE-17	10	<0.03	0.13	<0.03		
BDE-28	108	0.05	0.43	0.11	0.13	0.50
BDE-47	108	0.45	6.50	1.23	1.56	0.70
BDE-66	36	<0.06	0.32	<0.06		
BDE-85	13	<0.08	0.17	<0.08		
BDE-99	108	0.17	2.70	0.40	0.50	0.76
BDE-100	108	0.09	1.72	0.31	0.37	0.67
BDE-153	108	0.33	3.88	0.91	1.02	0.51
BDE-154	51	<0.08	0.26	<0.08		
BDE-183	105	<0.09	1.90	0.42	0.45	0.61
Total PBDEs		1.43	18.01	3.63		

From de Winter-Sorkina et al. (2003)

Table 70. PBDE concentrations in maternal serum collected at gestation week 20 (n = 8) and week 35 (n = 70)

	PBDE concentration (ng/g lipid weight) ^a		
	Mean	Median	Range
BDE-47	3.2	2.4	0.6–13
BDE-99	0.92	0.76	<0.12–4.3
BDE-100	0.69	0.69	0.11–2.9
BDE-153	4.5	4.5	0.79–39
BDE-154	1.5	1.1	0.26–6.

From Weiss et al. (2004a, 2004b)

^a Fat percentage: mean 0.77%, median 0.74%, range 0.4–1.8%.

9.3.13 Republic of Korea

Lee et al. (2002) reported a study of potential exposure of Korean municipal waste incinerator workers, with the comparison group taken from the general population living near the incinerator. The total value found for the total of five congeners was 41.577 and 24.695 ng/g lipid for the incinerator workers and general population, respectively (Table 73).

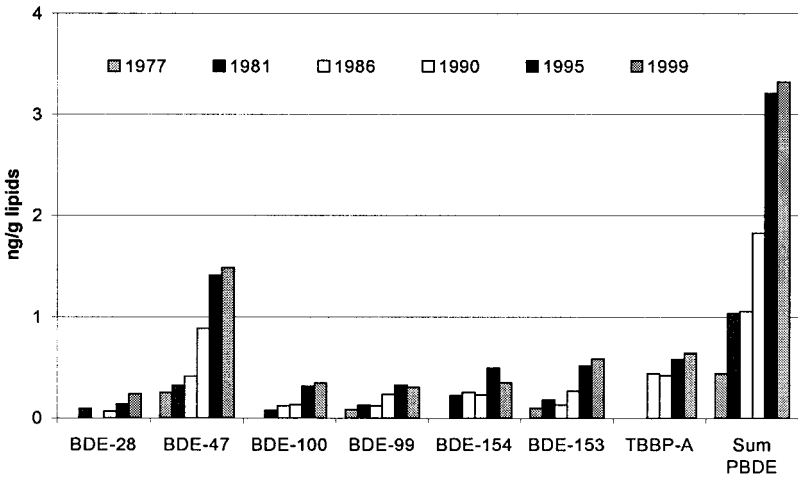
Table 71. Median concentrations and ranges of sum PBDEs in human milk from four different areas in Norway

	Sum PBDE ^a concentration (ng/g milk fat)			
	Rogaland (n = 17)	Telemark (n = 4)	Troms (n = 8)	Östfold (n = 3)
Median	2.47	1.66	2.52	1.95
Minimum	1.00	1.06	1.41	1.73
Maximum	10.79	2.55	10.56	3.76

From Polder et al. (2004)

^a Sum PBDEs: sum of BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154.

Figure 19. Concentration of individual PBDE congeners and sum PBDEs in pooled serum samples from Norwegian men age 40–50 years sampled between 1977 and 1999



Taken from Thomsen, C., Lundanes, E. & Becher, G. (2001) A time trend study on brominated flame retardants in serum samples from the general population in Norway. *Organohalogen Compd.*, **52**, 206–209.

9.3.14 Spain

Schumacher et al. (2004) analysed milk samples collected in 2002 from women living near and far from a hazardous waste incinerator in Spain. They concluded that living in the vicinity of the hazardous waste incinerator did not suggest any additional exposure to PBDEs (or PCBs) for the general population of the area. The PBDE data are given in Table 74.

Table 72. PBDEs in blood, Norway, $n = 123$

	#28	#47	#99	#100	#153	#154	#183	Total
Mean (ng/g lipid)	0.30	2.98	1.00	0.56	1.45	0.58	0.29	6.84
Median (ng/g lipid)	0.18	1.68	0.69	0.34	1.11	0.41	0.22	4.71
RSD ^a (%)	187	268	119	190	87	82	76	162
Minimum (ng/g lipid)	0.05	0.25	0.27	0.04	0.02	0.10	0.009	1.40
Maximum (ng/g lipid)	4.56	87.93	10.60	11.2	9.56	2.91	1.09	121.7
Number of detects	66	123	123	119	123	122	61	123

From Thomsen et al. (2004)

RSD, relative standard deviation of the mean

^a Seven samples have been excluded from calculation due to high blank value.

Table 73. Comparison of the concentrations of PBDE congeners in blood between this study and previous studies in general population ($n = 11$), Republic of Korea

Congener	Concentration (ng/g lipid based)	
	Waste incinerator workers	General population
BDE-33	1.163	
BDE-47	15.860	9.842
BDE-99	5.468	4.316
BDE-100	2.655	1.692
BDE-153	7.321	5.217
BDE-154	0.487	
BDE-183	8.603	3.628
Total PBDEs	41.557	24.695

From Lee et al. (2002)

9.3.15 Sweden

For Swedish human milk, decreasing levels of certain organochlorine compounds have been found by Norén & Meironyté Guvenius (2000). In contrast, levels for PBDEs have increased continuously since 1972. Recently, Meironyté Guvenius & Norén (2001) reported that after the peak level found for Swedish human milk in 1997, a decline for the years 1998–2000 was observed (Figure 20).

In 1998, Darnerud et al. (1998a) reported on PBDE values in 39 individual samples of breast milk from primiparous woman from Uppsala County (Table 75). The values are provided on both a fresh weight and lipid weight basis.

Further data for Uppsala County are given in Table 76.

Table 74. Levels of total PBDEs and percent fat in samples of human milk from women living near a hazardous waste incinerator, Catalonia, Spain

Sample code	Total PBDE concentration (ng/g fat)	Fat content (%)
1	5.6	3.2
2	2.5	3.1
3	3.7	1.6
4	2.6	1.6
5	1.8	3.0
6	1.5	1.7
7	1.2	4.1
8	1.5	1.6
9	1.4	3.0
10	1.4	4.7
11	1.0	3.6
12	6.6	2.5
13	2.3	4.1
14	1.3	4.3
15	1.7	3.4
Mean	2.4	3.0

From Schumacher et al. (2004)

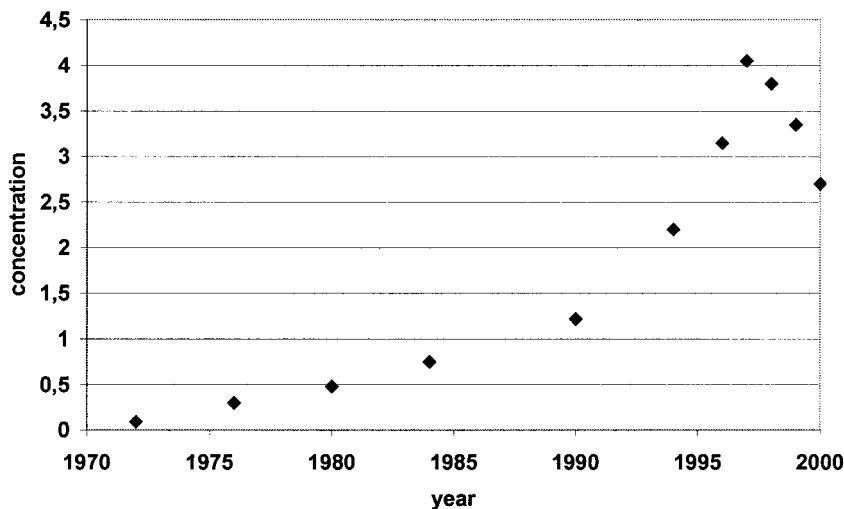
In the study of Lind et al. (2001), the authors stated that the PBDE levels in human milk are associated with factors other than age, body mass index, alcohol consumption, smoking habits and dietary intake of PBDEs.

Important information on the distribution of PBDEs in the general population was given by van Bavel et al. (2002). They collected blood from a cohort of 220 persons from Sweden. The distribution of selected samples is shown in Figure 21. A normal distribution can be seen between 1.0 and 13 ng/g lipid, with a mean value of 4.9 ng/g lipid. However, in 10 of the remaining samples, extremely high values for PBDEs were found, with one exceeding 1000 ng/g lipid.

9.3.16 United Kingdom

Kalantzi et al. (2004) collected human milk samples between late 2001 and early 2003 from 54 mothers in the United Kingdom. Of these, 27 originated from southeast England (London) and the other 27 from northwest England (Lancaster). For BDE-47 and Σ PBDEs, a difference could be found for the investigated areas, as presented in Table 77.

Figure 20. Mean concentration of PBDEs (ng/g lipid) in Swedish human milk from 1972 to 2000



Taken from Meironyté Guvenius, D. & Norén, K. (2001) Polybrominated diphenyl ethers in Swedish human milk. The follow-up study. In: *The Second International Workshop on Brominated Flame Retardants*. BFR 2001, 14–16 May 2001, Stockholm University, Stockholm, pp. 303–305.

Table 75. PBDE levels in breast milk from primiparous women in Uppsala County, Sweden

	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	Sum PBDEs
<i>pg PBDE/g fat weight</i>						
Mean	2516	717	475	648	70	4452
Median	1830	442	340	478	60	3373
Minimum	331	181	60	255	30	1139
Maximum	16 100	4470	5140	4320	270	28 170
<i>pg PBDE/g fresh weight</i>						
Mean	77	24	14	19	2.1	137
Median	58	16	10	14	1.5	102
Minimum	8	4	1.5	8	1.5	28
Maximum	358	222	114	96	6	626

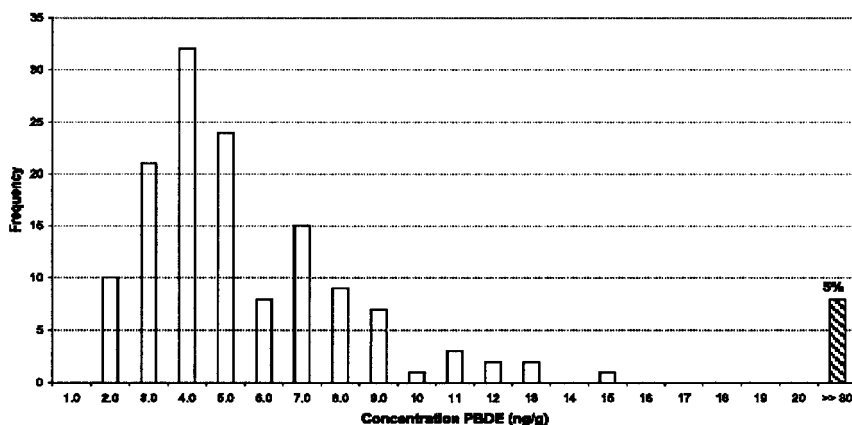
From Darnerud et al. (1998a)

Table 76. PBDE levels in breast milk from primiparous women (n = 93) in Uppsala County, Sweden

	PBDE concentration (ng/g fat weight)					
	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	ΣPBDEs
Mean	2.35	0.619	0.377	0.597	0.068	4.01
Median	1.78	0.432	0.269	0.496	0.060	3.15
Minimum	0.20	0.100	0.050	0.197	0.030	0.906
Maximum	16.1	4.47	5.14	4.32	0.270	28.2

From Lind et al. (2001)

Figure 21. The distribution of 143 samples rerun using negative chemical ionization to enhance LOD for PBDEs; 5% of the samples showed levels of PBDEs above 30 ng/g lipid



Taken from van Bavel, B., Hardell, L., Kittl, A., Lijedahl, M., Karlsson, M., Pettersson, A., Tysklind, M. & Lindström, G. (2002) High levels of PBDEs in 5 % of 220 blood samples from the Swedish population. *Organohalogen Compd.*, **58**, 161–164.

9.3.17 United States

The first data on PBDEs in adipose samples collected in 1987 were published by Stanley et al. (1991). The study essentially confirmed the presence of PBDEs in adipose tissue. The full-scan analysis demonstrated the presence of a hexa-BDE, which was estimated to exceed 1 ng/g lipid. The presence of other PBDEs was confirmed by additional HRMS–selected ion monitoring (SIM) analysis, although it was not possible to confirm the concentrations from this preliminary study due to a lack of standards for individual PBDE isomers.

Table 77. Comparison of concentrations of PBDE congeners in human milk from London and Lancaster, United Kingdom

Congener	Geometric mean (median; range) (ng/g milk fat)		Significance (P-value)
	London	Lancaster	
BDE-47	3.9 (4.6; 1.0–36)	1.8 (2.2; 0.1–17)	Yes
BDE-99	0.9 (1.0; ND–12)	0.8 (0.6; ND–6.8)	No
BDE-100	0.6 (0.5; 0.7–7.0)	0.5 (0.4; ND–4.5)	No
BDE-153	1.4 (1.2; ND–4.9)	1.4 (1.4; ND–3.5)	No
BDE-154	0.5 (0.5; ND–2.1)	0.4 (0.3; ND–2.4)	No
ΣPBDE	7.8 (8.1; 3.1–69)	4.6 (5.0; 0.3–34)	Yes

From Kalantzi et al. (2004)

ND, not detected

Approximately 10 years later, five adipose tissue samples were collected in the late 1990s from northern California women and analysed for PBDEs (She et al., 2000). The authors suggested that from these preliminary limited data, it was reasonable to conclude that the background level of PBDEs in the general population is in the low ng/g range (less than 100 ng/g). The data are given in Table 78.

Table 78. Concentration of PBDEs in human adipose tissue samples

Sample no.	% lipid	Concentration (ng/g lipid)			
		BDE-47	BDE-99	BDE-153	Total PBDEs
1	44.6	23	7.3	2.3	32.6
2	50.8	11	3.6	1.6	16.2
3	85.6	7.0	3.1	1.5	11.6
4	89	28	6.6	2.4	37
5	84.5	20	4.1	3.2	27.3
Mean	70.9	18	4.9	2.2	25.1
SD	21.3	8.6	1.9	0.69	

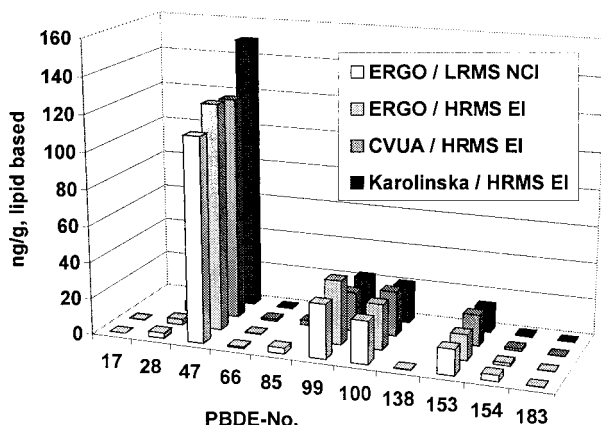
From She et al. (2000)

SD, standard deviation

Due to unexpectedly high PBDE concentrations found in a pooled human milk sample in the United States (Päpke et al., 2001), the pooled material was analysed in three laboratories with extensive experience in determination of halogenated contaminants in human samples: ERGO in Hamburg (O. Päpke), Chemical and Veterinary Control Laboratory in Münster (P. Fürst) and Karolinska

Institute in Stockholm (K. Norén). Comparing the HRMS results from the three laboratories, the differences in concentrations for most congeners are quite low, and the total PBDE concentrations of 204, 196 and 217 ng/g lipid are similar. Due to the relatively difficult determination of compounds with high boiling points such as PBDEs, the results of this comparison were helpful (Figure 22).

Figure 22. PBDEs in human milk (comparison of data from three laboratories with different analytical methods)



Adapted from Pöpke, O., Bathe, L., Bergman, Å., Fürst, P., Meironytė Guvenius, D., Herrmann, T. & Norén, K. (2001) Determination of PBDEs in human milk from the United States, comparison of results from three laboratories. *Organohalogen Compd.*, **52**, 197–200.

Congener BDE-47 occurred at the highest level, followed by BDE-99, BDE-100 and BDE-153. These compounds contributed approximately 61–69%, 11–17%, 10–13% and 5–9%, respectively, to the total PBDEs in the pooled milk sample in the United States. It is striking that a concentration of PBDEs as high as almost 200 ng/g lipid weight is indicated for human milk in the United States. The first study on individual samples of human milk in the United States was published by Schecter et al. (2004c). They analysed 47 milk samples collected in 2002. The data are given in Table 79 and in Figure 23. The mean and median values for total PBDEs are 73.9 and 34 ng/g lipid, respectively.

Archived serum samples from the United States have been analysed by Petreas et al. (2002) and Schecter et al. (2004b). They did not find significant concentrations of PBDEs in pooled samples collected in the 1960s and 1973, respectively.

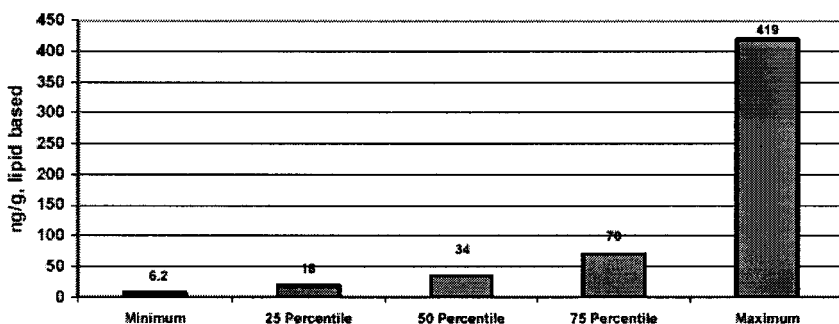
Sjödin et al. (2004a) analysed archived serum samples from the United States collected at four different time periods. The concentration of most of the PBDEs had significant positive correlations with time of sample collection, showing that PBDEs are increasing in serum collected in the United States. The data are given in Table 80.

Table 79. Levels of PBDE congeners in human milk from Texas, USA, in 2002, n = 47

	Age	Nursing (weeks)	Concentration (ng/g lipid)													Sum BDE
			17	28	47	66	77	85	99	100	138	153	154	183	209	
Mean	28.6	24.6	0.02	2.4	40.8	0.65	0.01	1.15	14.0	8.2	0.60	5.3	0.76	0.13	0.92	73.9
Median	29	20	0.01	1.2	18.4	0.14	NA	0.41	5.7	2.9	0.09	2.0	0.22	0.07	NA	34.0
SD	5.70	22.26	0.04	3.1	59.4	1.19	0.04	1.89	24.6	10.8	1.37	6.1	1.30	0.23	1.96	103.0
Minimum	20	2	ND	0.2	2.9	ND	ND	0.08	0.7	0.5	ND	0.4	0.06	ND	ND	6.2
Maximum	41	109	0.18	16.1	271.5	6.67	0.16	7.73	111.0	47.4	6.86	21.8	7.21	1.32	8.24	418.8

From Schecter et al. (2004c)

NA, not analysed; ND, not detected; SD, standard deviation

Figure 23. PBDEs in breast milk from Texas, USA, collected in 2002, n = 47

Taken from Schecter, A., Pavuk, M., Päpke, O., Ryan, J.J., Birnbaum, L. & Rosen, R. (2004c) Polybrominated diphenyl ethers (PBDEs) in US mothers' milk. *Environ. Health Perspect.*, **111** (14), 1723–1729.

Table 80. Concentration of selected PBDEs in archived serum pools from the United States, stratified according to 5-year collection periods

Compound	Median concentration (range) (ng/g lipid)			
	1985–1989 (n = 9)	1990–1994 (n = 14)	1995–1999 (n = 10)	2000–2002 (n = 7)
BDE-47	5.4 (<1–44)	28 (3.7–49)	46 (24–68)	34 (29–98)
BDE-85	<0.5 (<0.5–1.08)	0.61 (0.50–1.4)	0.78 (0.50–1.9)	0.70 (0.50–1.4)
BDE-99	<2 (<2–15)	10 (1.3–18)	13 (9.1–29)	11 (6.8–26)
BDE-100	0.81 (<0.5–7.3)	4.0 (0.63–7.7)	6.7 (3.8–14)	5.9 (3.5–18)
BDE-153	0.84 (<0.5–7.3)	1.6 (0.67–15)	4.2 (2.5–16)	7.3 (1.8–17)
BDE-154	<0.5 (<0.5–0.94)	<0.5 (<0.5–1.07)	0.88 (0.52–1.8)	0.95 (0.50–1.8)
ΣPBDEs	9.6 (4.6–74)	48 (7.5–86)	71 (42–120)	61 (47–160)

From Sjödin et al. (2004a)

She et al. (2004) analysed 16 recently collected human milk samples from residents of the Pacific Northwest of the United States. The total BDE values and the lipid content are given in Table 81.

The congener profiles for the 16 Northwest United States human milk samples, using means of individual PBDE congeners, are shown in Figure 24.

Table 81. Summary results of PBDE concentrations in 16 Northwest United States human milk samples

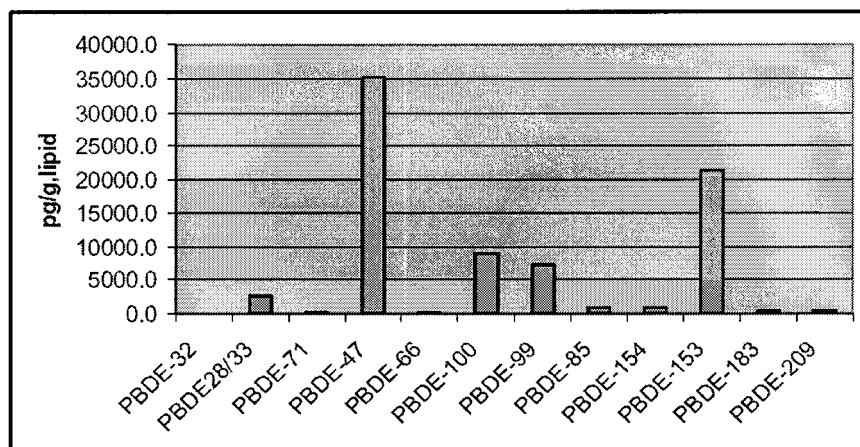
	Minimum	Maximum	Mean	Median	SD
% fat	2.78	5.06	4.19	4.50	0.69
ΣPBDEs ^a (ng/g lipid)	6.34	309	77.5	48.5	79.6

From She et al. (2004)

SD, standard deviation

^a Total of BDE-28/33, BDE-32, BDE-47, BDE-66, BDE-71, BDE-85, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, BDE-209.

Figure 24. Pattern of 12 PBDE congeners analysed in Northwest United States human milk, n = 16, mean



Taken from She, J., Holden, A., Sharp, M., Tanner, M., Williams-Derry, C. & Hooper, K. (2004) Unusual pattern of polybrominated diphenyl ethers (PBDEs) in US breast milk. *Organohalogen Compd.*, **66**, 3945–3950.

9.3.18 Summary

Human PBDE data are available for a number of countries. Typical values found for human samples such as human milk, blood and adipose tissue collected in various regions are shown in Tables 82 and 83.

9.4 Data on time trends for PBDEs

The first data on the observation of an increasing time trend for PBDE concentrations in humans were published by Norén & Meironyté Guvenius (1998). Subsequently, similar time trend data for PBDEs have been observed in many countries. In Table 84, observations on this time trend are summarized.

Table 82. PBDEs in human milk from Europe, North America and Asia

Country	Collection year	Sample type ^a ; number	Total PBDEs (ng/g, lipid)		References
			Median	Range	
Canada	1982	P; <i>n</i> = 200	<0.2	–	Ryan & Patry (2000)
	1986	P; <i>n</i> = 100	0.6	–	Ryan & Patry (2000)
	1992	I; <i>n</i> = 72	3.0	0.6–580	Ryan & Patry (2001)
	2001–2002	I; <i>n</i> = 98	22	0.8–956	Ryan et al. (2002); Pereg et al. (2003)
Canadian Arctic (Nunavik)	1989–1991	I; <i>n</i> = 20	1.7	ND–14	Pereg et al. (2003)
	1996–2000	I; <i>n</i> = 20	6.8	0.2–318	
Faroe Island	1987	P; <i>n</i> = 10	1.5	–	Fängström et al. (2004b)
	1994–1995	P; <i>n</i> = 10	3.6	–	
	1998–1999	P; <i>n</i> = 10	7.2	–	
Finland	1994–1998	I; <i>n</i> = 11	1.6	0.9–5.9	Strandman et al. (2000)
Germany	1992	P; <i>n</i> > 500	1.7	–	Fürst (2001)
	2000	I; <i>n</i> = 7	1.8	–	
	2002	I; <i>n</i> = 8	6.6	4.3–12	Weber & Hesecker (2004)
	2001–2003	I; <i>n</i> = 62	1.78	max. 7.25	Vieth et al. (2004)
Italy	1998–2001	P; <i>n</i> = 39	–	1.6–4.1	Ingelido et al. (2004)
Japan	late 1990s	I; <i>n</i> = 12	1.3	0.7–1.8	Ohta et al. (2002)
Japan	1973	P; <i>n</i> = 21	<0.1	–	Akutsu et al. (2003)
	1983	P; <i>n</i> = 19	0.6	–	
	1993	P; <i>n</i> = 35	2.3	–	
	2000	P; <i>n</i> = 27	1.4	–	
	1999	I; <i>n</i> = 13	1.6	0.6–4.0 (single sample = 291)	

Table 82. (contd)

Country	Collection year	Sample type ^a ; number	Total PBDEs (ng/g, lipid)		References
			Median	Range	
Mexico	not given	I; <i>n</i> = 7	2.1 (mean)	0.8–5.4	Lopez et al. (2004)
Netherlands	1998	I; <i>n</i> = 103	3.3	1.0–13	Baumann et al. (2003)
Norway	1993, 2001	P; <i>n</i> = 10–12	1.9, 2.9	–	Thomsen et al. (2003)
	2001	I; <i>n</i> = 9	2.8	2.0–10	
Spain	2002	I; <i>n</i> = 15	2.4	1.2–6.6	Schumacher et al. (2004)
Sweden	1972	P; <i>n</i> = 227	0.1	–	Meironyté Guvenius et al. (1998, 1999, 2003); Norén & Meironyté Guvenius (2000)
	1984–1985	P; <i>n</i> = 102	0.7	–	
	1994	P; <i>n</i> = 20	2.2	–	
	1997	P; <i>n</i> = 40	4.0	–	
	2000–2001	I; <i>n</i> = 15	2.1	0.6–7.7	
Sweden	1996–1999	I; <i>n</i> = 93	3.2	0.9–28	Lind et al. (2003)
	2000–2001	I; <i>n</i> = 31	2.9	1.5–1.8	
United Kingdom	2001–2002	I; <i>n</i> = 52	6.6	0.3–69	Kalantzi et al. (2004)
USA; New York	1997	I; <i>n</i> = 17	147	–	Ryan et al. (2002)
USA	2000	P; <i>n</i> = 20	196	–	Päpke et al. (2001)
USA; Texas	2002	I; <i>n</i> = 47	34	6.2–419	Schecter et al. (2003)
USA	2002–2003	I; <i>n</i> = 20	58	10–1080	Lunder & Sharp (2003)
USA; west coast	2003	I; <i>n</i> = 9	50	13–156	Northwest Environment Watch (2004)

max., maximum; ND, not detected

^a P = pooled sample, I = individual samples.

Table 83. Total PBDE concentration in different tissues from various countries

Country	Year of collection	Sample type	Total PBDE concentration (ng/g, lipid based)		n	Reference
			Mean	Range		
Australia	2003	Blood	11	6.7–18	10 pools (98–100 samples/pool)	Harden et al. (2004)
Belgium	Not given	Adipose	3.7	1.7–10.1	20	Covaci & Schepens (2001)
Canada	1994–1999	Blood	23.3	13.1–46.4		Ryan & van Oostdam (2004)
Czech Republic	2000–2001	Adipose	3.26/ 1.44	1.03–11.3 0.25–4.79	10/14	Crhova et al. (2002)
Finland	Unknown, from ongoing study	Adipose	11.7	6.49–21.7	10	Strandman et al. (1999)
Germany	1999	Blood	5.8	0.9–12.6	20	Schröter-Kermani et al. (2000)
Italy	2001	Blood	2.0		22	De Felip et al. (2003)
Japan	1970	Adipose	0.03	0.01–0.08	10	Choi et al. (2002)
	2000	Adipose	1.29	0.47–2.75	10	
Mexico	Not given	Blood	29.7	21.5–37.5	5	Lopez et al. (2004)
Republic of Korea	2001	Blood	24.7		11	Lee et al. (2002)
Spain	Not given	Adipose	1.4	0.2–5.8	13	Meneses et al. (1999)
USA	1998	Adipose	25.1	11.6–37.0	5	She et al. (2000)

Table 84. Total PBDE concentration in different tissues from various countries

Country	First year of collection	Latest year of collection	Sample type	Increasing effect	Reference
Canada	1982	2001–2002	Human milk	Strong effect	Ryan & Patry (2000); Ryan et al. (2002)
Faroe Islands	1987 (<i>n</i> = 10)	1998–1999 (<i>n</i> = 10)	Milk	Strong effect	Fängström et al. (2004a, 2004b)
Germany	1992	2000	Milk	Moderate effect	Schröter-Kermani et al. (2000)
				No effect	Fürst (2001)
Japan	1970 (<i>n</i> = 10)	2000 (<i>n</i> = 10)	Adipose	Strong effect	Choi et al. (2002)
Norway	1977	1999	Serum	Strong effect	Thomsen et al. (2001)
Sweden	1972	2001	Milk		Meironyté Guvenius & Norén (2001)
United States	1973	2003	Milk	Strong effect	Schechter et al. (2004c)
	1985–1989	2000–2002	Serum	Strong effect	Sjödin et al. (2004a)

10. DOSE-RESPONSE ANALYSIS AND ESTIMATION OF RISK

10.1 Contribution of above data to assessment of risk

10.1.1 Pivotal data from biochemical and toxicological studies

(a) DecaBDE

In repeat-dose short-term oral studies, DecaBDE appears to induce limited toxic effects in experimental animals. In a well conducted chronic feeding study in rodents (NTP, 1986), DecaBDE (94–97% purity) caused a variety of non-neoplastic and neoplastic organ changes, mainly in the liver and thyroid gland, when administered in the diet (2.5% or 5.0%) over 2 years. In rats, the incidence of neoplastic nodules (hepatoproliferative lesions) was significantly increased compared with controls in males (both dose groups) and females (high dose only), while in male mice, the combined incidence of hepatic adenomas and carcinomas was significantly increased (low dose group) along with thyroid gland follicular cell adenomas or carcinomas (combined) in both dose groups. Overall, NTP (1986) classified the cancer potential for DecaBDE as equivocal for male mice and

suggestive for rats. IARC estimated a TD_{50} value, defined as the chronic dose that results in one half of animals developing tumours, of 2220 mg/kg bw per day based on the male rat neoplastic nodule response (McGregor, 1992), but has placed DecaBDE in its Group 3 category (not classifiable as to its carcinogenicity to humans). Comparison of the TD_{50} value with an estimated lifetime average oral daily intake of 0.7 μ g/kg bw per day for DecaBDE (NAS, 2000) would suggest a low to negligible cancer risk (Gold et al., 1992).

Regarding non-cancer end-points, a recent study has suggested that BDE-209 can induce behavioural alterations in mice, similar to effects seen with less brominated congeners. Exposure of newborn NMRI male mice to a single oral BDE-209 dose of 20.1 mg/kg bw on PND 3 caused persistent changes in spontaneous behaviour with testing up to 6 months after dosing (Viberg et al., 2003b). No consistent effects were observed in mice with a dose of 2.22 mg/kg bw on PND 3 or similar doses when administered on PND 10 or PND 19. While the mode of action is not fully understood, it does not appear to require solely the presence of BDE-209 and/or metabolites in the brain. Similar amounts of radioactivity associated with a gavage dose of [$U-^{14}C$]BDE-209 were seen in the brains of neonatal mice 24 h and 7 days after dosing on either PND 3 or 10, whereas behavioural alterations were associated with dosing only on PND 3. In contrast to these findings, treatment of NMRI mice with BDE-99 on either PND 3 or PND 10 was effective in causing neurobehavioural alterations (Eriksson et al., 2002). Although this represents a single experimental observation with BDE-209 in one strain of mice, similar changes in spontaneous behaviour have been induced with lower brominated congeners in both NMRI and C57BL mice and Sprague-Dawley rats.

Timing of the dose in this experimental model appears to be critical for effects and is likely related to the brain growth spurt period, which in rodents occurs 2–3 weeks after parturition (peaks on PND 10). For the human infant, this critical period begins in the third trimester of pregnancy and extends into the first 2–3 years after birth (Eriksson & Talts, 2000).

Gestational exposure of rats to doses of BDE-209 up to 1000 mg/kg bw per day had no significant effect on fetal survival or development (Hardy et al., 2002). Based on fetal liver bromine analysis, it has been reported that there is no significant in utero transfer of decaBDE after maternal exposure to doses up to 1000 mg/kg bw per day during gestation (GD 6–15) (Norris et al., 1975a).

(b) *OctaBDE*

Initial investigations into the toxic potential of commercial OctaBDE suggested that target organs for effects would be liver, kidney and thyroid. Short-term (up to 14 days) and subchronic exposure of rats to dietary concentrations of OctaBDE from 100 to 10 000 mg/kg (equivalent to 7–9 to 1000 mg/kg bw per day) resulted in increased relative liver and thyroid weights and a variety of histopathological changes to the above-described target organs. Liver weight increases and microscopic changes were shown to be persistent in the highest dose group, even following a 12-month withdrawal period. The estimated lowest-observed-adverse-

effect level (LOAEL) from these feeding studies was 100 mg/kg diet or approximately 7–9 mg/kg bw per day. In a preliminary investigation with commercial OctaBDE (45.2% octa-, 47.4% nona- and 5.7% decaBDE; Norris et al., 1975b), bioaccumulation was evident, based on detected increases in the bromine content of both adipose tissue and liver that persisted following a 90-day recovery period. Overall, the database for OctaBDE is limited, with no chronic duration studies (e.g. cancer) available for evaluation. Based on the largely negative results from various mutagenicity/genotoxicity screening assays (bacterial mutagenicity, DNA repair, sister chromatid exchange, chromosomal aberrations), any cancer risk by a genotoxic mode of action is unlikely.

Short-term exposure of weanling rats to the OctaBDE commercial mixture DE-79 resulted in hepatic enzyme induction and decreases in both serum TT4 and TT3 levels, with BMDL estimates (20% decrease) of 5.29 and 11.98 mg/kg bw per day, respectively (Zhou et al., 2001). CYP2B enzyme induction was the most sensitive experimental end-point measured, with a BMDL of 0.40 mg/kg bw per day. This dose is in agreement with longer-duration exposure (90 days), where doses as low as 0.78 μ mol/kg bw per day (0.62 mg/kg bw per day) of a commercial OctaBDE mixture (8.5% hexaBDE, 45.1% heptaBDE, 30.7% octaBDE) resulted in increases in activity of *O*-ethyl-*O*-*p*-nitrophenyl phenylphosphonothioate detoxification and *p*-nitroanisole demethylation (Carlson, 1980b). The latter substrates have been shown to be preferentially metabolized by phenobarbital-inducible forms of cytochrome P450s.

Developmental toxicity studies have been conducted in rats and rabbits with commercial OctaBDE mixtures (DE-79 and Saytex 111) at doses ranging from 2.0 to 50 mg/kg bw per day during gestation (Great Lakes, 1986). Significant toxic effects on the developing fetus, including reduced weight gain and decreased live births per litter, were observed at doses greater than 10 mg/kg bw per day, while maternal toxicity (reduced gestational weight gain and increased relative liver weight) was seen at doses of 25 mg/kg bw per day and greater. A BMDL₅ of 8.7 mg/kg bw per day has been estimated for the reduced fetal weight gain in rats (VCCEP, 2003).

While no neurotoxicity studies have been conducted with commercial OctaBDE mixtures, BDE-153, one of the main congeners detected following oral exposure of male rats to DE-79 (Huwe et al., 2002), has been tested in a spontaneous activity protocol. A single oral dose of BDE-154 at 0.9 or 9.0 mg/kg bw (1.4 or 14 μ mol/kg bw) on PND 10 altered locomotor activity and spatial learning in adult mice (Viberg et al., 2003a). Similar effects were observed, albeit at higher doses, when neonatal mice were exposed to BDE-203 at 16.8 mg/kg bw and to BDE-206 at 18.5 mg/kg bw (Eriksson et al., 2004).

The lowest effective dose of 0.4 mg/kg bw per day for CYP2B induction in neonatal rats (Zhou et al., 2001) would not be considered toxicologically relevant of and by itself. Subchronic exposure to doses approximately 10-fold higher have been associated with relative liver weight increases and histological changes. A single BDE-153 dose of 0.45 mg/kg bw during a critical developmental period did not induce alterations in spontaneous behaviour in mice (Viberg et al., 2003a).

Significant time-dependent bioaccumulation of bromine in adipose and liver has been shown in rats after ingestion of OctaBDE at 0.1 mg/kg bw per day for up to 180 days (Norris et al., 1975b).

(c) *PentaBDE*

Unlike the higher brominated congeners that predominate in commercial DecaBDE and, to a certain extent, OctaBDE mixtures, PentaBDEs are readily bioavailable and persistent (bioaccumulation potential). Initial studies in rats with commercial PentaBDEs have shown that subchronic exposure to doses as low as 0.44 mg/kg bw per day results in hepatic enzyme induction that persists into a 30-day recovery period (Carlson, 1980a). At higher doses (greater than 2 mg/kg bw per day), relative liver weight increases along with hepatocytomegaly have been observed. Perinatal exposure (GD 6 to PND 21) of rats to DE-71 resulted in significant decreases in serum T4 levels in offspring, with BMDL₂₀ estimates of 0.94 mg/kg bw per day (Zhou et al., 2002). In a recent study (Stoker et al., 2004a), daily oral exposure of juvenile rats to DE-71 from PND 22 to PND 26 also resulted in decreases in serum TT4, with a BMDL₅ of 0.28 mg/kg bw per day. In adult C57BL/6 mice, acute doses of DE-71 as low as 0.8 mg/kg bw have been reported to decrease serum TT4. Gestational exposure of Wistar rats to BDE-99, a major congener found in PentaBDE mixtures, has also been shown to disrupt thyroid hormones in offspring at single doses as low as 60 µg/kg bw (Kuriyama et al., 2004a). In a similar experimental design, exposure of rats to BDE-47 on GD 6 also caused thyroid hormone changes in offspring at a dose of 140 µg/kg bw (Kuriyama et al., 2004b). These latter responses may be species-specific, as perinatal exposure of NMRI mice to multiple BDE-99 doses of 80 µmol/kg bw (45 mg/kg bw) had no effect on thyroid hormones of dams or offspring (Skarman et al., 2005). While thyroid histological changes and thyroid hormone perturbations by PentaBDEs are a consistent experimental observation, the significance of this end-point to humans is open to interpretation. In rodents, fluctuations in serum T4 are influenced by a number of mechanisms, including enhanced biliary excretion through induction of UDPGT and competition for TTR binding by hydroxylated BDE metabolites. In humans, TBG has the highest binding affinity and carries the majority of T4, while UDPGT is thought to be more easily inducible in rodents (Capen, 1997).

Neurobehavioural alterations in rodents have also been observed following exposure to the two main congeners found in PentaBDE commercial mixtures, BDE-47 and BDE-99. The calculation of BMDs for BDE-99, using doses of 0, 0.4, 0.8, 4.0, 8.0 or 16 mg/kg bw by gavage on PND 10 in mice, revealed lower-bound BMDs of 0.31, 0.85 and 1.2 mg/kg for a 10% change in total activity, locomotion and rearing, respectively (Sand et al., 2004). The authors of the primary study had suggested a NOEL of between 0.4 and 0.8 mg/kg bw (Viberg et al., 2004b).

Compared with rats, mice appear to be more sensitive to these effects, with BMDLs (total activity) for BDE-99 estimated at 0.31 mg/kg bw following a single neonatal exposure (Sand et al., 2004). However, gestational exposure of rats to BDE-47 (140 µg/kg bw) or BDE-99 (60 µg/kg bw) has also produced slightly increased locomotor activity in offspring at puberty. In contrast, perinatal exposure

of rats to DE-71 at doses up to 100 mg/kg bw per day (estimated BDE-99 dose of 50 mg/kg bw per day) had no effect on motor activity or spatial learning in the adult male offspring, but doses of 30 and 100 mg/kg bw per day were able to affect fear conditioning (cue-based performance) (Taylor et al., 2003).

While no reproductive toxicity studies are available for commercial Penta-BDEs or their major congeners, gestational exposure to BDE-99, while not affecting fertility, did cause significant decreases in spermatid numbers and daily sperm production in adult rat offspring. At a single dose of 60 µg/kg bw on GD 6, male offspring had a 31% decrease in daily sperm production (95% CI 25–38%), without associated changes in gonadotrophins (inhibin, LH, testosterone) or testis and seminal vesicle weights (Kuriyama et al., 2005). While not associated with a decline in fertility or follicle numbers, the same low dose of BDE-99 has been shown to induce ultrastructural degenerative changes in ovarian cells in the female offspring (Talsness et al., 2003).

(d) Benchmark dose calculations for endocrine and neurotoxic effects

BMDs have been calculated so far only in four studies (Zhou et al., 2001, 2002; Sand et al., 2004; Stoker et al., 2004a). The results are shown in Table 85. BMD values are available only for the technical mixtures DE-71 (mainly penta-BDE) and DE-79 (mainly octaBDE) and the congener BDE-99. Additional calculations may be useful for reduced TT3 and elevated TSH in male rats (Stoker et al., 2004a). Also, gene expression of the AR (Ceccatelli, 2004), decreased thyroid weights and reduced circulating estradiol in male offspring (Lilienthal et al., 2004) appear to be particularly sensitive to BDE-99 after gestational exposure, but in these studies only three dose levels were used.

10.1.2 Pivotal data from human clinical/epidemiological studies

Limited quantitative information is available for evaluation purposes from either clinical or epidemiological studies.

10.1.3 Biomarker studies

A number of detailed reviews have been conducted with various human tissues (serum/plasma, adipose and human milk) that provide evidence for the bio-accumulative potential of PBDEs. In general, lower brominated congeners such as BDE-47, BDE-99 and BDE-153, found in commercial PentaBDE mixtures, predominate. Preliminary analysis of serum and milk samples collected in North America indicated higher concentrations of PBDEs than in similar samples from European countries or Japan (Hites, 2004). Maternal blood samples ($n = 12$) collected in the United States in 2001 give median concentrations of sum PBDEs of 41 ng/g lipid, with congeners BDE-47 and BDE-99 representing approximately 82% of the total. In a larger survey of human milk collected in 2001 ($n = 47$; Texas, USA), the median sum PBDE concentration was 34 ng/g lipid, with the same two congeners accounting for 70% of the total. In Canadian human milk samples ($n = 98$) collected in 2001–2002, median concentrations of sum PBDEs are similar,

Table 85. BMD estimations for PBDEs

Dosing regimen	Species, strain, age	End-point	BMDL (lower bounds)	BMD	Reference
DE-71, oral, daily for 4 days	Weanling Long-Evans rats	Reduced TT4	6.95 mg/kg bw, 20% change	12.74 mg/kg bw, 20% change	Zhou et al. (2001)
		Reduced TT3	8.56 mg/kg bw	32.94 mg/kg bw	
DE-79, oral, daily for 4 days	Weanling Long-Evans rats	Reduced TT4	5.29 mg/kg bw	9.25 mg/kg bw	
		Reduced TT3	11.98 mg/kg bw	53.38 mg/kg bw	
DE-71, oral, daily from GD 6 to PND 21	Developing Long-Evans rats (F1)	Reduced TT4	0.94 mg/kg bw, 20% change	2.36 mg/kg bw, 20% change	Zhou et al. (2002)
DE-71, oral, daily from GD 6 to PND 21	Gravid Long-Evans rats (F0)	Reduced TT4	4.03 mg/kg bw	6.13 mg/kg bw	
DE-71, oral, daily from PND 23 to PND 27	Juvenile male Wistar rats	Reduced TT4	1.28 mg/kg bw, 5% change	1.42 mg/kg bw, 5% change	Stoker et al. (2004a)
DE-71, oral, daily from PND 23 to PND 53		Reduced TT4	0.94 mg/kg bw per day	0.91 mg/kg bw per day	
DE-71, oral, daily from PND 22 to PND 26	Juvenile female Wistar rats	Reduced TT4	0.28 mg/kg bw	0.37 mg/kg bw	
DE-71, oral, daily from PND 22 to PND 41		Reduced TT4	1.16 mg/kg bw	1.36 mg/kg bw	
BDE-99, oral, on PND 10	Infant C57BL mice	Altered total activity in adult males	0.31 mg/kg bw, 10% change	0.51 mg/kg bw, 10% change	Sand et al. (2004)

22.1 ng/g lipid, with the highest value being 956 ng/g lipid (Ryan, 2004). As with the other North American samples, BDE-47 and BDE-99 were almost 74% of the total. In comparison, in a limited sample of Swedish maternal blood samples collected in 2000, the sum PBDE concentration was 1.8 ng/g lipid, with BDE-47 and BDE-99 contributing 57% of the total. Similar results were found with human milk samples collected in Sweden in 2000.

10.2 General modelling considerations

10.2.1 Selection of data

A variety of experimental data sets from studies with limited dosing schedules that focused on either changes to thyroid hormones or behavioural alterations have been summarized (Tables 86–91).

Additional toxicological end-points for risk estimation include acute dosing of rats during gestation with BDE-47 and BDE-99 and subsequent effects on locomotor activity and sperm production observed in the adult offspring. Mechanistic data are limited for comparison with possible human relevancy.

10.2.2 Measure of exposure

Because PBDEs have long half-lives and tend to bioaccumulate, their hazard to health can be estimated only after consideration of intake over a period of months. Short-term variations in PBDE concentrations in foods have much less effect on overall intake than might be the case for other food contaminants. The distribution of long-term mean intake in various populations was calculated by the following procedure:

- The distributions of PBDE (total of the following congeners: 28, 47, 99, 100, 153 and 154) concentrations were constructed for various regions and food groups from the available data. The distributions were assumed to be log-normal. Data were available to construct such distributions for three regions only (Far East, North America and Western Europe). Since only summary data were available from most studies, two sets of distributions were derived, one representing lower-bound estimates of the concentrations (derived using data from studies where non-detects were set at zero), the other representing upper-bound estimates (derived using data from studies where non-detects were set at the LOD).
- Data on food consumption from the GEMS/Food regional diets were used to estimate mean consumption of six major food groups in each diet. A log-normal distribution was constructed from these data, with a GSD of 1.3 extrapolated from the results of the food consumption survey in the Netherlands in order to account for interindividual variation in consumption. The average contributions of the six basic food groups to total food consumption were derived for each diet.

Table 86. Effects of PBDE mixtures on thyroid hormones

Dosing regimen	Species, strain, sex, age	End-point	NOEL	LOEL	Reference
DE-71, single oral dose	Adult female C57BL/6J mice	Reduced TT4	–	0.8 mg/kg bw; no clear dose–response	Fowles et al. (1994)
DE-71, oral, daily for 14 days		Reduced TT4 and FT4	–	18 mg/kg bw	
Bromkal 70-5-DE, oral, daily for 14 days	Adult female C57BL/6N mice	Reduced TT4 and FT4	–	18 mg/kg bw	Hallgren et al. (2001)
	Adult female Sprague-Dawley rats	Reduced TT4 and FT4	–	18 mg/kg bw	
Bromkal 70-5-DE, oral, every 3rd day from GD 4 to PND 17 (10 doses)	NMRI mice dams	TT4 and FT4	80 µmol/kg bw	–	Skarman et al. (2005)
	NMRI mice offspring	Reduced TT4 and FT4 on PND 11	–	80 µmol/kg bw	
DE-71, oral, daily for 4 days	Weanling Long-Evans rats	Reduced TT4	10 mg/kg bw; BMDL 6.95 mg/kg bw	30 mg/kg bw; BMD 12.74 mg/kg bw	Zhou et al. (2001)
		Reduced TT3	30 mg/kg bw; BMDL 8.56 mg/kg bw	100 mg/kg bw; BMD 32.94 mg/kg bw	
DE-79, oral, daily for 4 days	Weanling Long-Evans rats	Reduced TT4	3 mg/kg bw; BMDL 5.29 mg/kg bw	10 mg/kg bw; BMD 9.25 mg/kg bw	
		Reduced TT3	30 mg/kg bw; BMDL 11.98 mg/kg bw	60 mg/kg bw; BMD 53.38 mg/kg bw	
DE-83R, oral, daily for 4 days	Weanling Long-Evans rats	Reduced TT4	100 mg/kg bw	>100 mg/kg bw	
		Reduced TT3	100 mg/kg bw	>100 mg/kg bw	

Table 86. (contd)

Dosing regimen	Species, strain, sex, age	End-point	NOEL	LOEL	Reference
DE-71, oral, daily from GD 6 to PND 21	Developing Long-Evans rats (F1)	Reduced TT4, TT3 unaffected	1 mg/kg bw; BMDL 0.94 mg/kg bw	10 mg/kg bw; BMD 2.36 mg/kg bw	Zhou et al. (2002)
	Long-Evans rat dams (F0)	Reduced TT4, TT3 unaffected	1 mg/kg bw; BMDL 4.03 mg/kg bw	10 mg/kg bw; BMD 6.13 mg/kg bw	
DE-71, oral, daily from PND 23 to PND 27	Juvenile male Wistar rats	Reduced TT4	3 mg/kg bw; BMDL 1.28 mg/kg bw	30 mg/kg bw; BMD 1.42 mg/kg bw	Stoker et al. (2004a)
		Reduced TT3	60 mg/kg bw	>60 mg/kg bw	
		Elevated TSH	60 mg/kg bw	>60 mg/kg bw	
DE-71, oral, daily from PND 23 to PND 53	Juvenile male Wistar rats	Reduced TT4	–; BMDL 0.94 mg/kg bw	3 mg/kg bw; BMD 0.91 mg/kg bw	
		Reduced TT3	3 mg/kg bw	30 mg/kg bw	
		Elevated TSH	3 mg/kg bw	30 mg/kg bw	
DE-71, oral, daily from PND 22 to PND 26	Juvenile female Wistar rats	Reduced TT4	3 mg/kg bw; BMDL 0.28 mg/kg bw	30 mg/kg bw; BMD 0.37 mg/kg bw	
		Reduced TT3	60 mg/kg bw	>60 mg/kg bw	
		Elevated TSH	60 mg/kg bw	>60 mg/kg bw	
DE-71, oral, daily from PND 22 to PND 41	Juvenile female Wistar rats	Reduced TT4	3 mg/kg bw; BMDL 1.16 mg/kg bw	30 mg/kg bw; BMD 1.36 mg/kg bw	
		Reduced TT3	60 mg/kg bw	>60 mg/kg bw	
		Elevated TSH	60 mg/kg bw	>60 mg/kg bw	

Table 86. (contd)

Dosing regimen	Species, strain, sex, age	End-point	NOEL	LOEL	Reference
Dust of technical-grade octaBDE mixture, inhalation, 6 h/day, 5 days/week for 90 days	Adult Charles River rats	Reduced TT4	12 mg/m ³	200 mg/m ³	WIL Research Laboratories (1984); Gill et al. (2004)
Technical-grade decaBDE mixture (77% purity), oral, for 30 days	Adult Sprague-Dawley rats	Thyroid hyperplasia	8 mg/kg bw	80 mg/kg bw	Norris et al. (1975a)
DE-79, oral, for 28 or 90 days	Adult Charles River rats	Thyroid hyperplasia	—	100 mg/kg diet (at both exposure durations)	IRDC (1976, 1977); Gill et al. (2004)

Table 87. Effects of PBDE congeners on thyroid hormones

Dosing regimen	Species, strain, sex, age	End-point	NOEL	LOEL	Reference
BDE-47, oral, daily for 14 days	Adult female C57BL/6N mice	Reduced TT4 and FT4	–	18 mg/kg bw	Hallgren et al. (2001)
BDE-47, oral, daily for 14 days	Adult female Sprague-Dawley rats	Reduced FT4	6 mg/kg bw	18 mg/kg bw	Hallgren & Darnerud (2002)
BDE-99, oral, every 3rd day from GD 4 to PND 17 (10 doses)	NMRI mice dams	TT4 and FT4	80 µmol/kg bw	–	Skarman et al. (2005)
	NMRI mice offspring	TT4 and FT4	80 µmol/kg bw	–	
BDE-47, oral, daily for 4 days	Juvenile female Long-Evans rats	Reduced TT4	3 mg/kg bw	10 mg/kg bw	Hedge et al. (2004)
		Reduced TT3	10 mg/kg bw	30 mg/kg bw	
BDE-99, oral, single dose on GD 6	Wistar rat offspring	Reduced TT4 in F1	–	60 µg/kg bw	Kuriyama et al. (2004a)
BDE-47, oral, single dose on GD 6	Wistar rat dams	Reduced TT4 on PND 1	140 µg/kg bw	700 µg/kg bw	Kuriyama et al. (2004b)
		Reduced TSH on PND 1	140 µg/kg bw	700 µg/kg bw	
BDE-47, oral, single dose on GD 6	Male Wistar rat offspring	Reduced TT3 on PND 1	140 µg/kg bw	700 µg/kg bw	Andrade et al. (2004)
		Reduced TT3 on PND 14	–	140 µg/kg bw	
		Reduced TSH on PND 14	–	140 µg/kg bw	
		Elevated TT4 on PND 22	140 µg/kg bw	700 µg/kg bw	
		Reduced TSH on PND 22	–	140 µg/kg bw	
BDE-99, subcutaneous, daily from GD 10 to GD 18	Long-Evans rat offspring	Decreased thyroid weight	–	1 mg/kg bw	Lilienthal et al. (2004)

Table 88. Effects of PBDE mixtures on steroid hormones and related effects

Dosing regimen	Species, strain, sex, age	End-point	NOEL	LOEL	Reference
DE-71, oral, daily for 14 days	Adult female C57BL/6J mice	Elevation of stress-induced corticosterone	–	18 mg/kg bw	Fowles et al. (1994)
DE-71, oral, daily from PND 22 to PND 41	Juvenile female Wistar rats	Delayed vaginal opening	30 mg/kg bw	60 mg/kg bw	Stoker et al. (2004a)
DE-71, oral, daily from PND 23 to PND 53	Juvenile male Wistar rats	Elevated serum prolactin	30 mg/kg bw	60 mg/kg bw	
		Delayed preputial separation	3 mg/kg bw	30 mg/kg bw	
		Ventral prostate, decreased weight	30 mg/kg bw	60 mg/kg bw	
		Seminal vesicle, decreased weight	30 mg/kg bw	60 mg/kg bw	

Table 89. Effects of PBDE congeners on steroid hormones and related effects

Dosing regimen	Species, strain, sex	End-point in F1 animals	NOEL	LOEL	Reference
BDE-47, oral, single dose on GD 6	Male Wistar rat offspring	Reduced FSH on PND 22	140 µg/kg bw	700 µg/kg bw	Andrade et al. (2004)
BDE-99, subcutaneous, daily from GD 10 to GD 18	Long-Evans rat offspring	Delayed vaginal opening	1 mg/kg bw	10 mg/kg bw	Ceccatelli (2004)
		Accelerated preputial separation	–	1 mg/kg bw	
		Ovary, increased weight	–	1 mg/kg bw	
		Dorsal prostate, increased weight	–	1 mg/kg bw	
		Ventral prostate, increased weight	–	Only at 1 mg/kg bw	
		Epididymis, decreased weight	–	1 mg/kg bw	
		Markedly reduced AR mRNA, ventral prostate	–	1 mg/kg bw	
		Elevated AR mRNA, dorsal prostate	–	1 mg/kg bw	
		Reduced ERα and ERβ mRNA, ventral prostate	–	1 mg/kg bw	
		Elevated ERα mRNA, dorsal prostate	1 mg/kg bw	10 mg/kg bw	
		Reduced ERβ mRNA, ventral prostate	–	1 mg/kg bw	
		Reduced IGF-I mRNA, ventral prostate	–	1 mg/kg bw	
		Reduced PR mRNA, uterus	–	1 mg/kg bw	
		ERβ mRNA elevated at low dose, reduced at high dose, uterus	–	1 mg/kg bw	

Table 89. (contd)

Dosing regimen	Species, strain, sex	End-point in F ₁ animals	NOEL	LOEL	Reference
BDE-99, subcutaneous, daily from GD 10 to GD 18	Long-Evans rat dams	Reduced serum estradiol	1 mg/kg bw	10 mg/kg bw	Lilienthal et al. (2004)
		Reduced serum 25-hydroxyvitamin D ₃	1 mg/kg bw	10 mg/kg bw	
	Male Long-Evans rat offspring	Markedly reduced serum estradiol	–	1 mg/kg bw	
		Reduced serum testosterone	–	1 mg/kg bw	
		Reduced anogenital distance	1 mg/kg bw	10 mg/kg bw	
		Feminization of sexually dimorphic behaviour	1 mg/kg bw	10 mg/kg bw	
	Female Long-Evans rat offspring	Reduced serum 1,25-dihydroxyvitamin D ₃	1 mg/kg bw	10 mg/kg bw	

Table 90. Effects of PBDE mixtures and congeners on neurobehavioural toxicity in mice

Dosing regimen	Species, strain	End-point in F1 animals	NOEL	LOEL	Reference
BDE-47 or BDE-99, oral, on PND 10	Infant NMRI mice	Altered locomotor activity and habituation in adult males	BDE-47: 0.7 mg/kg bw BDE-99: –	BDE-47: 10.5 mg/kg bw BDE-99: 0.8 mg/kg bw	Eriksson et al. (2001)
BDE-99, oral, on PND 3, 10 or 19	Infant NMRI mice	Altered locomotor activity and habituation in adult males	PND 3: – PND 10: – PND 19: 8 mg/kg bw	PND 3 and PND 10: 8 mg/kg bw PND 19: >8 mg/kg bw	Eriksson et al. (2002)
BDE-99, oral, on PND 10	Infant NMRI mice	Altered locomotor activity in response to cholinergic stimulation with nicotine	–	8 mg/kg bw	Viberg et al. (2002)
BDE-153, oral, on PND 10	Infant NMRI mice	Altered locomotor activity and habituation in adult males	0.45 mg/kg bw	0.9 mg/kg bw	Viberg et al. (2003a)
		Altered density of nicotinic receptors in hippocampus	0.9 mg/kg bw	9 mg/kg bw	
BDE-209, oral, on PND 3, 10 or 19	Infant NMRI mice	Altered locomotor activity and habituation in adult males	PND 3: 2.22 mg/kg bw; PND 10 or PND 19: 20.1 mg/kg bw	PND 3: 20.1 mg/kg bw PND 10 and PND 19: >20.1 mg/kg bw	Viberg et al. (2003b)
BDE-99, oral, on PND 10	Infant NMRI mice	Altered locomotor activity and habituation in adult males	0.4 mg/kg	12 mg/kg bw	Viberg et al. (2004a)
		Decreased density of nicotinic receptors in hippocampus	(lower dose not studied)	12 mg/kg bw	

Table 90. (contd)

Dosing regimen	Species, strain	End-point in F1 animals	NOEL	LOEL	Reference
BDE-99, oral, on PND 10	Infant C57BL mice	Altered locomotor activity and habituation in adults	0.4 mg/kg bw in both sexes	0.8 mg/kg bw in both sexes	Viberg et al. (2004b)
BDE-183, oral, on PND 3	Infant NMRI mice	Altered locomotor activity and habituation in adult males	<15.2 mg/kg bw	15.2 mg/kg bw	Eriksson et al. (2004)
BDE-203, oral, on PND 3 or 10	Infant NMRI mice	Altered locomotor activity and habituation in adult males	<16.8 mg/kg bw	16.8 mg/kg bw	
BDE-206, oral, on PND 10	Infant NMRI mice	Altered locomotor activity and habituation in adult males	<18.5 mg/kg bw	18.5 mg/kg bw	
BDE-203, oral, on PND 10	Infant NMRI mice	Altered learning and memory in adult males	<16.8 mg/kg bw	16.8 mg/kg bw	
BDE-206, oral, on PND 10	Infant NMRI mice	Altered learning and memory in adult males	<18.5 mg/kg bw	18.5 mg/kg bw	
BDE-99, oral, from GD 6 to PND 21	CD-1 Swiss mice	Delayed maturation of climbing in preweaning pups	6 mg/kg bw	30 mg/kg bw	Branchi et al. (2002)
		Altered locomotor activity in offspring	–	0.6 mg/kg bw, not at 30 mg/kg bw	

Table 91. Effects of PBDE mixtures and congeners on neurobehavioural toxicity in rats

Dosing regimen	Species, strain, sex	End-point in F1 animals	NOEL	LOEL	Reference
DE-71, oral, daily from GD 6 to PND 21	Long-Evans rat offspring	Impaired long-term potentiation in dentate gyrus	30 mg/kg bw	100 mg/kg bw	Gilbert et al. (2004)
DE-71, oral, daily from GD 6 to PND 21	Male Long-Evans rat offspring	Impaired cue-conditioned fear	5 mg/kg bw	30 mg/kg bw	Taylor et al. (2003)
BDE-99, subcutaneous, daily from GD 11 to GD 19	Female Wistar rat offspring	Increased locomotor activity	–	30 mg/kg bw	Wiegand et al. (2003)
BDE-99, subcutaneous, daily from GD 2 to GD 9 or from GD 11 to GD 19	Wistar rat offspring	Altered contents of proteins in the glutamate – nitric oxide – cGMP signal transduction system and increased extracellular cGMP after NMDA stimulation	–	30 mg/kg bw	
BDE-99, oral, on PND 10	Infant Sprague-Dawley rats	Altered locomotor activity and habituation in adult male offspring	0.8 mg/kg bw	8 mg/kg bw	Viberg et al. (2004c)
BDE-99, oral, on GD 6	Wistar rat male and female offspring	Increased locomotor activity	–	60 µg/kg bw	Kuriyama et al. (2004a)
BDE-47, oral, on GD 6	Female Wistar rat offspring	Increased locomotor activity on PND 70	–	140 µg/kg bw	Kuriyama et al. (2004c)
BDE-99, subcutaneous, daily from GD 10 to GD 18	Long-Evans rat offspring	Abolition of sexual dimorphism in PR mRNA in hypothalamus	–	1 mg/kg bw	Lichtensteiger et al. (2004)
	Female Long-Evans rat offspring	Markedly reduced mating behaviour	–	10 mg/kg bw	

Table 91. (contd)

Dosing regimen	Species, strain, sex	End-point in F1 animals	NOEL	LOEL	Reference
BDE-99, subcutaneous, daily from GD 10 to GD 18	Male Long-Evans rat offspring	Altered catalepsy after induction with haloperidol	–	1 mg/kg bw	Lilienthal et al. (2004)
		Altered reactivity after aversive stimulation	–	1 mg/kg bw	
BDE-99, subcutaneous, daily from GD 10 to GD 18	Male Long-Evans rat offspring	Impaired LTP in cortex and hippocampus, persistent after decline of fat tissue levels	–	1 mg/kg bw	Wiegand et al. (2003, 2004)

- The dietary intake of a particular population was assessed by combining the concentrations in food and food consumption distributions for that population with a Monte Carlo approach. In each Monte Carlo trial, the dietary intake was estimated by multiplying random values for food consumption and concentrations in various food groups. The concentrations were weighted according to the contribution of the food group to total food consumption. The estimates of intake were combined to form a distribution of long-term mean dietary intake for each population studied. The distributions are characterized by median, 80th-percentile and 90th-percentile intake.

The simulated intakes of PBDEs in the GEMS/Food regional diets are presented in Table 49 in section 7.3.2. These intakes are, however, likely to be overestimates, as it was generally not possible to determine whether the data on concentrations were derived from targeted surveys or whether they were truly random samples, and as the GEMS/Food regional diets are based on data on food supply (apparent consumption), which are known to overestimate food consumption by at least 15%.

More reliable estimates of intake, derived from national studies (see Table 47 in section 7.3.1), use national food consumption data rather than data on the food supply (apparent consumption) from the GEMS/Food regional diets.

The calculated contributions of various food categories to the intake of PBDEs showed that the largest fraction (>60%) is from food of animal origin in GEMS/Food regional and national diets.

Information was lacking on both the quality of data and geographical representativeness for some regions. More data are required on the occurrence of PBDEs in food products, particularly from geographical regions other than Europe and North America, so that more representative estimates of intake can be made for all regions.

The regional difference, in terms of exposure to PBDEs, is also apparent when considering intake by nursing infants. Based on a North American median human milk PBDE concentration of 30 ng/g lipid, total intake by a nursing infant can be estimated at 126 ng/kg bw per day (average 3.0% fat content of milk, 800 ml milk/day, 5.0 kg bw during nursing) (see Table 51 in section 7.3.3). For two of the main PBDE congeners detected in human milk, this would be approximately 73 ng/kg bw per day for BDE-47 and 22.5 ng/kg bw per day for BDE-99. In comparison, based on an average total PBDE concentration of 2.3 ng/g lipid found in German milk samples, estimated exposure by a nursing infant would be approximately 10-fold lower.

Limited biomarkers of exposure (internal dosing) are available from the identified experimental data sets. Pharmacokinetic studies indicate that lower brominated congeners such as BDE-47 and BDE-99 are readily bioavailable following oral exposure, whereas higher brominated congeners have limited potential for bioaccumulation. PBDE concentrations (lipid normalized) have been reported (McDonald, 2004) from a study in which rats were exposed by gavage to doses of

1.0 mg/kg bw per day from GD 6 to GD 21 (Taylor et al., 2003). In another study, plasma PBDE concentrations following oral exposure were also reported in relation to observed changes in thyroid hormone levels in rats (Darnerud et al., 2004). A total estimated exposure to BDE-47 of 2.7 mg (14 consecutive doses of 1 mg/kg bw per day; Hallgren & Darnerud, 2002) resulted in a plasma concentration of 28 µg/g lipid and had no effect on hepatic enzyme induction or thyroid hormones (TT4 or FT4). In comparison, exposure of rats to the commercial PentaBDE mixture Bromkal 70-5-DE at an estimated total dose of 48 mg (18 mg/kg bw per day for 14 days) resulted in a total plasma PBDE concentration of 463 µg/g lipid (sum BDE-28, BDE-47, BDE-66, BDE-99, BDE-100, BDE-138, BDE-153, BDE-154) and significant decreases in both FT4 and TT4.

10.2.3 Measure of response

The indicated data sets have been used to estimate benchmark doses (see Table 85 above).

10.3 Potency estimates

10.3.1 Potency estimates in humans based on epidemiological data

No studies are available in humans for evaluating either potency estimations or dose–response relationships.

10.3.2 Potency estimates in humans based on biomarkers

Biomarkers of exposure are available in the form of lipid-normalized serum or human milk values. For the purpose of risk estimations, comparison with any experimental biomarkers assumes that food is the main source of exposure. In a survey of human milk collected across Canada in 2002 ($n = 98$), the mean concentration of total PBDEs (sum of congeners 28, 47, 99, 100, 154, 154 and 183) was 60.4 ng/g lipid (Ryan, 2004). This represented an approximate 4-fold increase when compared with the previous human milk survey conducted in 1992 (mean = 15 ng/g). Five per cent of the 2002 sample set had a PBDE concentration of equal to or greater than 236 ng/g lipid, similar to a sample from the United States for which the 95% value was 378 ng/g lipid (Schechter et al., 2005).

However, while comparison of PBDE exposures in the United Kingdom suggests that diet is the major source, maximum inhalation exposure (96 ng/person per day) can equal that of average dietary intakes (90–107 ng/person per day) (Harrad et al., 2004).

10.3.3 Potency estimates in test species and basis for extrapolation to humans

Major congeners detected in foods and human tissue samples more closely resemble commercial PentaBDE and OctaBDE mixtures. Although sum PBDE results for human samples sometimes include highly brominated congeners such

as BDE-183 and BDE-209, they comprise only a minor component (less than 2% of total mass).

Only the DecaBDE commercial mixture has been subject to a chronic toxicity test, and the lowest concentration tested (2.5% in the diet) produced adverse effects. For PBDE commercial mixtures (PentaBDE and OctaBDE) whose congener patterns resemble residues found in food and human samples, there is limited toxicological information. Only short-term feeding studies (up to 13 weeks) have been conducted in rats, with liver, kidney and thyroid identified as target organs. Dose-related increases in relative liver weights and microscopic liver changes (hepatocellular enlargement with vacuolation) were noted in a study with a commercial OctaBDE mixture (DE-79) at 100 mg/kg diet or approximately 8 mg/kg bw per day. Similar effects were seen in a subchronic feeding study with a PentaBDE mixture (DE-71); dose-related increases in liver weights and histological changes (hypertrophy, slight degeneration and necrosis) were noted at the lowest dose level, 2 mg/kg bw per day. The effects were still partially evident in female animals at the lowest dose group after a 24-week recovery period. At higher doses (≥ 10 mg/kg bw per day), decreases in circulating thyroid hormones (T4) were observed. This latter observation is supported by a developmental toxicity study conducted in rats with the commercial PentaBDE (DE-71), in which decreases in serum T4 were seen in both fetuses and newborn pups at a maternal dose of 10 mg/kg bw per day (GD 6 to PND 21).

A number of additional preliminary studies were reviewed, available as extended abstracts involving acute dosing protocols on a single day during either gestation or lactation using mainly PentaBDE commercial mixtures (DE-71), BDE-47 or BDE-99. A variety of effects in both mice and rats were observed involving neurological development (behaviour, memory and activity), thyroid hormone perturbation and sexual maturation at doses as low as 60 μ g/kg bw. Due to a lack of mechanistic information and adequate dose-response relationships, a clear interpretation of the significance to human health could not be made at this time.

Developmental exposure of rats to the PentaBDE commercial mixture, DE-71, results in reductions in thyroid hormones in offspring, with an estimated BMDL₂₀ of 0.94 mg/kg bw per day. In comparison, exposure of weanling rats to DE-71 also resulted in thyroid hormone decreases, with a BMDL₅ estimate of 0.28 mg/kg bw per day. Differences in the two values are related not only to the exposure protocols but to the level of response for effect modelling (20% former, 5% latter). Based on a single exposure of newborn mice to BDE-99, a BMDL₁₀ for total activity changes was estimated at 0.31 mg/kg bw. Exposure during a similar critical developmental stage in humans can be compared based on PBDE concentrations in human milk. Median intake for total PBDEs by North American newborns would be on average 135 ng/kg bw per day, or approximately 2000-fold lower than the BMDL for a 5% decrease in thyroid hormones. Alternatively, the maximum concentration of total PBDEs found in human milk samples in the United States was approximately 420 ng/g, which would result in an estimated intake of 1.9 μ g/kg bw per day, or 147-fold less than the thyroid hormone BMDL. Based on results from a Canadian survey of human milk samples collected in 2001–2002, 5% of the sample population ($n = 98$) had total PBDE values equal to or greater

than 236 ng/g lipid (maximum 956.2 ng/g lipid). Comparison of this 95th percentile value with the estimated BMDL for thyroid hormone changes would give a difference of 226. Similarly, the BMDL₁₀ for total activity changes induced by BDE-99 in mice is approximately 940-fold greater than the estimated single-day exposure of 0.3 µg/kg bw by a nursing infant based on a BDE-99 concentration in human milk of 62.8 ng/g lipid (average of 95th percentile values from human milk surveys in the United States and Canada).

11. COMMENTS

11.1 Absorption, distribution, metabolism and excretion

The majority of detailed studies of the absorption, distribution, metabolism and excretion of PBDEs are limited to the individual congeners BDE-47, BDE-99 and BDE-209. The absorption of PBDEs is directly related to the extent of bromination of the parent diphenyl ether; as a general rule, greater substitution with bromine leads to a decrease in bioavailability. Intestinal absorption of deca-BDE is limited, with >90% of an orally administered dose being rapidly excreted in the faeces. For congeners with a lower degree of bromination (tetra- and penta-substituted), >80% of an orally administered dose is absorbed, with patterns of distribution in tissue being largely determined by lipid content. The metabolism of PBDEs consists of hydroxylation and methoxylation reactions and, in the case of congeners with a higher degree of bromination, oxidative debromination. Faecal excretion appears to be the predominant route of elimination; however, some differences exist between species. Urinary excretion of BDE-47 is a minor pathway in rats but is as important as faecal excretion in mice. Limited data were available regarding the half-lives of individual PBDE congeners; however, preliminary values in female rats exposed to a commercial pentaBDE mixture, Bromkal 70-5-DE, ranged from 30 to 90 days for the tetra- to hexa-substituted congeners.

Limited pharmacokinetic data were available for humans. On the basis of the observed increase in concentrations of PBDEs in tissue with time, PBDEs are absorbed and bioaccumulate.

11.2 Toxicological data

In the toxicological studies reviewed, PBDEs were administered by the oral (gavage or diet) route of exposure, unless otherwise stated.

The acute toxicity of mixtures of PBDEs is low in rodents. Generally, even at the highest doses (several grams per kilogram of body weight), there are no observable effects in standard tests for acute toxicity after exposure to decaBDE and octaBDE, although certain effects (increased mortality, behavioural symptoms and changes in gross pathology) are seen after exposure to pentaBDE at similar high doses. Induction of enzymes, changes in levels of hormones and neuro-behavioural effects are observed after bolus administration of mixtures of PBDEs (pentaBDE and octaBDE) and of specific congeners at considerably lower doses. In short-term studies of toxicity, the main effects of mixtures of PBDEs were seen in the liver, kidney and thyroid of both sexes. Enlargement of the liver is a common

finding, which may be connected to increased activity of microsomal enzymes in the liver. Histological changes occur in liver (enlargement, "round bodies," vacuolization, necrosis), kidney (hyaline degenerative cytoplasmic changes) and thyroid (hyperplasia). In short-term studies, effects on thyroid hormone, vitamin A homeostasis and microsomal enzymes were observed at doses of 1–10 mg/kg bw per day.

The only long-term study with PBDEs was conducted with the decaBDE mixture. In this NTP study of carcinogenicity (NTP, 1986), decaBDE (purity, 94–99%; brominated dioxins and furans reported not to be detected), given in the diet at high concentrations (2.5% or 5%) for 111–113 weeks, significantly increased the combined incidence of hepatocellular adenomas and carcinomas in male mice, but not in female mice. In spite of an increase in follicular cell hyperplasia, the incidence of thyroid follicular cell adenoma/carcinoma was not significantly increased. In male and female rats, the incidence of liver adenomas, but not hepatocellular carcinomas, was increased. Other effects, such as liver hypertrophy, granulomas, thrombosis and degeneration, thyroid follicular cell hypertrophy and lymphoid hyperplasia, were also noted. The Committee concluded that evidence for the carcinogenicity of decaBDE in experimental animals was limited and noted that no information was available on the carcinogenic potential of the other PBDE mixtures.

The results of the majority of tests for genotoxicity performed *in vitro* (point mutations, chromosomal aberrations, unscheduled DNA synthesis, sister chromatid exchange) and limited data from tests *in vivo* (chromosomal aberration) indicated that PBDE mixtures and individual congeners are not genotoxic.

The developmental toxicity of deca-, octa- and pentaBDE mixtures has been studied in rats and rabbits. In rats, preparations of pure decaBDE (purity, 97–98%) had no effects on developmental parameters, while decaBDE of lower purity (decaBDE, 77.4%; nonaBDE, 21.8%; octaBDE, 0.8%) caused fetotoxic effects. Exposure to commercial octaBDE mixtures (Saytex 111 and DE-79) produced developmental toxicity as indicated by increased numbers of late resorptions, reduced fetal weight, severe oedemas, reduced ossification of skull bones and bent rib and limb bones at a dose range of 10–50 mg/kg bw per day; only slight maternal toxicity (decreased body weight) was observed at doses of 25–50 mg/kg bw per day. A pentaBDE mixture (Saytex 115) has been tested in only one study, with no clear adverse effects at a dose of 100 mg/kg bw per day.

In rabbits given a commercial octaBDE mixture (Saytex 111) during gestation, no major fetotoxic effects were observed, but an increase in the incidence of delayed ossification of sternebrae was seen at 15 mg/kg bw per day.

The Committee concluded that the embryo and fetus may be more sensitive to PBDEs than maternal animals and that exposure to octaPBDE mixtures causes an increase in the incidence of developmental abnormalities.

11.3 Special studies

Studies with purified PBDE congeners *in vitro* have shown lack of activation of the Ah receptor at doses 6 orders of magnitude higher than the half-maximal effective concentration (EC_{50}) of TCDD, suggesting that some toxicity data may be confounded by the presence of traces of impurities that are potent agonists of the Ah receptor.

In studies with the commercial PBDE mixtures PentaBDEs (Bromkal 70-5-DE and DE-71), OctaDBE (DE-79) and DecaBDE (DE-83R), various strains and both sexes of adult mice and rats have been used and acute or short-term dosing schedules applied to examine effects on thyroid hormone homeostasis. In the majority of studies, concentrations of TT4 and, in some cases, FT4 in the blood were found to be suppressed, with almost no corresponding alteration in TSH. DE-79 was reported to be more potent than DE-71, while no effects were found after exposure to DE-83R. When pregnant rats were given DE-71 at maternal doses of ≥ 3 mg/kg bw per day, circulating concentrations of T4 in the offspring were found to be reduced until weaning, with recovery of T4 values within 2 weeks thereafter. In juvenile rats given DE-71, reductions in serum concentrations of T4 were similar in both sexes, but concentrations of TSH were elevated and serum concentrations of T3 were significantly decreased only in males. Plasma concentrations of TT4 and FT4 were decreased in adult female mice and rats given Bromkal 70-5-DE at a dose of 18 mg/kg bw per day for 2 weeks. At doses at which circulating concentrations of T4 were decreased (>1 mg/kg bw per day), the activities of UDPGT and EROD were often found to be increased, suggesting that Ah receptor-dependent effects are most likely to be mediated by contamination of commercial PBDE mixtures with dioxin-like compounds. A similar observation was also made in studies with individual PBDE congeners (BDE-47 and BDE-99).

Of the individual congeners, only BDE-47, BDE-99 and BDE-209 have been studied. With regard to effects on the concentrations and activities of thyroid hormones, the available data indicated that BDE-209 is much less potent than BDE-47 and BDE-99, but lack of data precluded a comparison of the potencies of BDE-47 and BDE-99. In general, the results of studies with individual congeners indicated that their effects on thyroid hormones were similar to those observed with mixtures. The most pronounced effects were reduced concentrations of circulating TT4 and FT4. TSH was not affected in the majority of studies.

Recent studies, available as extended abstracts, showed that the offspring (both males and females) of rats given a single oral dose of BDE-99 (60 μ g/kg bw) or BDE-47 (140 μ g/kg bw) on day 6 of gestation had altered concentrations of T3 and T4 during the weaning period. Serum concentrations of TSH were also reduced during lactation. These alterations in thyroid hormones recovered during postnatal development. In general, examination of effects on the thyroid after maternal exposure to mixtures of PBDEs or to individual congeners demonstrated that the offspring were more susceptible than the dams.

While competitive inhibition of the binding of T4 to TTR by hydroxylated metabolites of PBDE is thought to be one of the mechanisms responsible for decreases in circulating concentrations of thyroid hormones in rats, the

significance of this for human exposure is questionable. TBG, which is absent in rats, is the main thyroid hormone transport protein in humans. Metabolites of PBDE have been shown to have limited binding affinity to human TBG. A general observation by the Committee was the apparent lack of consistency in the results of a number of experimental studies measuring thyroid hormone changes; significant decreases in serum concentrations of T4 were observed in the absence of corresponding effects on TSH. There was insufficient information about the effects of PBDEs on feedback mechanisms in the hypothalamus and pituitary. In a number of studies in which the effects of PBDE congeners or mixtures on thyroid hormones were measured, induction of hepatic EROD was also observed, which could indicate the presence of dioxin-like contaminants. Alterations in thyroid hormones are also a sensitive response in experimental animals exposed to dioxin-like chemicals. The available data were considered to be insufficient to determine the mechanism for the reported effects on thyroid hormones and the possible role of pure PBDEs in altering delivery of maternal thyroid hormones across the placental barrier to the developing embryo/fetus and into the brain.

Possible effects of PBDEs on steroid hormones and steroid-related end-points have been reported in a limited number of studies (mainly in extended abstracts) with a commercial PentaBDE mixture (DE-71) and two congeners, BDE-47 and BDE-99. In weanling rats treated by oral administration with a commercial PentaBDE mixture (DE-71) for 20 days (female) or 31 days (male), the onset of puberty was delayed in both sexes at doses of 30–60 mg/kg bw per day. After a single oral dose of BDE-47 (700 µg/kg bw) on day 6 of gestation, decreased serum concentrations of FSH were seen in male offspring. With the same exposure protocol, BDE-99 was recently reported to reduce sperm production at a dose of 60 µg/kg bw. Induction of hepatic EROD was observed in all these experiments; therefore, Ah receptor-mediated effects by possible dioxin-like contaminants could not be excluded.

In rats given BDE-99 at doses as low as 1 mg/kg bw per day by subcutaneous administration during days 10–18 of gestation, decreases in the circulating concentrations of sex steroid hormones (estradiol and testosterone) were observed in weanling and adult male offspring. Anogenital distance was reduced in male offspring, and reproductive organ weights were altered in both sexes. The onset of puberty was delayed in females and accelerated in males, while there was a marked reduction in the expression of AR mRNA in the ventral prostate on PND 120. In the same study, exposure to a technical mixture of PCBs (Aroclor 1254), known to possess dioxin-like activity, at a dose of 30 mg/kg bw per day did not affect several of these end-points, indicating that contamination of the BDE-99 with dioxin-like compounds was unlikely to account for these observations.

The majority of investigations examining neurotoxicity *in vivo* involved oral exposure of mice and rats to individual congeners. In almost all experiments in mice, individual congeners (e.g. BDE-47, BDE-99, BDE-153, BDE-183, BDE-203, BDE-206 and BDE-209) given to neonates as a single oral dose on a specific postnatal day produced changes in activity patterns and habituation, which became more pronounced with ageing. Essentially identical results were observed in the same laboratory with two different strains of mice, in both sexes, and also in

rats. In general, the congeners with a lower degree of bromination appeared to be more potent than the congeners with a higher degree of bromination. Most of the neurotoxicological examinations were performed in rats treated with BDE-99 during gestation. Decreases in LTP in the cortex and hippocampus, as well as influences on sexually dimorphic brain structures, reductions in mating behaviour and feminization of sweet preference behaviour, were reported at doses of ≥ 1 mg/kg bw per day administered subcutaneously. As some of these end-points were not affected by administration of Aroclor 1254 at higher doses, this would indicate that mechanisms similar to those for dioxins are unlikely to be involved. Impaired hippocampal LTP and conditioned behaviour were also detected in the offspring of female rats treated with the PentaBDE mixture (DE-71) at oral doses of 30–100 mg/kg bw per day from day 6 of gestation to PND 21. Altered locomotor activity was reported in the offspring of female rats given a single oral dose of BDE-47 (140 or 700 μ g/kg bw) or BDE-99 (60 or 300 μ g/kg bw) on day 6 of gestation. Because of the preliminary nature of these findings, an interpretation of their significance for human health could not be made.

11.4 Observations in humans

No clinical observations have been reported in humans after oral ingestion of PBDEs. Although several studies have been conducted in workers exposed occupationally to PBDEs, these subjects were also exposed to other substances, making it difficult to attribute any observed effects solely to PBDEs. Therefore, the Committee did not consider these studies to be useful for evaluation of the potential health effects of dietary exposure to PBDEs. In a case-control study, elevated concentrations of BDE-47 were found in the adipose tissue of patients with NHL (incident cases), but the etiological significance of this association is uncertain. In a study of adult male consumers of Baltic fish, plasma concentrations of BDE-47 were inversely related to concentrations of TSH and were not related to the concentrations of any of the thyroid hormones measured, suggesting that exposure to BDE-47 via frequent consumption of fish does not impair thyroid function in adult men.

The Committee concluded that the available studies in humans were not adequate to evaluate whether exposure to PBDEs, at the levels studied, is associated with adverse health effects.

In human milk collected in Sweden between 1972 and 1997, the concentrations of PBDEs increased, doubling every 5 years, resulting in current concentrations in the low nanogram per gram of lipid range. Recent investigations with human milk from other European countries showed similar levels of contamination.

Analysis of a limited number of samples of human serum collected between 1985 and 1999 in the United States also showed an increase in concentrations of PBDEs over time.

Analysis of a limited number of recently collected human samples (blood, milk, adipose) from North America has indicated that average concentrations of PBDEs are 10–20 times higher than those in samples collected in European

countries. The reason for the higher values found for North America was not thought to be solely related to dietary intake. The significance of pathways of exposure other than food, such as indoor air and indoor dust, is currently under investigation.

Generally, lipid-based concentrations are similar in different human samples, such as milk, blood and adipose tissue.

The typical pattern of congeners found in humans is normally dominated by BDE-47, followed by BDE-99 and the hexabrominated congener BDE-153. Preliminary results indicated that congener BDE-153 is becoming more prominent in European samples.

11.5 Analytical methods

GC-HRMS using the isotope dilution technique (^{13}C -labelled standards) has been found to be the most reliable method for the determination of PBDE congeners in food and environmental samples, as well as in samples of human tissues.

The total number of possible PBDE congeners is 209. For reasons of occurrence in food and human samples and analytical capability, only a limited number of congeners has been measured in recent years. This number ranged between three and nine congeners (BDE-28, BDE-47, BDE-66, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, BDE-209). With increasing analytical power and availability of standards, the number of individual congeners measured in food and human samples could be increased.

The physical and chemical properties of the BDE-209 congener are such that great demands are made of the analytical method, including sample preparation, extraction and cleanup, as well as final chromatographic separation. The problems encountered during the analysis of PBDE congeners of high relative molecular mass are associated with thermal instability and sensitivity to light rather than with their high boiling points.

Typical limits of detection for tetra/pentaBDEs range from 0.005 to 0.05 ng/g, depending on lipid content and sample size.

The Committee noted that as DecaBDE was the only commercial formulation currently marketed in Europe and North America, analytical methods should include the determination of this fully brominated congener.

11.6 Effects of processing

No data were available on the effect of processing on concentrations of PBDEs in foods.

11.7 Prevention and control

As with other lipophilic contaminants, control of PBDE residues in animal feed is likely to have an impact on the concentrations of PBDEs found in meat, poultry, farmed fish and other animal-derived products. Additional investigations should also consider the significance of exposure from other non-food sources (indoor air, dust) as a means of control.

11.8 Levels and pattern of food contamination

The Committee reviewed data available on concentrations of PBDEs in foods (Table 92). Some of the data were from TDS conducted either at the national level (Finland, Netherlands and Sweden) or at the regional level within a given country (e.g. Vancouver and Whitehorse in Canada, and Catalonia in Spain), while others were from more limited, market basket surveys targeting special foods, e.g. foods of animal origin or fish and seafood, or were from grab samples collected from local markets (Canada, Germany, Japan, United Kingdom, United States). The data from the Canadian TDS and Special Fish and Seafood Survey and the TDS from the Netherlands and Sweden were available in reports published by the respective national agencies, while the data from the other studies were available in published scientific journals or were submitted by local governments. Concentration data were available for individual congeners or their sum. The patterns of congeners detected were not uniform across the various foods tested and were different from those present in any one commercial mixture.

In general, the available data on concentrations of PBDEs in food for the various countries have not covered the entire diets in these countries or are based on a small number of samples. Thus, the currently available data do not allow a comprehensive assessment to be made of contamination in all foods. Differences in concentrations were detected in samples of similar foods collected from various geographical areas.

11.9 Dietary intake assessment

Preliminary estimates of mean intake of PBDEs, based on a limited number of samples from Canada, some European countries, Japan and the United States, as reported in published studies and reports, range from 13 to 113 ng/day (Table 92). Fish and shellfish were the main contributors to total intakes of PBDEs in the European countries and Japan, while meats, poultry and products of these foods were the major contributors to the total intakes of PBDEs in Canada and the United States.

Estimates of regional intakes for the European and North American region were made using the GEMS/Food regional diets and concentration data from studies summarized in Table 92. Table 93 summarizes the food consumption data used in this estimation and the estimated mean intakes of total PBDEs for these regions. Although the North American diet is included under the European diet in GEMS/Food, intake estimates for the North American and European regions were

Table 92. Summary of available data on concentrations of PBDEs and associated national intakes

Country	Type of study	Foods	PBDEs measured	Data reported	Concentrations detected (ng/g fresh weight)	Consumption data	Reported mean intake of PBDEs (ng/day)
Canada	Food basket survey	Foods of animal origin	NA	NA	NA	National estimates	44
	TDS (1998)	About 50 foods representing the total diet	Total PBDEs (28, 47, 99, 100, 153, 154, 183)	Level/food sample	Range of means/food group 0.024 (dairy) to 0.333 (egg — 1 sample)	National estimates	38
	TDS, Vancouver (2002)	About 50 foods representing the total diet	Total PBDEs (28, 47, 99, 100, 153, 154, 183)	Level/food sample	Range of means/food group 0.035 (dairy) to 0.680 (fish)	National estimates	30
	Special Fish & Seafood Survey (2002)	70 samples of farmed and wild fish	Total PBDEs (28, 47, 99, 100, 153, 154, 183)	N, Mean, SE, SD, Min, Max per species and source	Range of means: 0.2–2.2 (farmed) 0.1–0.6 (wild)	NA	NA
Finland	Market basket survey (1997–1999)	228 foods grouped in 10 market baskets (about 4000 samples)	Total PBDEs (47, 99, 100, 153, 154)	Level/basket	Range of means/food group 0.009 (other) to 0.85 (fish)	1997 Dietary Survey of Finnish Adults	43
	Total diet basket	228 foods	Total PBDEs (47, 99, 100, 153, 154)	Level in total diet basket	0.043	1997 Dietary Survey of Finnish Adults	44

Table 92. (contd)

Country	Type of study	Foods	PBDEs measured	Data reported	Concentrations detected (ng/g fresh weight)	Consumption data	Reported mean intake of PBDEs (ng/day)
Germany	Samples collected from German markets (2001–2003)	Fish, meats, dairy (607 samples)	Total PBDEs (28, 47, 99, 100, 153, 154)	<i>N</i> , Number of detects; Number of non-detects; Mean concentration (positive samples); Range	Range of means/food group 0.030 (dairy) to 1.45 (fish and shellfish)	NA	NA
	Fish samples collected from German markets (2004)	13 fish samples	17, 28, 47, 66, 77, 99, 100, 153, 154, 183, 209, and total	Data/congener per sample	Mean: 0.66 Range: 0.01–2.87	NA	NA
Japan	TDS	13 food groups (two composites each for fish, meats and eggs, and milk and milk products, one composite for each of the remaining food groups)	47, 49, 66, 99, 100, 119, 153, 154, 183	Total PBDE/food group	Range of levels/food group (for detects) 0.009 (dairy) to 1.26 (fish) (ND = LOD)	National Nutrition Survey	113

Table 92. (contd)

Country	Type of study	Foods	PBDEs measured	Data reported	Concentrations detected (ng/g fresh weight)	Consumption data	Reported mean intake of PBDEs (ng/day)
Japan (contd)	Duplicate-diet study	Duplicate meals collected from six subjects for 2–3 days	47, 49, 66, 99, 100, 119, 153, 154, 183	Data/congener per person	Mean: 0.29 Range: 0.003–0.081	Total diet of six subjects for 2–3 days	68
	Market basket survey	Fish, shellfish, meats & vegetables (26 samples)	28, 47, 99, 100, 153, 154	Total PBDE per sample	Range of means/food group 0.030 (meats & poultry) to 0.91 (fish and shellfish)	NA	NA
Netherlands	TDS (2001–2002)	84 samples (dairy, eggs, meats, animal fats, fish, oil)	28, 47, 99, 100, 153, 154, 71, 77, 190, 209	Data per congener per sample	Range of means/food group ND (eggs) to 6.82 (fish and shellfish)	Data from 6250 individuals in Dutch National Food Consumption Survey	13
Spain	Market basket, in Catalonia (2000)	Fish, meats, dairy, vegetables, cereals, fats and oils ($n = 54$)	Tetra-, penta-, hexa-, hepta-, octaBDE	Total PBDE per food group and congener level/entire diet	Range of means/food group 0.001 (other foods) to 0.46 (fats and oils)	Consumption data for Catalonia	82
Sweden	TDS	Fish, meats, dairy, fats and oils ($n = 20$ composite samples)	47, 99, 100, 153, 154	Total PBDE level per sample	Range of means/food group: 0.04 (eggs) to 1.884 (fish and shellfish) (ND = LOD/2)	National Consumption Survey (1997–98)	Adults aged 17–74 years Females: 41 Males: 47 (ND = LOD/2)

Table 92. (contd)

Country	Type of study	Foods	PBDEs measured	Data reported	Concentrations detected (ng/g fresh weight)	Consumption data	Reported mean intake of PBDEs (ng/day)
Sweden (contd)	Market basket	Fish, meat, dairy products, eggs, fats/oils, pastry	Sum of congeners 47, 99, 100, 153, 154	NA	NA	Based on production	51 (ND = LOD/2)
UK	Targeted study	Fish (18 samples)	Total PBDEs	Range per fish type and location	Range 12–53 (control location) 59–288 (target location)	Standard portion sizes	Maximum intakes Control: 9 ng/kg bw per day Target: 56 ng/kg bw per day
USA	Duplicate-diet study	Total diet of 10 individuals	Sum of 47, 99, 100, 153, 154	NA	NA	10 individuals	90
	Market basket survey (2003)	32 food samples (fish, meats, dairy)	17, 28, 47, 66, 77, 85, 99, 100, 138, 153, 154, 183, 209	Total PBDEs per sample	Range 0.0009 (fats and oils), 1.487 (fish and shellfish)	USDA 1994–1996 Continuing Survey of Food Intakes by Individuals	Females: 1.4 ng/kg bw per day Males: 2.0 ng/kg bw per day
	Grab samples from local supermarkets (Texas)	15 meat samples	17, 28, 47, 66, 85, 99, 100, 138, 153, 154, 183, 209	Individual congeners and total PBDEs per sample	Mean: 0.58 Range: ND–2.86	NA	NA

Table 92. (contd)

Country	Type of study	Foods	PBDEs measured	Data reported	Concentrations detected (ng/g fresh weight)	Consumption data	Reported mean intake of PBDEs (ng/day)
USA (contd)	Grab samples from local supermarkets (Texas)	11 dairy samples	17, 28, 47, 66, 85, 99, 100, 138, 153, 154, 183, 209	Individual congeners and total PBDEs per sample	Mean: 0.17 Range: 0.03–0.66	NA	NA
	Samples from supermarkets in nine cities	48 bacon and meat trimmings	28/33, 47, 85, 99, 100, 153, 154, 183, 209	Mean and range for each congener and total PBDEs per food type	Mean: 0.20 (bacon), 1.07 (meat trimmings)	NA	NA
	Samples from local markets (California)	Nine meat samples	26 congeners	Individual congeners and total PBDEs per sample	Mean: 0.60 Range: 0.16–2.52	NA	NA
United States and EU	Review paper	Various fish species	47, 99, 100, 153, 154	<i>N</i> for each study, Mean concentration for each congener and total PBDEs	Range of means (ng/g lipid): European studies: 6.31–515 USA studies: 12.1–7200	NA	NA

Max, maximum; Min, minimum; *N*, number of samples; NA, not available or analysis not conducted; ND, not detected and counted as zero unless otherwise stated; SD, standard deviation; SE, standard error; USDA, United States Department of Agriculture

derived separately in light of the potential differences between concentrations of PBDEs detected in foods in Europe and North America. The estimated mean intakes of PBDEs for the European and North American regions were 2.2 and 3.6 ng/kg bw per day, respectively. Consumption of fish contributed most to European intake estimates, while meats and poultry contributed most to the North American intake estimates. No data on concentrations of PBDEs were available for countries in the following GEMS/Food regions: Africa, the Middle East or Latin America, and limited data were available for the Far East. The Committee derived estimates of international intake for these regions using the GEMS/Food regional diets and assuming that concentrations of PBDEs in food in these regions were equal to the average levels of contamination derived from European and North American data.¹ Estimated intakes for Africa, the Middle East, Latin America and the Far East were 1.5, 1.3, 2.1 and 1.2 ng/kg bw per day, respectively. Fish and shellfish contributed most to estimated intakes in the African, Latin American and Far Eastern regions, while fats and oils contributed most to the estimates for the Middle East. It should be noted that these estimates were only rough approximations since they were based on concentration data from other regions.

Table 93. Estimated intakes of total PBDEs in GEMS/Food regional diets

Food group	Consumption (g/day)	Estimated intake of PBDEs (ng/day) ^a	
		European diet ^b	North American diet ^c
Dairy and products	336	10	24
Eggs	38	2	8
Fats and oils	49	13	47
Fish and shellfish	47	84	40
Meat and poultry	217	17	66
Other foods	826	6	29 ^d
Total (ng/day)		131	213
Total (ng/kg bw per day) ^e		2.2	3.6

^a Non-detects set at zero.

^b Concentration data from Finland, Germany, Netherlands, Spain and Sweden were used in the estimation.

^c United States and Canada.

^d Limited data were available for this region; therefore, the combined data from the other regions were used instead.

^e Based on a body weight of 60 kg.

¹ For the Far Eastern region, limited data were available for some food groups and were used in combination with data from the North American and European regions for the remaining food groups.

A regional difference was apparent when considering intake by breastfeeding infants. Based on a median concentration of PBDEs of approximately 23 ng/g of lipid in human milk ($n = 145$), intake by a breastfeeding infant in North America was estimated at 120 ng/kg bw per day (average fat content of milk, 3.0%; 750 ml of milk per day; 5.0 kg bw during nursing). In comparison, based on a median concentration of PDBEs of 1.8 ng/g of lipid in samples of human milk, estimated intake for a breastfeeding infant in Germany would be approximately 10 ng/kg bw per day.

The Committee recognized the preliminary nature of the data on concentrations of PBDEs in food and human milk, which adds considerable uncertainty to the intake estimates.

11.10 Dose-response analysis

Only the commercial DecaBDE mixture has been tested in a long-term study of toxicity; the lowest concentration tested (2.5% in the diet) produced adverse effects. Limited toxicological information was available for commercial PBDE mixtures (PentaBDE and OctaBDE) whose congener patterns resemble those of residues found in food and human tissues. Only short-term feeding studies (up to 13 weeks) have been conducted in rats, with liver, kidney and thyroid being identified as target organs. Dose-related increases in relative liver weights and microscopic liver changes (hepatocellular enlargement with vacuolation) were noted in a study with a commercial OctaBDE mixture (DE-79) at a concentration of 100 mg/kg of diet (approximately 8 mg/kg bw per day). Similar effects were seen in a short-term feeding study with a commercial PentaBDE mixture (DE-71); dose-related increases in liver weights and histological changes (hypertrophy, slight degeneration and necrosis) were noted at the lowest dose, 2 mg/kg bw per day. The effects were still partially evident in females at the lowest dose after a 24-week recovery period. At higher doses (≥ 10 mg/kg bw per day), decreases in concentrations of circulating thyroid hormones (T4) were observed. The latter observation was supported by the results of a study of developmental toxicity in rats given the commercial PentaBDE mixture (DE-71); decreases in serum concentrations of T4 were seen in both fetuses and newborn pups at a maternal dose of 10 mg/kg bw per day administered on day 6 of gestation to PND 21.

The Committee also reviewed a number of preliminary studies of acute toxicity involving dosing with mainly commercial PentaBDE mixtures, BDE-47 or BDE-99 on a single day during gestation or lactation. In mice and rats, there were a variety of effects involving neurological development (behaviour, memory and activity), thyroid hormone perturbation and sexual maturation at doses as low as 60 μ g/kg bw. Owing to a lack of mechanistic information and adequate data on dose-response relationships, a clear interpretation of the significance to human health could not be made at the present time.

12. EVALUATION

For non-genotoxic substances, the Committee would normally allocate a provisional maximum tolerable daily intake (PMTDI) or provisional tolerable weekly intake (PTWI) based on the NOEL for the most sensitive adverse effect; however, the available data on PBDEs were not adequate for such an approach because:

- PBDEs represent a complex group of related chemicals, and the pattern of PBDE congeners in food is not clearly defined by a single commercial mixture.
- Data are inadequate to establish a common mechanism of action that would allow a single congener to be used as a surrogate for total exposure or, alternatively, as the basis for establishing toxic equivalence factors.
- There is no systematic database on toxicity including long-term studies on the main congeners present in the diet, using standardized testing protocols, that could be used to define a NOEL for individual PBDEs of importance.
- Several of the reported effects are biological outcomes for which the toxicological significance remains unclear.
- Studies with purified PBDE congeners *in vitro* have shown a lack of activation of the Ah receptor; however, many of the adverse effects reported are similar to those found with dioxin-like contaminants, suggesting that some toxicity data may be confounded by the presence of traces of impurities that are potent agonists of the Ah receptor.

DecaBDE was the only brominated diphenyl ether for which a long-term study of toxicity was available. A complete hazard characterization for this PBDE will become increasingly important, as at the time of the present evaluation it was the primary commercial mixture in use worldwide.

The limited toxicity data suggested that for the more toxic PBDE congeners, adverse effects would be unlikely to occur in rodents at doses of less than approximately 100 µg/kg bw per day. The current estimates of dietary intake were approximately 0.004 µg/kg bw per day, while intake by breastfeeding infants could be up to 0.1 µg/kg bw per day for the sum of all measured PBDE congeners, including the less toxic ones. In consequence, there appeared to be a large margin of exposure (MOE) for a non-genotoxic compound, which, despite the inadequacy of the data on toxicity and intake, gave reassurance that intakes of PBDEs are not likely to be a significant health concern. The Committee noted that, as with related bioaccumulative persistent contaminants (PCBs, dioxins), a more appropriate dose metric for interspecies comparison of risk would be a measure of the internal dose. For the majority of PBDEs studied, however, the data from experimental animals or on concentrations in human tissue were insufficient to allow a comparison with external dose.

12.1 Recommendations

Although no specific recommendations for PBDEs were made by the Committee, it was noted that as DecaBDE was the only commercial formulation currently marketed in Europe and North America, analytical methods should include the determination of this fully brominated congener.

The Committee also considered that continuing studies of PBDEs in samples from humans, including human milk, would be useful in assessing the overall exposure to PBDEs in foods and other possible sources.

13. REFERENCES

- Akutsu, K., Kitagawa, M., Nakazawa, H., Makino, T., Iwazaki, K., Oda, H. & Hori, S. (2003) Time-trend (1973–2000) of polybrominated diphenyl ethers in Japanese mother's milk. *Chemosphere*, **53** (6), 645–654.
- Alaee, M. & Wenning, R.J. (2002) The significance of brominated flame retardants in the environment: current understanding, issues and challenges. *Chemosphere*, **46** (5), 579–582.
- Alaee, M., Sergeant, D.B., Ikononou, M.G. & Luross, J.M. (2001) A gas chromatography/high-resolution mass spectrometry (GC/HRMS) method for determination of polybrominated diphenyl ethers in fish. *Chemosphere*, **44** (6), 1489–1495.
- Alaee, M., Arias, P., Sjödin, A. & Bergman, A. (2003) An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environ. Int.*, **29** (6), 683–689.
- Andersson, Ö. & Blomkvist, G. (1981) Polybrominated aromatic pollutants found in fish in Sweden. *Chemosphere*, **10**, 1051–1060.
- Andrade, A.J.M., Kuriyama, S.N., Akkoc, Z., Talsness, C. & Chahoud, I. (2004) Effects of low dose PBDE 47 exposure on thyroid hormone status and serum concentrations of FSH and inhibin B in male rats. *Organohalogen Compd.*, **66**, 3907–3912.
- Ashizuka, Y., Nakagawa, R., Hori, T., Tobiishi, K. & Iida, T. (2004) Levels of polybrominated diphenyl ethers and polybrominated dioxins in fish, total diet study food groups and Japanese meals. *Organohalogen Compd.*, **66**, 2553–2559.
- Asplund, L., Athanasiadou, M., Sjödin, A., Bergman, A. & Børjeson, H. (1999) Organohalogen substances in muscle, egg and blood from healthy Baltic salmon (*Salmo salar*) and Baltic salmon that produce offspring with the M74 syndrome. *Ambio*, **28**, 67–76.
- ATSDR (2004) *Toxicological Profile for Polybrominated Biphenyls and Polybrominated Diphenyl Ethers*. Atlanta, Georgia: United States Department of Health and Human Services, Agency for Toxic Substances and Disease Registry.
- Bahn, A.K., Mills, J.L., Snyder, P.J., Gann, P.H., Houten, L., Bialik, O., Hollmann, L. & Utiger, R.D. (1980) Hypothyroidism in workers exposed to polybrominated biphenyls. *N. Engl. J. Med.*, **302** (1), 31–33.
- Ballschmitter, K.H. & Zell, M. (1980) Analysis of polychlorinated biphenyls (PCBs) by glass capillary chromatography. *Fresenius Z. Anal. Chem.*, **302**, 20–31.
- Baumann, B., Hijman, W., van Beuzekom, S., Hoogerbrugge, R., Houweling, D. & Zeilmaker, M. (2003) PBDEs in human milk from the Dutch 1998 monitoring programme. *Organohalogen Compd.*, **61**, 187–190.

- Behnisch, P.A., Hosoe, K. & Sakai, S.I. (2003) Brominated dioxin-like compounds: in vitro assessment in comparison to classical dioxin-like compounds and other polyaromatic compounds. *Environ. Int.*, **29**, 861–877.
- BFRIP (1990) *Brominated Flame Retardants. A Review of Recent Research*. Arlington, Virginia: Brominated Flame Retardant Industry Panel (Unpublished Report No. III/4143/90). As cited in IPCS (1994).
- BIBRA (1977) *BIBRA Report*. Surrey: British Industrial Biological Research Association (Project No. 193/1/77).
- Björklund, J., Tollbäck, P. & Österman, C. (2003) Evaluation of the gas chromatographic column system for the determination of polybrominated diphenyl ethers. *Organohalogen Compd.*, **63**, 361–364.
- Bocio, A., Llobet, J.M., Domingo, J.L., Corbella, J., Teixido, A. & Casas, C. (2003) Polybrominated diphenyl ethers (PBDEs) in foodstuffs: human exposure through the diet. *J. Agric. Food Chem.*, **51** (10), 3191–3195.
- Boon, J.P., Lewis, W.E., Tjoen-A-Choy, M.R., Allchin, C.R., Law, R.J., de Boer, J., Ten Hallers-Tjabbes, C.C. & Zegers, B.N. (2002) Levels of polybrominated diphenyl ether (PBDE) flame retardants in animals representing different trophic levels of the North Sea food web. *Environ. Sci. Technol.*, **36** (19), 4025–4032.
- Branchi, I., Alleva, E. & Costa, L.G. (2002) Effects of perinatal exposure to a polybrominated diphenyl ether (PBDE 99) on mouse neurobehavioural development. *Neurotoxicology*, **23**, 375–384.
- Breslin, W.J., Kirk, H.D. & Zimmer, M.A. (1989) Teratogenic evaluation of a polybromodiphenyl oxide mixture in New Zealand white rabbits following oral exposure. *Fundam. Appl. Toxicol.*, **12** (1), 151–157.
- BSEF (2003) *Major Brominated Flame Retardants Volume Estimates*. Bromine Science and Environmental Forum (<http://www.bsef-site.com>).
- Buitenhuis, C., Cenijn, P.C., van Velzen, M., Lilienthal, H., Malmberg, T., Bergman, Å., Gutleb, A.C., Legler, J. & Brouwer, A. (2004) Effects of prenatal exposure to hydroxylated PCB metabolites and some brominated flame retardants on the development of rats. *Organohalogen Compd.*, **66**, 3586–3592.
- Burreau, S., Broman, D. & Zebuhr, Y. (1999) *Biomagnification Quantification of PBDEs in Fish Using Stable Nitrogen Isotope*. Poster presentation at the 19th International Symposium on Halogenated Environmental Organic Pollutants and POPs, 12–17 September 1999, Venice.
- CAC (2003) *Report of the Thirty-fifth Session of the Codex Committee on Food Additives and Contaminants, Arusha, Tanzania, 17–21 March 2003*. Rome: Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ALINORM 03/12A; <http://www.codexalimentarius.net/web/archives.jsp?year=03>).
- Capen, C.C. (1997) Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. *Toxicol. Pathol.*, **25** (1), 39–48.
- Carlson, G.P. (1980a) Induction of xenobiotic metabolism in rats by brominated diphenyl ethers administered for 90 days. *Toxicol. Lett.*, **6**, 207–212.
- Carlson, G.P. (1980b) Induction of xenobiotic metabolism in rats by short-term administration of brominated diphenyl ethers. *Toxicol. Lett.*, **5**, 19–25.
- Ceccatelli, S. (2004) *Effects of Polybrominated Diphenyl Ether (PBDE) and PCB on the Development of Reproductive Organs and Estrogen-Regulated Gene Expression in the Rat* [Ph.D. thesis]. Zurich: University of Zurich.

- Cheek, A.O., Kow, K., Chen, J. & McLachlan, J.A. (1999) Potential mechanisms of thyroid disruption in humans: interaction of organochlorine compounds with thyroid receptor transthyretin and thyroid-binding globulin. *Environ. Health Perspect.*, **107**, 273–278.
- Chen, G. & Bunce, N.J. (2003) Polybrominated diphenylethers as Ah receptor agonists and antagonists. *Toxicol. Sci.*, **76**, 310–320.
- Chen, G., Konstantinov, A.D., Chittim, B.G., Joyce, E.M., Bols, N.C. & Bunce, N.J. (2001) Synthesis of polybrominated diphenylethers and their capacity to induce CYP1A1 by the Ah receptor mediated pathway. *Environ. Sci. Technol.*, **35**, 3749–3756.
- Choi, J.W., Fujimaki, S., Kitamura, K., Hashimoto, S., Ito, H., Suzuki, N., Sakai, S.I. & Morita, M. (2002) Polybrominated dibenzo-*p*-dioxins (PBDDs), dibenzofurans (PBDFs) and diphenylethers (PBDEs) in Japanese human adipose tissue. *Organohalogen Compd.*, **58**, 169–171.
- Christensen, J.H., Glasius, M., Pecseli, M., Platz, J. & Pritzl, G. (2002) Polybrominated diphenyl ethers (PBDEs) in marine fish and blue mussels from southern Greenland. *Chemosphere*, **47** (6), 631–638.
- Chui, Y.C., Hansell, M.M., Addison, F. & Law, F.C.P. (1985) Effects of chlorinated diphenyl ethers on the mixed-function oxidases and ultrastructure of rat and trout liver. *Toxicol. Appl. Pharmacol.*, **81**, 287–294.
- CMA (1996) *Chromosome Aberrations in Human Peripheral Blood Lymphocytes*. Rockville, Maryland: Microbiological Associates Inc. (Study No. G96A063.342; Chemical Manufacturers Association).
- Covaci, A. & Schepens, P. (2001) Simplified method for determination of organochlorine pollutants in human serum by solid-phase disk extraction and gas chromatography. *Chemosphere*, **43** (4–7), 439–447.
- Covaci, A., de Boer, J., Ryan, J.J., Voorspoels, S. & Schepens, P. (2002a) Distribution of organobrominated and organochlorinated contaminants in Belgian human adipose tissue. *Environ. Res.*, **88** (3), 210–218.
- Covaci, A., de Boer, J., Ryan, J.J., Voorspoels, S. & Schepens, P. (2002b) Determination of polybrominated diphenyl ethers and polychlorinated biphenyls in human adipose tissue by large-volume injection-narrow-bore capillary gas chromatography/electron impact low-resolution mass spectrometry. *Anal. Chem.*, **74** (4), 790–798.
- Crhova, S., Cerna, M., Grabic, R., Tomsey, T. & Ocelka, T. (2002) Polybrominated flame retardants in human adipose tissue in Czech Republic inhabitants. The pilot study. *Organohalogen Compd.*, **58**, 241–244.
- Crofton, K.M., Taylor, M.M., Hedge, J.M., Gilbert, M.E. & DeVito, M.J. (2003) Developmental exposure to polybrominated diphenyl ethers; disruption of thyroid homeostasis, hepatic metabolism and neurobehavioral development. In: *9th Meeting of the International Neurotoxicology Association, Dresden*, Abstract 65, p. 65 (<http://www.neurotoxicology.org>).
- CVUA (2001) *Annual Report (2001)*. Chemisches und Veterinaruntersuchungsamt Freiburg.
- Darnerud, P.O. & Risberg, S. (2005) Tissue localization of tetra- and pentabromodiphenyl ether congeners (BDE-47, -85 and -100) in perinatal and adult C57BL mice. *Chemosphere* (in press).
- Darnerud, P.O., Atuma, S., Aune, M., Cnattingius, S., Wernroth, M.L. & Wicklund-Glynn, A. (1998a) Polybrominated diphenyl ethers (PBDEs) in breast milk from primiparous women in Uppsala County, Sweden. *Organohalogen Compd.*, **35**, 411–414.

- Darnerud, P.O., Eriksen, G., Johannesson, T., Larsen, P. & Viluksela, M. (1998b) *Polybrominated Diphenyl Ethers: Food Contamination and Potential Risks*. Copenhagen: Nordic Council of Ministers (Thema Nord 503).
- Darnerud, P.O., Atuma, S., Aune, M., Becker, W., Wicklund-Glynn, A. & Petersson-Grewe, K. (2000) New Swedish estimate on dietary intake of PBDE (a brominated flame retardant), dioxins, PCBs and DDT derived from market basket data. *Toxicol. Lett.*, **116** (Suppl.), 28 (abstract).
- Darnerud, P.O., Eriksen, G., Johannesson, T., Larsen, P. & Viluksela, M. (2001) Polybrominated diphenyl ethers: occurrence, dietary exposure, and toxicology. *Environ. Health Perspect.*, **109** (Suppl. 1), 49–68.
- Darnerud, P.O., Aune, M., Larsson, L. & Hallgren, S. (2004) Serum PBDE levels in exposed rats in relation to effects on thyroxine homeostasis. *Organohalogen Compd.*, **66**, 3977–3981.
- Dead Sea Bromide Works (1984) *Penta-bromo-diphenyl-ether. Assessment of Its Mutagenic Potential in Histidine Auxotrophs of Salmonella typhimurium*. Life Sciences Research Ltd. (Unpublished Report No. 84/DSB006/064). As cited in EU (2001).
- de Boer, J. & Denneman, M. (1998) Polychlorinated diphenylethers: origin, analysis, distribution, and toxicity in the marine environment. *Rev. Environ. Contam. Toxicol.*, **157**, 131–144.
- de Boer, J., van der Zande, T.E., Pieters, H., Ariese, F., Schipper, C.A., van Brummelen, T. & Vethaak, A.D. (2001) Organic contaminants and trace metals in flounder liver and sediment from the Amsterdam and Rotterdam harbours and off the Dutch coast. *J. Environ. Monit.*, **3** (4), 386–393.
- De Felip, E., Pöpke, O., Herrmann, T., Cardelli, M., Ingelido, A.M., Porpora, M.G. & di Domenico, A. (2003) PBDE levels in Italian nulliparous women of reproductive age. *Organohalogen Compd.*, **61**, 287–290.
- de Winter-Sorkina, R., Bakker, M.I., van Donkersgoed, G. & van Klaveren, J.D. (2003) *Dietary Intake of Brominated Flame Retardants by the Dutch Population*. Bilthoven: National Institute for Public Health and the Environment (RIVM Report 310305001/2003).
- de Wit, C.A. (2002) An overview of brominated flame retardants in the environment. *Chemosphere*, **46**, 583–624.
- Dodder, N.G., Strandberg, B. & Hites, R.A. (2002) Concentrations and spatial variations of polybrominated diphenyl ethers and several organochlorine compounds in fishes from the northeastern United States. *Environ. Sci. Technol.*, **36** (2), 146–151.
- el Dareer, S.M., Kalin, J.R. & Tillery, K.F. (1987) Disposition of decabromobiphenyl ether in rats dosed intravenously or by feeding. *J. Toxicol. Environ. Health*, **22**, 405–415.
- Eriksson, P. & Talts, U. (2000) Neonatal exposure to neurotoxic pesticides increases adult susceptibility: a review of current findings. *Neurotoxicology*, **21** (1–2), 37–47.
- Eriksson, P., Jakobsson, E. & Fredriksson, A. (2001) Brominated flame retardants: A novel class of developmental neurotoxicants in our environment? *Environ. Health Perspect.*, **109**, 903–908.
- Eriksson, P., Viberg, H., Jakobsson, E., Örn, U. & Fredriksson, A. (2002) A brominated flame retardant, 2,2',4,4',5-pentabromodiphenyl ether: uptake, retention, and induction of neurobehavioral alterations in mice during a critical phase of neonatal brain development. *Toxicol. Sci.*, **67**, 98–103.

- Eriksson, P., Johansson, N., Viberg, H., Fischer, C. & Fredriksson, A. (2004) Comparative developmental neurotoxicity of flame retardants, polybrominated flame retardants and organophosphorous compounds, in mice. *Organohalogen Compd.*, **66**, 3163–3165.
- Ethyl Corporation (1985) *Embryo/Fetal Toxicity and Teratogenic Potential of Saytex 115 Administered Orally Via Gavage to Sprague-Dawley Rats, Presumed Pregnant Rats*. Horsham, Pennsylvania: Argus Research Laboratories Inc. (Protocol No. 305-002).
- EU (2001) *European Union Risk Assessment Report: Pentabromophenyl (Diphenyl Ether, Pentabromo Derivative; CAS No. 32534-81-9*. Brussels: European Union.
- EU (2003) *European Union Risk Assessment Report: Diphenyl Ether, Octabromo Derivative. CAS No: 32536-52-0, EINECS No: 251-087-9*. Brussels: European Union.
- EU (2004) *European Union Risk Assessment Report: Update of the Risk Assessment Addendum of Bis(pentabromophenyl)ether*. Brussels: European Union.
- Evandri, M.G., Mastrangelo, S., Costa, L.G. & Bolle, P. (2003) In vitro assessment of mutagenicity and clastogenicity of BDE-99, a pentabrominated diphenyl ether flame retardant. *Environ. Mol. Mutagen.*, **42** (2), 85–90.
- Fängström, B., Strid, A., Athanassiadis, I., Grandjean, P., Weihe, P. & Bergman, Å. (2004a) A retrospective study of PBDEs in human milk from the Faroe Islands. In: *The Third International Workshop on Brominated Flame Retardants*. BFR 2004, Toronto, Ontario, 6–9 June 2004, pp. 33–36 (<http://www.bfr2004.com>).
- Fängström, B., Strid, A., Athanassiadis, I., Grandjean, P., Weihe, P. & Bergman, Å. (2004b) A retrospective time trend study of PBDEs and PCBs in human milk from the Faroe Islands. *Organohalogen Compd.*, **66**, 2829–2833.
- Fattore, E., Filipsson, A., Hanberg, A., Bergendorff, A. & Håkansson, H. (2001) Toxicity of a technical mixture of polybrominated diphenyl ethers following 28 days of oral exposure in male and female rats. *Organohalogen Compd.*, **53**, 357–360.
- Fernlof, G., Gadhasson, I., Podra, K., Damerud, P.O. & Thuvander, A. (1997) Lack of effects of some individual polybrominated diphenyl ether (PBDE) and polychlorinated biphenyl (PCB) congeners on human lymphocyte functions in vitro. *Toxicol. Lett.*, **90** (2–3), 189–197.
- Food Standards Australia New Zealand (2004) Submission to JECFA: *Individual Dietary Records Approach: PBDEs*. Australian National Nutrition Survey (Information Sheet 4).
- Fowles, J.R., Fairbrother, A., Baecher-Steppan, L. & Kerkvliet, N. (1994) Immunologic and endocrine effects of the flame-retardant pentobromodiphenyl ether (DE-71) in C57BL/6J mice. *Toxicology*, **86**, 49–61.
- Fürst, P. (2001) Organochlorine pesticides, dioxins, PCBs and polybrominated biphenyl ethers in human milk from Germany in the course of time. *Organohalogen Compd.*, **52**, 185–188.
- Garner, C.E. & Matthews, H.B. (1998) The effect of chlorine substitution on the dermal absorption of polychlorinated biphenyls. *Toxicol. Appl. Pharmacol.*, **149** (2), 150–158.
- Gilbert, M.E., Sui, I. & Crofton, K.M. (2004) Developmental exposure to polybrominated diphenyl ethers impairs synaptic transmission and LTP in hippocampus. *Toxicologist*, **78** (1-S), 1915.
- Gill, U., Chu, I., Ryan, J.J. & Feeley, M. (2004) Polybrominated diphenyl ethers: human tissue levels and toxicology. *Rev. Environ. Contam. Toxicol.*, **182**, 55–96.
- Gillner, M. & Jakobsson, E. (1996) Structure–affinity relationships for thyroid and dioxin receptor binding of halogenated naphthalenes and diphenylethers. *Organohalogen Compd.*, **29**, 220–221.

- Gold, L.S., Slone, T.H., Stern, B.R., Manley, N.B. & Ames, B.N. (1992) Rodent carcinogens: setting priorities. *Science*, **258**, 261–265.
- Great Lakes (1974) *Toxicity Data on DBDPO (DE-83). Acute Oral Toxicity in the Albino Rat*. Prepared by International Research and Development Corporation for Great Lakes Chemical Corporation.
- Great Lakes (1975) *Acute Toxicity Studies of Pentabromodiphenyl Ether, 345-76A in Rats and Rabbits*. Prepared by International Research and Development Corporation for Great Lakes Chemical Corporation (Report No. 2764-025).
- Great Lakes (1986) *Toxicity Data of Octabromo-diphenyloxyde (DE-79). A Range Finding Teratology Study in Rats with DE-79. Final Report*. Hazelton Laboratories, prepared for Great Lakes Chemical Corporation. As cited in EU (2003).
- Great Lakes (1987) *Toxicity Data of Octabromo-diphenyloxyde (DE-79)*. West Lafayette, Indiana: Great Lakes Chemical Corporation. As cited in IPCS (1994).
- Great Lakes (1990) *Great Lakes DE-79. Product Information*. West Lafayette, Indiana: Great Lakes Chemical Corporation. As cited in IPCS (1994).
- Great Lakes (1999) *Toxicity Data of Octabromo-diphenyloxyde (DE-79). In Vitro Mammalian Chromosome Aberration Test. Final Report*. Prepared by BioReliance for Great Lakes Chemical Corporation. As cited in EU (2003).
- Haglund, P.S., Zook, D.R., Buser, H.-R. & Hu, J. (1997) Identification and quantification of polybrominated diphenyl ethers and methoxy-polybrominated diphenyl ethers in Baltic biota. *Environ. Sci. Technol.*, **31** (11), 3281–3287.
- Hagmar, L., Sjödin, A., Höglund, P., Thuresson, K., Rylander, L. & Bergman, Å. (2000) Biological half-lives of polybrominated diphenylethers and tetrabromobisphenol A in exposed workers. *Organohalogen Compd.*, **47**, 198–201.
- Hagmar, L., Bjork, J., Sjödin, A., Bergman, A. & Erfurth, E.M. (2001) Plasma levels of persistent organohalogenes and hormone levels in adult male humans. *Arch. Environ. Health*, **56** (2), 138–143.
- Hakk, H. & Letcher, R.J. (2003) Metabolism in the toxicokinetics and fate of brominated flame retardants — a review. *Environ. Int.*, **29** (6), 801–828.
- Hakk, H., Huwe, J.K. & Lorentzen, M. (2001) A mass balance study of a commercial pentabromodiphenyl ether mixture in male Sprague-Dawley rats. *Organohalogen Compd.*, **52**, 5–8.
- Hakk, H., Larsen, G.L. & Klasson-Wehler, E. (2002) Tissue disposition, excretion, and metabolism of 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) in male Sprague-Dawley rats. *Xenobiotica*, **32**, 369–382.
- Hale, R.C., La Guardia, M.J., Harvey, E.P., Gaylor, M.O., Mainor, T.M. & Duff, W.H. (2001) Flame retardants. Persistent pollutants in land-applied sludges. *Nature*, **412** (6843), 140–141.
- Hallgren, S. & Darnerud, P.O. (1998) Effects of polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) on thyroid hormone levels and enzyme activities in rats. *Organohalogen Compd.*, **35**, 391–394.
- Hallgren, S. & Darnerud, P.O. (2002) Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) in rats — testing interactions and mechanisms for thyroid hormone effects. *Toxicology*, **177** (2–3), 227–243.
- Hallgren, S., Sinjari, T., Hakansson, H. & Darnerud, P.O. (2001) Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroid hormone and vitamin A levels in rats and mice. *Arch. Toxicol.*, **75**, 200–208.

- Hanberg, A., Ståhlberg, M., Georgellis, A., de Wit, C. & Ahlborg, U. (1991) Swedish dioxin survey: evaluation of the H-4IIE bioassay for screening environmental samples for dioxin-like enzyme induction. *Pharmacol. Toxicol.*, **69**, 442–449.
- Hardell, L., Lindström, G., van Bavel, B., Wingfors, H., Sundelin, E. & Liljegren, G. (1998) Concentrations of the flame retardant 2,2',4,4'-tetrabrominated diphenyl ether in human adipose tissue in Swedish persons and the risk for non-Hodgkin's lymphoma. *Oncol. Res.*, **10**, 429–432.
- Hardell, L., Eriksson, M., Lindström, G., van Bavel, B., Lind, A., Carlberg, M. & Liljegren, G. (2001) Case-control study on concentrations of organohalogen compounds and titers of antibodies to Epstein-Barr virus antigens in the etiology of non-Hodgkin lymphoma. *Leuk. Lymphoma*, **42**, 619–629.
- Harden, F., Toms, L.M., Ryan, J.J. & Müller, J. (2004) Determination of the levels of polybrominated diphenylethers (PBDEs) in pooled blood sera obtained from Australians aged 31–45 years. In: *The Third International Workshop on Brominated Flame Retardants*. BFR 2004, Toronto, Ontario, 6–9 June 2004, pp. 59–62 (<http://www.bfr2004.com>).
- Hardy, M.L., Schroeder, R., Biesemeier, J. & Manor, O. (2002) Prenatal oral (gavage) developmental toxicity study of decabromodiphenyl oxide in rats. *Int. J. Toxicol.*, **21**, 83–91.
- Harrad, S., Wijesekera, R., Hunter, S., Halliwell, C. & Baker, R. (2004) Preliminary assessment of U.K. human dietary and inhalation exposure to polybrominated diphenyl ethers. *Environ. Sci. Technol.*, **38**, 2345–2350.
- Health Canada (2004a) *Concentrations (ppt, based on wet wt.) of Polybrominated Biphenyl Ethers (PBDEs) from Total Diet Study in Whitehorse, 1998*. Ottawa, Ontario: Health Canada, Food Program (http://www.hc-sc.gc.ca/food-aliment/cs-ipc/fr-ra/e_pbde_conc_whitehorse98.html).
- Health Canada (2004b) *Concentrations (ppt, based on wet wt.) of Polybrominated Biphenyl Ethers (PBDEs) from Total Diet Study in Vancouver, 2002*. Ottawa, Ontario: Health Canada, Food Program (http://www.hc-sc.gc.ca/food-aliment/cs-ipc/fr-ra/e_pbde_conc_vancouver2002.html).
- Health Canada (2004c) *Fish and Seafood Survey — 2002*. Ottawa, Ontario: Health Canada, Food Program (http://www.hc-sc.gc.ca/food-aliment/cs-ipc/fr-ra/e_seafood_survey.html).
- Health Canada (2004d) *Dietary Intakes of Polybrominated Diphenyl Ethers (PBDEs) for All Ages Canadians from Total Diet Study in Whitehorse, 1998*. Ottawa, Ontario: Health Canada, Food Program (http://www.hc-sc.gc.ca/food-aliment/cs-ipc/fr-ra/e_pbde_intake_whitehorse98.html).
- Health Canada (2004e) *Dietary Intakes of Polybrominated Diphenyl Ethers (PBDEs) for All Ages Canadians from Total Diet Study in Vancouver, 2002*. Ottawa, Ontario: Health Canada, Food Program (http://www.hc-sc.gc.ca/food-aliment/cs-ipc/fr-ra/e_pbde_intake_vancouver2002.html).
- Health Canada (2004f) *Screening Assessment Report. Polybrominated Diphenyl Ethers (PBDEs) (Tetra-, Penta-, Hexa-, Hepta-, Octa-, Nona- and Deca-Congeners) (CAS Nos. 40088-47-9, 32534-81-9, 36483-60-0, 68928-80-3, 32536-52-0, 63936-56-1, 1163-19-5)*. Ottawa, Ontario: Health Canada, Existing Substances Division (http://www.ec.gc.ca/CEPARRegistry/documents/subs_list/HC_PBDEs_f.pdf).
- Hedge, J.M., Crofton, K.M., Laws, S.C., DeVito, M.J., Ross, D.G. & Das, P.C. (2004) 2,2',4,4'-Tetrabromodiphenyl ether (PBDE-47) alters thyroid function in rats. *Toxicologist*, **78** (1-S), 1909.

- Helleday, T., Tuominen, K.L., Bergman, A. & Jenssen, D. (1999) Brominated flame retardants induce intragenic recombination in mammalian cells. *Mutat. Res.*, **439** (2), 137–147.
- Herrmann, T., Schilling, B. & Pöpke, O. (2003) Photolysis of PBDEs in solvents by exposure to daylight in routine laboratory procedure. *Organohalogen Compd.*, **63**, 361–364.
- Herzke, D., Kallenborn, R., Nygard, T. & Sandanger, T. (2001) Species dependent distribution of polybrominated biphenyls and diphenylethers in eggs of Norwegian birds of prey. In: *The Second International Workshop on Brominated Flame Retardants*. BFR 2001, 14–16 May 2001, Stockholm University, Stockholm, pp. 321–324.
- Hirai, T., Fujimine, Y., Watanabe, S., Hata, J. & Watanabe, S. (2003) Concentration of polybrominated diphenyl ethers (PBDEs) in human samples in Japan. *Organohalogen Compd.*, **61**, 51–154.
- Hites, R. (2004) Polybrominated diphenyl ethers in the environment and in people: a meta-analysis of concentrations. *Environ. Sci. Technol.*, **38** (4), 945–956.
- Hites, R., Foran, J., Schwager, S., Knuth, B., Hamilton, C. & Carpenter, D. (2004) Global assessment of polybrominated diphenyl ethers in farmed and wild salmon. *Environ. Sci. Technol.*, **38** (19), 4945–4949.
- Holden, A., She, J., Tanner, M., Lunder, S., Sharp, R. & Hooper, K. (2003) PBDEs in San Francisco Bay area: measurements in fish. *Organohalogen Compd.*, **61**, 255–258.
- Howie, L., Dickerson, R., Davis, D. & Safe, S. (1990) Immunosuppressive and mono-oxygenase induction activities of polychlorinated diphenyl ether congeners in C57BL/6N mice: quantitative structure–activity relationships. *Toxicol. Appl. Pharmacol.*, **105**, 254–263.
- Huwe, J. (2004) Polybrominated diphenyl ethers in meat samples collected from supermarkets across the US. In: *The Third International Workshop on Brominated Flame Retardants*. BFR 2004, Toronto, Ontario, 6–9 June 2004, pp. 41–44 (<http://www.bfr2004.com>).
- Huwe, J.K., Hakk, H. & Lorentzen, M. (2002) A mass balance feeding study of a commercial octabromodiphenyl ether mixture in rats. *Organohalogen Compd.*, **58**, 229–232.
- IARC (1990) Decabromodiphenyl oxide. In: *Some Flame Retardants and Textile Chemicals, and Exposures in the Textile Manufacturing Industry*. Lyon: International Agency for Research on Cancer, pp. 73–84 (IARC Monographs on the Evaluation of Carcinogenic Risk to Humans, Vol. 48).
- Ikononou, M.G., Rayne, S., Fischer, M., Fernandez, M.P. & Cretney, W. (2002) Occurrence and congener profiles of polybrominated diphenyl ethers (PBDEs) in environmental samples from coastal British Columbia, Canada. *Chemosphere*, **46** (5), 649–663.
- Ingelido, A.M., Di Domenico, A., Ballard, T., De Felip, E., Dellatte, E., Ferri, F., Fulgenzi, A.R., Herrmann, T., Iacovella, N., Minero, R., Pöpke, O. & Porpora, M. (2004) Levels of polybrominated diphenyl-ethers in milk from Italian women living in Rome and Venice. *Organohalogen Compd.*, **66**, 2722–2728.
- IPCS (1994) *Brominated Diphenyl Ethers*. Geneva: World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 162).
- IPCS (1997) *Flame Retardants: A General Introduction*. Geneva: World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 192).
- IRDC (1976) *Decabromodiphenyl Ether and Octabromodiphenyl Ether. A 28-Day Toxicity Study in Rats*. Submitted by the International Research and Development Corporation to the United States Environmental Protection Agency under TSCA Section 8D (OTS0523322).

- IRDC (1977) *Octabromodiphenyl Ether. Thirteen Week Feeding Study in Rats*. Submitted by the International Research and Development Corporation to the United States Environmental Protection Agency under TSCA Section 8D (OTS0522297).
- ISCCCL (1977) *Tardex 50 Ames Test*. Unpublished report, Consultox Laboratories Inc. I.S.C. Chemicals Limited (Project No. CL 77:178). As cited in EU (2001).
- Jackson, J.A., Diliberto, J.J. & Birnbaum, L.S. (1993) Estimation of octanol–water partition coefficients and correlation with dermal absorption for several polyhalogenated aromatic hydrocarbons. *Fundam. Appl. Toxicol.*, **21**, 334–344.
- Jacobs, M., Covaci, A. & Schepens, P. (2002) Investigation of selected persistent organic pollutants in farmed Atlantic salmon (*Salmo salar*), salmon aquaculture feed, and fish oil compartments of feed. *Environ. Sci. Technol.*, **36**, 2797–2805.
- Jansson, B., Anderson, R., Asplund, L., Bergman, A., Litzen, K., Reutergardh, L., Sellstrom, U., Uvemo, U.-B., Wahlberg, C. & Wideqvist, U. (1991) Multiresidue method for the gas-chromatographic analysis of some polychlorinated and polybrominated pollutants in biological samples. *Fresenius Z. Anal. Chem.*, **340**, 439–445.
- Johansson, F., Allkvist, A., Erixon, K., Malmvarn, A., Nilsson, R., Bergman, A., Helleday, T. & Jenssen, D. (2004) Screening for genotoxicity using the DRAG assay: investigation of halogenated environmental contaminants. *Mutat. Res.*, **563** (1), 35–47.
- Johnson, A. & Olson, N. (2001) Analysis and occurrence of polybrominated diphenyl ethers in Washington state freshwater fish. *Arch. Environ. Contam. Toxicol.*, **41** (3), 339–344.
- Jones-Otazo, H.A., Clarke, J.P., Diamond, M.L., Archbold, J.A., Ferguson, G., Harner, T., Richardson, G.M., Ryan, J.J. & Wilford, B. (2005) Is house dust the missing exposure pathway for PBDEs? An analysis of the urban fate and human exposure to PBDEs. *Environ. Sci. Technol.*, **39** (14), 5121–5130.
- Kalantzi, O.I., Martin, F.L., Thomas, G.O., Alcock, R.E., Tang, H.R., Drury, S.C., Carmichael, P.L., Nicholson, J.K. & Jones, K.C. (2004) Different levels of polybrominated diphenyl ethers (PBDEs) and chlorinated compounds in breast milk from two U.K. regions. *Environ. Health Perspect.*, **112** (10), 1085–1091.
- Kalk (1978) *Ames Metabolic Activation Test to Assess the Potential Mutagenic Effect of Bromkal 70-5 DE*. Unpublished report, Huntington Research Centre. Chemische Fabrik Kalk GmbH (Report No. 86-9000004000). As cited in EU (2001).
- Kalk (1982) *CFK Bromkal® Branschütz Ausrüstungen*. Chemische Fabrik Kalk GmbH (Information Sheet No. 3000-7/82). As cited in IPCS (1994).
- Kato, Y., Ikushiro, S., Haraguchi, K., Yamazaki, T., Ito, Y., Suzuki, H., Kimura, R., Yamada, S., Inoue, T. & Degawa, M. (2004) A possible mechanism for decrease in serum thyroxine level by polychlorinated biphenyls in Wistar and Gunn rats. *Toxicol. Sci.*, **81**, 309–315.
- Khera, K.S. (1984) Maternal toxicity — a possible factor in fetal malformations in mice. *Teratology*, **29** (3), 411–416.
- Kiviranta, H., Ovaskainen, M.L. & Vartiainen, T. (2004) Market basket study on dietary intake of PCDD/Fs, PCBs, and PBDEs in Finland. *Environ. Int.*, **30**, 923–932.
- Klasson-Wehler, E., Mörrck, A. & Hakk, H. (2001) Metabolism of polybrominated diphenyl ethers in the rat. In: *The Second International Workshop on Brominated Flame Retardants*. BFR 2001, 14–16 May 2001, Stockholm University, Stockholm, pp. 93–97.
- Knoth, W., Mann, W., Meyer, R. & Nebhut, J. (2002) Polybrominated diphenyl ethers in house dust. *Organohalogen Compd.*, **58**, 213–216.
- Knoth, W., Mann, W., Meyer, R. & Nebhut, J. (2003) Polybrominated diphenyl ethers in indoor dust. *Organohalogen Compd.*, **61**, 207–210.

- Kociba, R.J., Frauson, L.O., Humiston, C.G., Norris, J.M., Wade, C.E., Lisowe, R.W., Quast, J.F., Jersey, G.C. & Jewett, G.L. (1975) Results of a two-year dietary feeding study with decabromodiphenyl oxide (DBDPO) in rats. *J. Combust. Toxicol.*, **2**, 267–285.
- Kodavanti, P.R.S. & Derr-Yellin, E.C. (2002) Differential effects of polybrominated diphenyl ethers and polychlorinated biphenyls on [³H]arachidonic acid release in rat cerebellar granule neurons. *Toxicol. Sci.*, **68**, 451–457.
- Koistinen, J., Sanderson, J.T., Giesy, J.P., Nevalainen, T. & Paasivirta, J. (1996) Ethoxyresorufin-O-deethylase induction potency of polychlorinated diphenyl ethers in H4IIE rat hepatoma cells. *Environ. Toxicol. Chem.*, **15**, 2028–2034.
- Körner, W. & Hagenmaier, H. (1990) PCDD/PCDF formation in smoked, fried and broiled meat and fish. *Organohalogen Compd.*, **4**, 243–248.
- Kuriyama, S.N., Talsness, C., Wittfohr, W. & Chahoud, I. (2004a) Exposure to an environmentally relevant dose of PBDE 99 disrupts thyroid hormone homeostasis and causes neurobehavior disturbances in rat offspring. *Toxicologist*, **78** (1-S), 1908.
- Kuriyama, S.N., Fidalgo-Nieto, A.A., Grande, S.W., Akkoc, Z., de Souza, C.A.M. & Chahoud, I. (2004b) Thyroid hormone levels and hepatic enzyme activity in lactating dams after gestational exposure to low dose PBDE 47. *Organohalogen Compd.*, **66**, 3901–3906.
- Kuriyama, S.N., Talsness, C. & Chahoud, I. (2004c) Sex-dependent behavioral changes in rat offspring after in utero administration of a single low dose PBDE 47. *Organohalogen Compd.*, **66**, 3893–3900.
- Kuriyama, S.N., Talsness, C.E., Grote, K. & Chahoud, I. (2005) Developmental exposure to low dose PBDE 99: 1. Effects on male fertility and neurobehavior in rat offspring. *Environ. Health Perspect.*, **113** (2), 149–154.
- Lee, S.-J., Kim, B., Kim, H. & Chang, Y.-S. (2002) Human blood levels of polybrominated diphenylethers in Korea. *Organohalogen Compd.*, **58**, 205–208.
- Leonards, P., Santillo, D., Bridgen, K., van der Veen, I., Heselingen, J., de Boer, J. & Johnston, P. (2001) Brominated flame retardants in office dust samples. In: *The Second International Workshop on Brominated Flame Retardants*. BFR 2001, 14–16 May 2001, Stockholm University, Stockholm, pp. 299–302.
- Lepom, P., Karasyova, T. & Sawal, G. (2002) Occurrence of PBDEs in freshwater fish from Germany. *Organohalogen Compd.*, **58**, 209–212.
- Leung, H.-W. & Paustenbach, D.J. (1994) Techniques for estimating the percutaneous absorption of chemicals due to occupational and environmental exposure. *Appl. Occup. Environ. Hyg.*, **9**, 187–197.
- Lichtensteiger, W., Ceccatelli, R., Faass, O., Fleischmann, I. & Schlumpf, M. (2003) Effects of PBDE and PCB on neuroendocrine ontogeny and sex hormone target gene expression. In: *9th Meeting of the International Neurotoxicology Association, Dresden*, p. 143 (abstract) (<http://www.neurotoxicology.org>).
- Lichtensteiger, W., Faass, O., Ceccatelli, R. & Schlumpf, M. (2004) Developmental exposure to PBDE 99 and PCB affects estrogen sensitivity of target genes in rat brain regions and female sexual behavior. *Organohalogen Compd.*, **66**, 3965–3970.
- Lilienthal, H., Hack, A., Roth-Härer, A., Altmann, L., Winneke, G. & Wiegand, H. (2004) Developmental neurotoxicity of polybrominated diphenyl ethers (PBDE): Steroid-dependent behavior, sexual development and circulating steroids. *Toxicologist*, **78** (1-S), 1905.
- Lind, Y., Atuma, S., Aune, M., Bjerselius, R., Darnerud, P.O., Cnattingius, S. & Glynn, A. (2001) Polybrominated diphenyl ethers (PBDEs) in breast milk from Uppsala women — extension and updating of data. In: *The Second International Workshop on Brominated*

- Flame Retardants*. BFR 2001, 14–16 May 2001, Stockholm University, Stockholm, p. 222.
- Lind, Y., Aune, M., Atuma, S., Besker, W., Gjerselius, R., Glynn, A. & Darnerud, P.O. (2002) Food intake of the brominated flame retardants PBDEs and HBCD in Sweden. *Organohalogen Compd.*, **58**, 181–184.
- Lind, Y., Darnerud, P.O., Atuma, S., Aune, M., Becker, W., Bjerselius, R., Cnattingius, S. & Glynn, A. (2003) Polybrominated diphenyl ethers in breast milk from Uppsala County, Sweden. *Environ. Res.*, **93** (2), 186–194.
- Lopez, D., Athanasiadou, M., Athanassiadis, I., Estrada, L. & Bergmann, A. (2004) A preliminary study on PBDEs and HBCDD in blood and milk from Mexican women. In: *The Third International Workshop on Brominated Flame Retardants*. BFR 2004, Toronto, Ontario, 6–9 June 2004, pp. 483–487 (<http://www.bfr2004.com>).
- Luksemburg, W., Wenning, R., Patterson, A. & Meier M. (2004) Levels of polybrominated diphenyl ethers (PBDEs) in fish, beef and fowl purchased in food markets in northern California, USA. In: *The Third International Workshop on Brominated Flame Retardants*. BFR 2004, Toronto, Ontario, 6–9 June 2004, pp. 479–482 (<http://www.bfr2004.com>).
- Lunder, S. & Sharp, R. (2003) *Mothers' Milk: Record Levels of Toxic Fire Retardants Found in American Mothers' Breast Milk*. Washington, D.C.: Environmental Working Group (http://www.ewg.org/reports_content/mothersmilk/pdf/mothersmilk_final.pdf).
- Luross, J.M., Alaei, M., Sergeant, D.B., Cannon, C.M., Whittle, D.M., Solomon, K.R. & Muir, D.C. (2002) Spatial distribution of polybrominated diphenyl ethers and polybrominated biphenyls in lake trout from the Laurentian Great Lakes. *Chemosphere*, **46** (5), 665–672.
- Madina, F., Giordano, G., Fattori, V., Vitalone, A., Branchi, I., Capone, F. & Costa, L.G. (2004) Differential in vitro neurotoxicity of the flame retardant PBDE-99 and of the PCB Aroclor 1254 in human astrocytoma cells. *Toxicol. Lett.*, **154**, 11–21.
- Manchester-Neesvig, J.B., Valters, K. & Sonzogni, W.C. (2001) Comparison of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in Lake Michigan salmonids. *Environ. Sci. Technol.*, **35** (6), 1072–1077.
- Mariussen, E. & Fonnum, F. (2003) The effect of brominated flame retardants on neurotransmitter uptake into rat brain synaptosomes and vesicles. *Neurochem. Int.*, **43**, 533–542.
- Marsh, G., Bergman, A., Bladh, L.G., Gillner, M. & Jakobsson, E. (1998) Synthesis of p-hydrobromodiphenyl ethers and binding to the thyroid receptor. *Organohalogen Compd.*, **37**, 305–308.
- Mayer, R. (1998) Polychlorinated dibenzo-p-dioxins and dibenzofurans in smoked meat products. *Organohalogen Compd.*, **38**, 139–142.
- Mayer, R. & Jahr, D. (1998) *Lebensmittelchemie*, **52**, 100–104.
- McDonald, T.A. (2004) Distribution of PBDE levels among U.S. women: estimates of daily intake and risk of developmental effects. In: *The Third International Workshop on Brominated Flame Retardants*. BFR 2004, Toronto, Ontario, 6–9 June 2004, pp. 443–446 (<http://www.bfr2004.com>).
- McGregor, D.B. (1992) Chemicals classified by IARC: their potency in tests for carcinogenicity in rodents and their genotoxicity and acute toxicity. In: Vainio, H., Magee, P., McGregor, D.B. & McMichael, A.J., eds., *Mechanisms of Carcinogenesis in Risk Identification*. Lyon, International Agency for Research on Cancer, pp. 323–352 (IARC Scientific Publications No. 116).

- Meerts, I.A.T.M., Luijks, E.A.C., Marsh, G., Jakobsson, E., Bergman, Å. & Brouwer, A. (1998) Polybrominated diphenylethers (PBDEs) as Ah-receptor agonists and antagonists. *Organohalogen Compd.*, **37**, 147–150.
- Meerts, I.A.T.M., van Zanden, J.J., Luijks, E.A.C., van Leeuwen-Bol, I., Marsh, G., Jakobson, E., Berman, A. & Brouwer, A. (2000) Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. *Toxicol. Sci.*, **56**, 95–104.
- Meerts, I.A.T.M., Letcher, R.J., Hoving, S., Marsh, G., Bergman, A., Lemmen, J.G., van der Burg, B. & Brouwer, A. (2001) In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs, and polybrominated bisphenol A compounds. *Environ. Health Perspect.*, **109**, 399–407.
- Meirionyté Guvenius, D. & Norén, K. (2001) Polybrominated diphenyl ethers in Swedish human milk. The follow-up study. In: *The Second International Workshop on Brominated Flame Retardants*. BFR 2001, 14–16 May 2001, Stockholm University, Stockholm, pp. 303–305.
- Meirionyté Guvenius, D., Bergman, A. & Norén, K. (1998) Analysis of polybrominated diphenyl ethers in human milk. *Organohalogen Compd.*, **35**, 387–390.
- Meirionyté Guvenius, D., Norén, K. & Bergman, A. (1999) Analysis of polybrominated diphenyl ethers in Swedish human milk. A time-related study, 1972–1997. *J. Toxicol. Environ. Health*, **A58**, 329–341.
- Meirionyté Guvenius, D., Bergman, A. & Norén, K. (2001) Polybrominated diphenylethers in Swedish human liver and adipose tissue. *Arch. Environ. Contam. Toxicol.*, **40**, 564–570.
- Meirionyté Guvenius, D., Aronsson, A., Ekman-Ordeberg, G., Bergman, A. & Noren, K. (2003) Human prenatal and postnatal exposure to polybrominated diphenyl ethers, polychlorinated biphenyls, polychlorobiphenylols, and pentachlorophenol. *Environ. Health Perspect.*, **111** (9), 1235–1241.
- Meneses, M., Wingfors, H., Schuhmacher, J.L., Lindstrom, G. & Bavel, B. (1999) Polybrominated diphenyl ethers detected in human adipose tissue from Spain. *Chemosphere*, **39**, 2271–2273.
- Mörck, A. & Klasson-Wehler, E. (2001) Metabolism of decabromodiphenyl ether (BDE-209) in the rat. *Organohalogen Compd.*, **52**, 9–12.
- Mörck, A., Hakk, H., Örn, U. & Klasson-Wehler, E. (2003) Decabromodiphenyl ether in the rat — absorption, distribution, metabolism and excretion. *Drug Metab. Dispos.*, **31** (7), 900–907.
- Mundy, W.R., Freudenreich, T.M., Crofton, K.M. & De Vito, M.J. (2004) Accumulation of PBDE-47 in primary cultures of rat neocortical cells. *Toxicol. Sci.*, **82**, 164–169.
- NAS (2000) *Toxicological Risks of Selected Brominated Flame Retardant Chemicals. Decabromodiphenyl Oxide*. Washington, D.C.: National Academy of Sciences, National Academy Press.
- Norén, K. & Meirionyté Guvenius, D. (1998) Contaminants in Swedish human milk. Decreasing levels of organochlorine and increasing levels of organobromine compounds. *Organohalogen Compd.*, **358**, 1–4.
- Norén, K. & Meirionyté Guvenius, D. (2000) Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20–30 years. *Chemosphere*, **40** (9–11), 1111–1123.
- Norris, J.M., Ehrmantraut, J.W., Gibbons, C.L., Kociba, R.J., Schwetz, B.A., Rose, J.Q., Humistone, C.G., Jewett, G.L., Crummett, W.B. & Gehring, P.J. (1973) Toxicological

- and environmental factors involved in the selection of decabromodiphenyl oxide as a fire retardant chemical. *Appl. Polym. Symp.*, **22**, 195–219.
- Norris, J.M., Ehrmantraut, J.W., Kociba, R.J., Schwetz, B.A., Rose, J.Q., Humiston, C.G., Jewett, G.L., Crummett, W.B., Gehring, P.J. & Tirsell, J.B. (1975a) Evaluation of decabromodiphenyl oxide as a flame-retardant chemical. *Chem. Hum. Health Environ.*, **1**, 100–116.
- Norris, J.M., Kociba, R.J., Schwetz, B.A., Rose, J.Q., Humiston, C.G., Jewett, G.L., Gehring, P.J. & Mailhes, J.B. (1975b) Toxicology of octabromodiphenyl and decabromodiphenyl oxide. *Environ. Health Perspect.*, **11**, 153–161.
- Norstrom, R.J., Simon, M., Moisey, J., Wakeford, B. & Weseloh, D.V. (2002) Geographical distribution (2000) and temporal trends (1981–2000) of brominated diphenyl ethers in Great Lakes herring gull eggs. *Environ. Sci. Technol.*, **36** (22), 4783–4789.
- Northwest Environment Watch (2004) *Flame Retardants in Puget Sound Residents. First Round of Results from a Study on Toxic Body Burdens*. Seattle, Washington: Northwest Environment Watch, February (http://www.northwestwatch.org/pollution/WA_PBDEs.pdf).
- NTP (1986) *Toxicology and Carcinogenesis Studies of Decabromodiphenyl Oxide (CAS No 1163-19-5) in F344/N Rats and B6C3F1 Mice (Feed Studies)*. Research Triangle Park, North Carolina: United States Department of Health and Human Services, Public Health Service, National Institutes of Health, National Toxicology Program (NTP Technical Report Series No. 309).
- Nylund, K., Kierkegaard, A., Eriksson, U., Asplund, L., Bignert, A. & Olsson, M. (2001) Spatial distribution of some polybrominated diphenyl ethers and hexabromocyclododecane in herring along the Swedish coast. In: *The Second International Workshop on Brominated Flame Retardants*. BFR 2001, 14–16 May 2001, Stockholm University, Stockholm, pp. 349–352.
- Öberg, K., Warman, K. & Öberg, T. (2002) Distribution and levels of brominated flame retardants in sewage sludge. *Chemosphere*, **48** (8), 805–809.
- Ohta, S., Ishizuka, D., Nishimura, H., Nakao, T., Aozasa, O., Shimidzu, Y., Ochiai, F., Kida, T., Nishi, M. & Miyata, H. (2002) Comparison of polybrominated diphenyl ethers in fish, vegetables, and meats and levels in human milk of nursing women in Japan. *Chemosphere*, **46**, 689–696.
- Örn, U. (1997) *Synthesis of Polybrominated Diphenyl Ethers and Metabolism of 2,2',4,4'-Tetrabromo[¹⁴C]diphenyl Ether* [Licentiate Thesis]. Stockholm: Stockholm University.
- Örn, U. & Klasson-Wehler, E. (1998) Metabolism of 2,2',4,4'-tetrabromodiphenyl ether in rat and mouse. *Xenobiotica*, **28**, 199–211.
- PAI (1984) *Initial Submission: Acute Oral Toxicity in Rats (14 Days) of Saytex 115 (Pentabromodiphenyl oxide)*. Submitted to the United States Environmental Protection Agency under TSCA Section FYI. OTS0000972. Pharmakon Associates, Inc. As cited in ATSDR (2004).
- Päpke, O., Bathe, L., Bergman, Å., Fürst, P., Meironyté Guvenius, D., Herrmann, T. & Norén, K. (2001) Determination of PBDEs in human milk from the United States, comparison of results from three laboratories. *Organohalogen Compd.*, **52**, 197–200.
- Päpke, O., Fürst, P. & Herrmann, T. (2004) Determination of polybrominated diphenyl ethers (PBDEs) in biological tissues with special emphasis on QC/QA measures. *Talanta*, **63**, 1203–1211.

- Pereg, D., Ryan, J.J., Ayotte, P., Muckle, G., Patry, B. & Dewailly, E. (2003) Temporal and spatial changes of brominated diphenyl ethers (BDEs) and other POPs in human milk from Nunavik (Arctic) and southern Quebec. *Organohalogen Compd.*, **61**, 127–130.
- Peters, A.K., Van Londen, K., Bergman, A., Bohonowych, J., Denison, M.S., Van den Berg, M. & Sanderson, T.S. (2004) Effects of polybrominated diphenyl ethers (PBDEs) on basal and TCDD-induced ethoxyresorufin (EROD) activity and cytochrome P450 1A1 expression in MCF7, HepG2 and H4IIE cells. *Toxicol. Sci.*, **82** (2), 488–496.
- Petreas, M., She, J., Brown, F.R., Winkler, J., Visita, P., Li, C., Chand, D., Dhaliwal, J., Rogers, E., Zhao, G. & Charles, M. (2002) High PBDE concentrations in Californian human and wildlife populations. *Organohalogen Compd.*, **58**, 177–180.
- Petroske, E., Zaylskie, R.G. & Feil, V.J. (1997) The effect of cooking on dioxin and furan concentrations in beef. *Organohalogen Compd.*, **33**, 436–439.
- Petroske, E., Zaylskie, R.G. & Feil, V.J. (1998) Reduction in polychlorinated dibenzodioxin and dibenzofuran residues in hamburger meat during cooking. *J. Agric. Food Chem.*, **46**, 3280–3284.
- Pirard, C., de Pauw, E. & Focant, J.F. (2003) Levels of selected PBDE and PCBs in Belgian human milk. *Organohalogen Compd.*, **61**, 263–266.
- Polder, A., Thomsen, C., Bescher, G., Skaare, J., Løken, K. & Eggesbø, M. (2004) The Norwegian Human Milk Study HUMIS: Variation in levels of chlorinated pesticides, PCBs and PBDEs in Norwegian breast milk. *Organohalogen Compd.*, **66**, 2476–2482.
- Prevedouros, K., Jones, K.C. & Sweetman, A.J. (2004) Estimation of the production, consumption, and atmospheric emissions of pentabrominated diphenyl ether in Europe between 1970 and 2000. *Environ. Sci. Technol.*, **38** (12), 3224–3231.
- Rahman, F., Langford, K.H., Scrimshaw, M.D. & Lester, J.N. (2001) Polybrominated diphenyl ether (PBDE) flame retardants. *Sci. Total Environ.*, **275**, 1–17.
- Rayne, S., Ikonomou, M.G. & Antcliffe, B. (2003) Rapidly increasing polybrominated diphenyl ether concentrations in the Columbia River system from 1992 to 2000. *Environ. Sci. Technol.*, **37** (13), 2847–2854.
- Rice, C.P., Chernyak, S.M., Begnoche, L., Quintal, R. & Hickey, J. (2002) Comparisons of PBDE composition and concentration in fish collected from the Detroit River, MI and Des Plaines River, IL. *Chemosphere*, **49** (7), 731–737.
- Richards, R.G., DiAugustine, R.P., Petrusz, P., Clark, G.C. & Sebastian, J. (1996) Estradiol stimulates tyrosine phosphorylation of the insulin-like growth factor-1 receptor and insulin receptor substrate-1 in the uterus. *Proc. Natl. Acad. Sci. U.S.A.*, **93** (21), 12002–12007.
- Rowell, P., Yagminas, A., Chu, I. & Arnold, D.L. (2004) 28 day gavage study with a technical mixture of lower polybrominated diphenyl ethers in Sprague-Dawley rats. In: *The Third International Workshop on Brominated Flame Retardants*. BFR 2004, Toronto, Ontario, 6–9 June 2004, pp. 419–423 (<http://www.bfr2004.com>).
- Rozman, K.K. (1991) Letter to the Editor. *Toxicol. Appl. Pharmacol.*, **108**, 568–569.
- Ryan, J.J. (2004) Polybrominated diphenyl ethers (PBDEs) in human milk; occurrence worldwide. In: *The Third International Workshop on Brominated Flame Retardants*. BFR 2004, Toronto, Ontario, 6–9 June 2004, pp. 17–22 (<http://www.bfr2004.com>).
- Ryan, J.J. & Patry, B. (2000) Determination of brominated diphenyl ethers (BDEs) and levels in Canadian milks. *Organohalogen Compd.*, **47**, 57–60.
- Ryan, J.J. & Patry, B. (2001) Body burdens and food exposure in Canada for polybrominated diphenylethers (BDEs). *Organohalogen Compd.*, **51**, 226–229.

- Ryan, J.J. & van Oostdam, J. (2004) Polybrominated diphenyl ethers (PBDEs) in maternal and cord blood plasma of several northern Canadian populations. *Organohalogen Compd.*, **66**, 2579–2585.
- Ryan, J.J., Patry, B., Mills, P. & Beaudoin, N. (2002) Recent trends in levels of brominated diphenyl ethers in human milk from Canada. *Organohalogen Compd.*, **58**, 173–176.
- Safe, S. (1990) Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *Crit. Rev. Toxicol.*, **21**, 51–88.
- Sand, S., von Rosen, D., Eriksson, P., Frederiksson, A., Viberg, H., Victorin, K. & Falk Felipsson, A. (2004) Dose–response modeling and benchmark calculations from spontaneous behavior data on mice neonatally exposed to 2,2',4,4',5-pentabromodiphenyl ether. *Toxicol. Sci.*, **81**, 491–501.
- Sandholm, A. (2003) *Metabolism of Some Polychlorinated Biphenyl and Polybrominated Diphenyl Ether Congeners in the Rat* [Doctoral dissertation]. Stockholm: Stockholm University, Department of Environmental Chemistry.
- Sandholm, A., Emanuelsson, B.-M. & Klasson-Wehler, E. (2003) Bioavailability and half-life of decabromodiphenyl ether (BDE-209) in the rat. *Xenobiotica*, **33** (11), 1149–1158.
- Schecter, A.J., Päpke, O., Dellarco, M. & Olson, J.R. (1996) A comparison of dioxins and dibenzofurans in cooked and uncooked food. *Organohalogen Compd.*, **28**, 166–170.
- Schecter, A., Päpke, O. & Dellarco, M. (1997) Dioxin, dibenzofuran, and PCB congeners in cooked and uncooked foods. *Organohalogen Compd.*, **33**, 462–466.
- Schecter, A., Dellarco, M., Päpke, O. & Olson, J. (1998) A comparison of dioxins, dibenzofurans and coplanar PCBs in uncooked and broiled ground beef, catfish and bacon. *Chemosphere*, **37**, 1723–1730.
- Schecter, A., Pavuk, M., Päpke, O., Ryan, J.J., Birnbaum, L. & Rosen, R. (2003) Congener specific measurements of polybrominated diphenyl ethers in 47 individual milk samples from nursing mothers in the USA. *Organohalogen Compd.*, **61**, 13–16.
- Schecter, A., Päpke, O., Tung, K.C., Staskal, D. & Birnbaum, L. (2004a) Polybrominated diphenyl ethers contamination of United States food. *Environ. Sci. Technol.*, **38** (20), 5306–5311.
- Schecter, A., Päpke, O., Staskal, D., Tung, K.C., Ryan, J.J., Rosen, R. & Birnbaum, L. (2004b) PBDE contamination of U.S. food and human milk; and PBDE, PCDD/F, PCB, and [sic] levels in U.S. human blood (1973 and 2003). In: *The Third International Workshop on Brominated Flame Retardants*. BFR 2004, Toronto, Ontario, 6–9 June 2004, pp. 27–32 (<http://www.bfr2004.com>).
- Schecter, A., Pavuk, M., Päpke, O., Ryan, J.J., Birnbaum, L. & Rosen, R. (2004c) Polybrominated diphenyl ethers (PBDEs) in US mothers' milk. *Environ. Health Perspect.*, **111** (14), 1723–1729.
- Schecter, A., Päpke, O., Tung, K.C., Joseph, J., Dahlgren, J. & Harris, T.R. (2005) Polybrominated diphenylether (PBDE) flame retardants in the US population: current levels, temporal trends, and comparison with dioxins, dibenzofurans and polychlorinated biphenyls. *J. Occup. Environ. Med.*, **47** (3), 199–211.
- Schlabach, M., Fjeld, E. & Brevik, E. (2001) PBDEs and other persistent organic pollutants in Norwegian freshwater fish. In: *The Second International Workshop on Brominated Flame Retardants*. BFR 2001, 14–16 May 2001, Stockholm University, Stockholm, pp. 371–374.

- Schröter-Kermani, C., Helm, D., Herrmann, T. & Päpke, O. (2000) The German environmental specimen bank — application in trend monitoring of polybrominated diphenyl ethers in human blood. *Organohalogen Compd.*, **47**, 49–52.
- Schumacher, M., Kiviranta, H., Varitainen, T. & Domingo, L.L. (2004) Concentrations of PCBs and PBDEs in breast milk of women from Catalonia, Spain. *Organohalogen Compd.*, **66**, 2560–2566.
- Sellström, U. (1996) *PBDEs in the Swedish environment* [Licentiate Thesis]. Stockholm: Stockholm University, Institute of Applied Research (ITM Report, 1996:45).
- Sellström, U., Jansson, B., Kierkegaard, A., de Wit, C., Odsjo, T. & Olsson, M. (1993) Polybrominated diphenyl ethers (PBDE) in biological samples from the Swedish environment. *Chemosphere*, **26** (9), 1703–1718.
- Sellström, U., Söderström, G. & Tysklind, M. (1998) Photolytic debromination of decabromodiphenyl ether (DeBDE). *Organohalogen Compd.*, **35**, 447–450.
- She, J., Winkler, J., Visita, P., McKinney, M. & Petreas, M. (2000) Analysis of PBDEs in seal blubber and human breast adipose samples. *Organohalogen Compd.*, **47**, 53–56.
- She, J., Holden, A., Sharp, M., Tanner, M., Williams-Derry, C. & Hooper, K. (2004) Unusual pattern of polybrominated diphenyl ethers (PBDEs) in US breast milk. *Organohalogen Compd.*, **66**, 3945–3950.
- Sjödin, A. (2000) *Occupational and Dietary Exposure to Organohalogen Substances with Special Emphasis on Polybrominated Diphenyl Ethers* [Thesis]. Stockholm: Stockholm University.
- Sjödin, A., Jakobsson, E., Kierkegaard, A., Marsh, G. & Sellström, U. (1998) Gas chromatographic identification and quantification of polybrominated diphenyl ethers in a commercial product, Bromkal 70-5DE. *J. Chromatograph. A*, **822** (1), 83–89.
- Sjödin, A., Hagmar, L., Klasson-Wehler, E., Kronholm-Diab, K., Jakobsson, E. & Bergman, A. (1999) Flame retardant exposure: polybrominated diphenyl ethers in blood from Swedish workers. *Environ. Health Perspect.*, **107** (8), 643–648.
- Sjödin, A., Patterson, D.G., Jr., & Bergman, Å. (2003) A review on human exposure to brominated flame retardants — particularly polybrominated diphenyl ethers. *Environ. Int.*, **29**, 829–839.
- Sjödin, A., Jones, R., Focant, J.-F., Lapeza, C., Wang, R., Needham, L. & Patterson, D. (2004a) Retrospective time-trend study of polybrominated diphenyl ether and polybrominated and polychlorinated biphenyl levels in human serum from the United States. *Environ. Health Perspect.*, **112**, 654–658.
- Sjödin, A., Päpke, O., McGahee, E., Jones, R., Focant, J.F., Pless-Mulloli, T., Tooms, L.M., Wang, R., Needham, L.L., Herrmann, T. & Patterson, D. (2004b) Concentration of polybrominated diphenyl ethers (PBDEs) in household dust from various countries — inhalation a potential route of human exposure. *Organohalogen Compd.*, **66**, 3817–3822.
- Skarman, E., Darnerud, P.O., Öhrvik, H. & Oskarsson, A. (2005) Reduced thyroxine levels in mice perinatally exposed to polybrominated diphenyl ethers. *Environ. Toxicol. Pharmacol.*, **19**, 273–281.
- Stanley, J., Cramer, P., Thornburg, K., Remmers, J., Breen, J.J. & Schwemberger, J. (1991) Mass spectral confirmation of chlorinated and brominated diphenylethers in human adipose tissues. *Chemosphere*, **23**, 1185–1195.
- Stapelton, H., Dodder, N., Schantz, M. & Wise, S. (2004) Measurement of the flame retardants polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDD) in house dust. *Organohalogen Compd.*, **66**, 3740–3744.

- Staskal, D.H., Diliberto, J.J., DeVito, M.J. & Birnbaum, L.S. (2005) Toxicokinetics of BDE 47 in female mice: effect of dose, route of exposure, and time. *Toxicol. Sci.*, **83**, 215–223.
- Stoker, T.E., Laws, S.C., Crofton, K.M., Hedge, J.M., Ferrell, J.M. & Cooper, R.L. (2004a) Assessment of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture, in the EDSP male and female pubertal protocols. *Toxicol. Sci.*, **78**, 144–155.
- Stoker, T.E., Cooper, R.L., Lambright, C.S., Wilson, V.S. & Gray, L.E. (2004b) In vivo and in vitro anti-androgenic effects of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture. *Toxicologist*, **78** (1-S), 573.
- Strandman, T., Koistinen, J., Kiviranta, H., Vuorinen, P., Tuomisto, J. & Vartiainen, T. (1999) Levels of some polybrominated diphenyl ethers in fish and human adipose tissue in Finland. *Organohalogen Compd.*, **40**, 355–357.
- Strandman, T., Koistinen, J. & Vartiainen, T. (2000) Polybrominated diphenyl ethers (PBDEs) in placenta and human milk. *Organohalogen Compd.*, **47**, 61–64.
- Takasuga, T., Tsuji, H. & Nagayama, J. (2002) Gender specific dynamics of PCDD/Fs, PCBs, PBDEs and organochlorines in blood of Japanese families over two-year study period. *Organohalogen Compd.*, **58**, 297–300.
- Talsness, C.E., Shakibaei, M., Kuriyama, S., de Souza, C. & Chahoud, I. (2003) Ultra-structural changes in the ovaries of adult offspring following a single maternal exposure to low dose 2,2',4,4',5-pentabromodiphenyl ether. *Organohalogen Compd.*, **61**, 88–91.
- Taylor, M.M., Hedge, J.M., Gilbert, M.E., DeVito, M.J. & Crofton, K.M. (2003) Perinatal exposure to a polybrominated diphenyl ether mixture (DE-71): Disruption of thyroid homeostasis and neurobehavioral development. *Toxicologist*, **77** (1-S), 602.
- Thomsen, C., Lundanes, E. & Becher, G. (2001) A time trend study on brominated flame retardants in serum samples from the general population in Norway. *Organohalogen Compd.*, **52**, 206–209.
- Thomsen, C., Frøshaug, M., Leknes, H. & Becher, G. (2003) Brominated flame retardants in breast milk from Norway. *Organohalogen Compd.*, **64**, 33–36.
- Thomsen, C., Frøshaug, M., Becher, G., Kvalem, H.E., Knutsen, H., Alexander, J., Bergsten, C. & Meltzer, H.M. (2004) PBDEs in serum from persons with varying consumption of fish and game. In: *The Third International Workshop on Brominated Flame Retardants*. BFR 2004, Toronto, Ontario, 6–9 June 2004, pp. 37–40 (<http://www.bfr2004.com>).
- Thuvander, A. & Darnerud, P.O. (1999) Effects of polybrominated diphenyl ether (PBDE) and polychlorinated biphenyl (PCB) on some immunological parameters after oral exposure in rats and mice. *Toxicol. Environ. Chem.*, **70**, 229–242.
- Tollbäck, P., Björklund, J. & Östman, C. (2003) Evaluation of gas chromatographic injection techniques for PBDE. *Organohalogen Compd.*, **61**, 49–52.
- Tritscher, A., Stadler, R., Scanlan, F., Collingro, C. & Pöpke O. (2003) Determination of polychlorinated diphenylethers in samples of raw cow's milk, fish and egg. *Organohalogen Compd.*, **61**, 131–134.
- UK COT (2004) *COT Statement on Brominated Flame Retardants in Fish from the Skerne-Tees Rivers System*. United Kingdom Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment.
- US EPA (1986) *Brominated Diphenyl Ethers. Chemical Hazard Information Profile*. Washington, D.C.: United States Environmental Protection Agency. As cited in IPCS (1994).
- US EPA (1990a) *Ames Metabolic Activation Test to Assess the Potential Mutagenic Effect of MUSTER 13 with Cover Letter Dated 031290*. Unpublished laboratory report,

- Huntingdon Research Centre. Office of Toxic Substances, United States Environmental Protection Agency (Document No. 86-9000004040).
- US EPA (1990b) EPA/OTS. *Ames Metabolic Activation Test to Assess the Potential Mutagenic Effect of MUSTER 82 with Cover Letter Dated 031290*. Unpublished laboratory report, Huntingdon Research Centre. Office of Toxic Substances, United States Environmental Protection Agency (Document No. 86-900000403).
- US EPA (1990c) *Ames Metabolic Activation Test to Assess the Potential Mutagenic Effect of MUSTER 84 with Cover Letter Dated 031290*. Unpublished laboratory report, Huntingdon Research Centre. Office of Toxic Substances, United States Environmental Protection Agency (Document No. 86-900000402).
- US EPA (2003) *EPA Method 1614, Brominated Diphenyl Ethers in Water, Soil, Sediment and Tissue by HRGC/HRMS (draft)*. Washington, D.C.: United States Environmental Protection Agency.
- van Bavel, B., Hughes, J., Davis, S., Wingfors, H. & Lindström, G. (1999) Fast screening for PCBs, pesticides, and brominated flame retardants in biological samples by SFE-LC in combination with GC-TOF. *Organohalogen Compd.*, **40**, 293–296.
- van Bavel, B., Hardell, L., Kittl, A., Lijedahl, M., Karlsson, M., Petterson, A., Tysklind, M. & Lindström, G. (2002) High levels of PBDEs in 5 % of 220 blood samples from the Swedish population. *Organohalogen Compd.*, **58**, 161–164.
- Van den Berg, M., Birnbaum, L., Bosveld, A.T., Brunstrom, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., van Leeuwen, F.X., Liem, A.K., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F. & Zacharewski, T. (1998) Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Perspect.*, **106** (12), 775–792.
- VCCEP (2003) *Voluntary Children's Chemical Evaluation Program Pilot Tier 1: Assessment of the Potential Health Risks to Children Associated with Exposure to the Commercial Octabromodiphenyl Ether Product*. CAS No. 32536-52-0 ([http://www.tera.org/peer/VCCEP/OctaPenta/Octabromodiphenyl%20Ether%20VCCEP%20Tier%201_Main%20Report%20\(04-21-03\).pdf](http://www.tera.org/peer/VCCEP/OctaPenta/Octabromodiphenyl%20Ether%20VCCEP%20Tier%201_Main%20Report%20(04-21-03).pdf)).
- Viberg, H. (2004) *Neonatal Developmental Neurotoxicity of Brominated Flame Retardants, the Polybrominated Diphenyl Ethers (PBDEs)*. Acta Universitatis Upsaliensis: Comprehensive summaries of Uppsala dissertations from the Faculty of Science and Technology. 62 pp. (ISBN 91-554-6053-4).
- Viberg, H., Fredriksson, A. & Eriksson, P. (2002) Neonatal exposure to the brominated flame retardant 2,2',4,4',5-pentabromodiphenyl ether causes altered susceptibility in the cholinergic transmitter system in the adult mouse. *Toxicol. Sci.*, **67**, 104–107.
- Viberg, H., Fredriksson, A. & Eriksson, P. (2003a) Neonatal exposure to polybrominated diphenyl ether (PBDE 153) disrupts spontaneous behaviour, impairs learning and memory, and decreases hippocampal cholinergic receptors in adult mice. *Toxicol. Appl. Pharmacol.*, **192**, 95–106.
- Viberg, H., Fredriksson, A., Jakobsson, E., Örn, U. & Eriksson, P. (2003b) Neurobehavioral derangements in adult mice receiving decabrominated diphenyl ether (PBDE 209) during a defined period of neonatal brain development. *Toxicol. Sci.*, **76**: 112–120.
- Viberg, H., Fredriksson, A. & Eriksson, P. (2004a) Neonatal exposure to the brominated flame retardant, 2,2',4,4',5-pentabromodiphenyl ether, decreases cholinergic nicotinic receptors in the hippocampus and affects spontaneous behavior in the adult mouse. *Environ. Toxicol. Pharmacol.*, **17**, 61–65.

- Viberg, H., Fredriksson, A. & Eriksson, P. (2004b) Investigations of strain and/or gender differences in developmental neurotoxic effects of polybrominated diphenyl ethers in mice. *Toxicol. Sci.*, **81**, 344–353.
- Viberg, H., Fredriksson, A. & Eriksson, P. (2004c) Comparative developmental neurotoxicity of PBDE 99 in two different mouse strains and rat. *Toxicologist*, **78** (1-S), 1907.
- Vieth, B., Herrmann, T., Mielke, H., Ostermann, B., Pöpke, O. & Rüdiger, T. (2004) PBDE levels in human milk: the situation in Germany and potential influencing factors — a controlled study. *Organohalogen Compd.*, **66**, 2643–2648.
- Vijverberg, H. & van den Berg, M. (2004) Letter to the editor. *Toxicol. Sci.*, **79** (1), 205–206.
- von Meyerinck, L., Huftnagel, B., Schmoldt, A. & Bente, H.F. (1990) Induction of rat liver microsomal cytochrome P-450 by the pentabromo diphenyl ether Bromkal 70 and half-lives of its components in the adipose tissue. *Toxicology*, **61**, 259–274.
- Weber, H. & Hesecker, H. (2004) Analysis of polybrominated diphenyl ethers in breast milk of German mothers — results of a pilot study. *Fresenius Environ. Bull.*, **13** (4), 356–360.
- Weiss, J., Meijer, L., Sauer, P., Linderholm, L., Athanasiadis, I. & Bergman, A. (2004a) PBDE and HBCDD levels in blood from Dutch mothers and infants. In: *The Third International Workshop on Brominated Flame Retardants*. BFR 2004, Toronto, Ontario, 6–9 June 2004, pp. 71–74 (<http://www.bfr2004.com>).
- Weiss, J., Meijer, L., Sauer, P., Linderholm, L., Athanasiadis, I. & Bergman, A. (2004b) PBDE and HBCDD levels in blood from Dutch mothers and infants — analysis of a Dutch Groningen infant cohort. *Organohalogen Compd.*, **66**, 2677–2682.
- WHO (1998) *GEMS/Food Regional Diets (Regional Per Capita Consumption of Raw and Semi-processed Agricultural Commodities)*. Geneva: World Health Organization, Global Environment Monitoring System Food Contamination Monitoring and Assessment Programme (WHO/FSF/FOS/98.3; http://www.who.int/foodsafety/publications/chem/regional_diets/en/).
- WHO (2000) *Methodology for Exposure Assessment of Contaminants and Toxins in Food*. Report of a Joint FAO/WHO Workshop, 7–8 June 2000. Geneva: World Health Organization (WHO/SDE/PHE/FOS/00.5).
- Wicklund-Glynn, A., Darnerud, P.O., Andersson, Ö., Atuma, S., Johansson, H., Linder, C.E. & Becker, W. (1996) *Revised Fish Consumption Advisory Regarding PCBs and Dioxins*. Uppsala: National Food Administration (Report 4/96).
- Wiegand, H., Costa, L.G., Eriksson, P., Felipo, V., Lichtensteiger, W., Alleva, E., Altmann, L., Branchi, I., Canales, J.J., Ceccatelli, R., Bordini, F., Erceg, S., Faass, O., Fleischmann, I., Frederiksson, A., Lilienthal, H., Llansola, M., Montoliu, C., Pettersson, A., Saez, R., Santucci, D., Schlumpf, M., Silvestrini, B., Smolnikar, K. & Viberg, H. (2003) *Developmental Neurotoxicity of Polybrominated Diphenyl Ethers (PBDE): Mechanisms and Effects. Final Report*. Brussels: European Commission (EU Project QLK4-CT-1999-1562).
- Wiegand, H., Altmann, L. & Lilienthal, H. (2004) Developmental neurotoxicity of PBDEs: impairment of synaptic plasticity in rat cortex and hippocampus. *Toxicologist*, **78** (1-S), 1906.
- Wijesekera, R., Halliwell, C., Hunter, S. & Harrad, S. (2002) A preliminary assessment of UK human exposure to polybrominated diphenyl ethers (PBDEs). *Organohalogen Compd.*, **55**, 239–242.
- Wilford, B.H., Harner, T., Zhu, J.P., Shoeib, M. & Jones, K.C. (2004) Passive sampling survey of polybrominated diphenyl ether flame retardants in indoor and outdoor air in

- Ottawa, Canada: Implications for sources and exposure. *Environ. Sci. Technol.*, **38** (20), 5312–5318.
- Wilford, B.H., Shoeib, M., Harner, T., Zhu, J.P. & Jones, K.C. (2005) Polybrominated diphenyl ethers in indoor dust in Ottawa, Canada: Implications for sources and exposure. *Environ. Sci. Technol.*, **39** (18), 7027–7035.
- WIL Research Laboratories (1984) *90-Day Dietary Study in Rats with Pentabromo Diphenyl Oxide Including Recovery Periods of 6, 12 and 24 Weeks. Final Report*. Ashland, Ohio: WIL Research Laboratories (Project 12042).
- Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K. & Speck, W. (1987) *Salmonella* mutagenicity tests: III. Results from the testing of 255 chemicals. *Environ. Mutagen.*, **9** (Suppl. 9), 1–11.
- Zennegg, M., Kohler, M., Gerecke, A.C. & Schmid, P. (2003) Polybrominated diphenyl ethers in whitefish from Swiss lakes and farmed rainbow trout. *Chemosphere*, **51** (7), 545–553.
- Zhou, T., Ross, D.G., DeVito, M.J. & Crofton, K.M. (2001) Effects of short-term in vivo exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats. *Toxicol. Sci.*, **61**, 76–82.
- Zhou, T., Taylor, M.M., DeVito, M.J. & Crofton, K.M. (2002) Developmental exposure to brominated diphenyl ethers results in thyroid hormone disruption. *Toxicol. Sci.*, **66**, 105–116.

APPENDIX 1: LIST OF COMMON BROMINATED DIPHENYL ETHER (BDE) CONGENERS

Congener number	Bromine substitution pattern
BDE-0	diphenyl ether
BDE-1	2-monobromodiphenyl ether
BDE-2	3-monobromodiphenyl ether
BDE-3	4-monobromodiphenyl ether
BDE-7	2,4-dibromodiphenyl ether
BDE-8	2,4'-dibromodiphenyl ether
BDE-10	2,6-dibromodiphenyl ether
BDE-11	3,3'-dibromodiphenyl ether
BDE-12	3,4-dibromodiphenyl ether
BDE-13	3,4'-dibromodiphenyl ether
BDE-15	4,4'-dibromodiphenyl ether
BDE-17	2,2',4-tribromodiphenyl ether
BDE-25	2,3',4-tribromodiphenyl ether
BDE-28	2,4,4'-tribromodiphenyl ether
BDE-30	2,4,6-tribromodiphenyl ether
BDE-32	2,4',6-tribromodiphenyl ether
BDE-33	2',3,4-tribromodiphenyl ether
BDE-35	3,3',4-tribromodiphenyl ether
BDE-37	3,4,4'-tribromodiphenyl ether
BDE-39	3,4',5-tribromodiphenyl ether
BDE-47	2,2',4,4'-tetrabromodiphenyl ether
BDE-51	2,2',4,6'-tetrabromodiphenyl ether
BDE-66	2,3',4,4'-tetrabromodiphenyl ether
BDE-71	2,3',4',6-tetrabromodiphenyl ether
BDE-75	2,4,4',6-tetrabromodiphenyl ether
BDE-77	3,3',4,4'-tetrabromodiphenyl ether
BDE-85	2,2',3,4,4'-pentabromodiphenyl ether
BDE-99	2,2',4,4',5-pentabromodiphenyl ether
BDE-100	2,2',4,4',6-pentabromodiphenyl ether
BDE-101	2,2',4,5,5'-pentabromodiphenyl ether
BDE-105	2,3,3',4,4'-pentabromodiphenyl ether

Appendix 1 (contd)

Congener number	Bromine substitution pattern
BDE-116	2,3,4,5,6-pentabromodiphenyl ether
BDE-119	2,3',4,4',6-pentabromodiphenyl ether
BDE-126	3,3',4,4',5-pentabromodiphenyl ether
BDE-128	2,2',3,3',4,4'-hexabromodiphenyl ether
BDE-138	2,2',3,4,4',5'-hexabromodiphenyl ether
BDE-140	2,2',3,4,4',6'-hexabromodiphenyl ether
BDE-151	2,2',3,5,5',6-hexabromodiphenyl ether
BDE-153	2,2',4,4',5,5'-hexabromodiphenyl ether
BDE-154	2,2',4,4,5,6'-hexabromodiphenyl ether
BDE-155	2,2',4,4',6,6'-hexabromodiphenyl ether
BDE-166	2,3,4,4',5,6-hexabromodiphenyl ether
BDE-172	2,2',3,3',4,5,5'-heptabromodiphenyl ether
BDE-176	2,2',3,3',4,6,6'-heptabromodiphenyl ether
BDE-181	2,2',3,4,4',5,6'-heptabromodiphenyl ether
BDE-183	2,2',3,4,4',5,6-heptabromodiphenyl ether
BDE-185	2,2',3,4,4',5',6-heptabromodiphenyl ether
BDE-189	2,3,3',4,4',5,5'-heptabromodiphenyl ether
BDE-190	2,3,3',4,4',5,6-heptabromodiphenyl ether
BDE-192	2,3,3',4,5,5',6-heptabromodiphenyl ether
BDE-197	2,2',3,3',4,4',6,6'-octabromodiphenyl ether
BDE-203	2,2',3,4,4',5,5',6-octabromodiphenyl ether
BDE-206	2,2',3,3',4,4',5,5',6-nonabromodiphenyl ether
BDE-209	2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether

POLYCYCLIC AROMATIC HYDROCARBONS

First draft prepared by

**D. Benford,¹ A. Agudo,² C. Carrington,³ T. Hambridge,⁴ R. van Leeuwen,⁵
M. Rao,⁶ W. Slob,⁵ M.C. de Figueiredo Toledo⁷ and R. Walker⁸**

¹ **Food Standards Agency, London, United Kingdom**

² **Catalan Institute of Oncology, Unit of Epidemiology and Cancer
Registration, L'Hospitalet de Llobregat, Spain**

³ **Center for Food Safety and Applied Nutrition, Food and Drug
Administration, College Park, Maryland, USA**

⁴ **Food Standards Australia New Zealand, Canberra, ACT, Australia**

⁵ **National Institute of Public Health and the Environment (RIVM), Bilthoven,
The Netherlands**

⁶ **Central Laboratories Unit, United Arab Emirates University, Al Ain, United
Arab Emirates**

⁷ **State University of Campinas, Campinas São Paulo, Brazil**

⁸ **Ash, Aldershot, Hampshire, United Kingdom**

Explanation.....	565
Biological data	567
Biochemical aspects	567
Absorption, distribution and excretion	567
Biotransformation	569
Effects on enzymes and other parameters.....	572
Toxicological studies	574
Acute toxicity	574
Short-term studies of toxicity.....	576
Long-term studies of toxicity and carcinogenicity	580
Genotoxicity	592
Reproductive toxicity	598
Special studies	599
Observations in humans.....	603
Introduction	603
Biomarkers of exposure	603
Biomarkers of effect	605
Epidemiological studies.....	606
Analytical methods	609
Chemistry	609
Introduction	609
Physical and chemical properties.....	609
Description of analytical methods.....	611
Introduction	611
Screening methods	612
Quantitative methods	612
Sampling protocols.....	617

Routes of PAH contamination in food.....	617
Environmental contamination	617
Contamination during processing and cooking of food.....	618
Grilling	618
Smoking	619
Drying and roasting	619
Other minor routes of contamination	620
Levels and patterns of contamination of food commodities	620
Surveillance data on PAHs in foods	620
Meat and meat products	621
Fish and other marine foods	626
Vegetables	630
Fruits and confections	634
Cereals and cereal products	634
Beverages.....	634
Oils, fats and related products	644
Dairy products	650
Profiles of PAHs in food	654
Conclusion and recommendations on analysis of PAHs in foods	656
Intake estimates	656
Dietary intake estimates	656
Introduction and background to intake estimates	656
Methods	659
International estimates of intake	661
Estimates of non-dietary exposure	684
Comparative estimates of intake from different sources	685
Summary of intake estimates	686
Conclusions on dietary intake	689
Prevention and control	690
Dose-response analysis and estimation of carcinogenic risk	691
Contribution of above data to assessment of risk.....	691
Pivotal data from biochemical and toxicological studies.....	691
Pivotal data from human clinical/epidemiological studies.....	692
Biomarker studies	692
General modelling considerations	692
Selection of data	693
Potency estimates.....	697
The surrogate approach.....	697
The equivalency factor approach	700
Comments.....	704
Absorption, distribution, metabolism and excretion	704
Toxicological data	705
Observations in humans.....	707
Analytical methods	707
Sampling protocols.....	708
Sources and occurrence in foods.....	708
Effects of processing and cooking of food.....	709

Prevention and control of PAHs in foods.....	709
Levels and pattern of food contamination.....	709
Dietary intake assessment	710
Dose-response analysis	717
Approaches for mixtures of PAHs	718
Evaluation.....	719
Recommendations	720
References	720

1. EXPLANATION

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds containing two or more fused aromatic rings. Hundreds of individual PAHs may be formed during incomplete combustion or pyrolysis of organic matter, industrial processes and cooking and food processing.

At its thirty-seventh meeting, the Committee evaluated benzo[a]pyrene (Annex 1, references 94, 95) and recognized that it was one member of a family of PAHs that should be considered as a class. The Committee concluded that, for the purpose of the evaluation, the most significant toxicological effect of benzo[a]pyrene was carcinogenicity. The Committee noted that the estimated average daily intake of benzo[a]pyrene by humans was about 4 orders of magnitude lower than that reported to be without effect on the incidence of tumours in rats given diets containing benzo[a]pyrene. However, at that time the Committee was unable to establish a tolerable intake for benzo[a]pyrene, on the basis of the available data. The Committee noted that the large differences between the estimated intakes in humans and the doses producing tumours in animals suggested that any effects on human health were likely to be small. Despite this, the Committee concluded that the considerable uncertainties in the estimation required that efforts should be made to minimize human exposure to benzo[a]pyrene as far as was practicable.

At its present meeting, in response to a request from the Codex Committee on Food Additives and Contaminants (CCFAC) at its Thirty-fifth session (CAC, 2003), the Committee reviewed all information relevant to the toxicology, epidemiology, intake assessment, analytical methodology, formation, fate and occurrence of PAHs in food. Two documents were particularly important in this evaluation: the opinion of the European Union Scientific Committee on Food on the risks to human health posed by PAHs (EC, 2002), and the International Programme on Chemical Safety (IPCS) Environmental Health Criteria document on selected non-heterocyclic PAHs (IPCS, 1998). The present Committee used these assessments as the starting point for its evaluation and also took into account newer studies that were considered to be informative for the evaluation.

The 33 compounds considered in the present evaluation are listed in Table 1. These are the 33 PAHs selected for consideration by the IPCS and the Scientific Committee on Food on the basis of available information on their occurrence and toxic effects.

Table 1. PAHs considered in the present evaluation

Common name	CAS name	CAS registry No.
Acenaphthene	Acenaphthylene	83-32-9
Acenaphthylene	Acenaphthylene, 1,2-dihydro	208-96-8
Anthanthrene	Dibenzo[<i>def,mno</i>]chrysene	191-26-4
Anthracene	Anthracene	120-12-7
Benz[<i>a</i>]anthracene	Benz[<i>a</i>]anthracene	56-55-3
Benzo[<i>a</i>]fluorene	11 <i>H</i> -Benzo[<i>a</i>]fluorene	238-84-6
Benzo[<i>b</i>]fluorene	11 <i>H</i> -Benzo[<i>b</i>]fluorene	243-17-4
Benzo[<i>b</i>]fluoranthene	Benz[<i>e</i>]acephenanthrylene	205-99-2
Benzo[<i>ghi</i>]fluoranthene	Benzo[<i>ghi</i>]fluoranthene	203-12-3
Benzo[<i>j</i>]fluoranthene	Benzo[<i>j</i>]fluoranthene	205-82-3
Benzo[<i>k</i>]fluoranthene	Benzo[<i>k</i>]fluoranthene	207-08-9
Benzo[<i>ghi</i>]perylene	Benzo[<i>ghi</i>]perylene	191-24-2
Benzo[<i>c</i>]phenanthrene	Benzo[<i>c</i>]phenanthrene	195-19-7
Benzo[<i>a</i>]pyrene	Benzo[<i>a</i>]pyrene	50-32-8
Benzo[<i>e</i>]pyrene	Benzo[<i>e</i>]pyrene	192-91-2
Chrysene	Chrysene	218-01-9
Coronene	Coronene	191-07-1
Cyclopenta[<i>cd</i>]pyrene	Cyclopenta[<i>cd</i>]pyrene	27208-37-3
Dibenz[<i>a,h</i>]anthracene	Dibenz[<i>a,h</i>]anthracene	53-70-3
Dibenzo[<i>a,e</i>]pyrene	Naphtho[1,2,3,4- <i>def</i>]chrysene	192-65-4
Dibenzo[<i>a,h</i>]pyrene	Dibenzo[<i>b,def</i>]chrysene	189-64-0
Dibenzo[<i>a,i</i>]pyrene	Benzo[<i>rst</i>]pentaphene	189-55-9
Dibenzo[<i>a,l</i>]pyrene	Dibenzo[<i>def,p</i>]chrysene	191-30-0
Fluoranthene	Fluoranthene	206-44-0
Fluorene	9 <i>H</i> -Fluorene	86-73-7
Indeno[1,2,3- <i>cd</i>]pyrene	Indeno[1,2,3- <i>cd</i>]pyrene	193-39-5
5-Methylchrysene	Chrysene, 5-methyl-	3697-24-3
1-Methylphenanthrene	Phenanthrene, 1-methyl	932-69-9
Naphthalene	Naphthalene	91-20-3
Perylene	Perylene	198-55-0
Phenanthrene	Phenanthrene	85-01-8
Pyrene	Pyrene	129-00-0
Triphenylene	Triphenylene	217-59-4

CAS, Chemical Abstracts Service

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Absorption

PAHs may be absorbed via the respiratory tract, skin and gastrointestinal tract. Only the latter is considered in detail here. The key determinants of gastrointestinal absorption are aqueous solubility and lipophilicity.

Rapid absorption of benzo[a]pyrene has been reported following intragastric administration to rats. The highest levels were found in the thoracic lymph 3–4 h after administration (Rees et al., 1971).

In a study with Sprague-Dawley rats, the presence of bile was found to increase intestinal absorption of PAHs such as benzo[a]pyrene and 7,12-dimethyl-benzanthracene to a greater degree than that of anthracene and pyrene (Rahman et al., 1986).

Absorption of radiolabelled benzo[a]pyrene was investigated in Wistar rats following oral administration in solution, emulsion or various food components. The bioavailability was in the range of 20–50%, increasing with increasing content of lipophilic components in the foods. Absorption from triolein and soya bean oil resulted in a greater area under the curve, compared with absorption from less lipophilic foods (Kawamura et al., 1988).

Radiolabelled benzo[a]pyrene or phenanthrene in milk was fed to catheterized Large White pigs. Radioactivity was detectable within 1 h and peaked at 5–6 h, returning to background levels by 24 h. The peak radioactivity was higher for phenanthrene than for benzo[a]pyrene, despite a 3-fold lower dose (Laurent et al., 2001). In a subsequent study, also in pigs, absorption of phenanthrene was reported to occur mainly within 1–3 h, whereas benzo[a]pyrene was mainly absorbed 3–6 h after feeding (Laurent et al., 2002).

Absorption of phenanthrene and pyrene was about 75% following administration by gavage to lactating goats, whereas absorption of benzo[a]pyrene was 12% (Grova et al., 2002).

(b) Distribution

Following intravenous administration in the rat, radiolabelled benzo[a]pyrene was rapidly cleared from the blood, with a half-life of less than 1 min. The rate of clearance was increased when the rats were pretreated with benzo[a]pyrene or phenobarbital, both of which induce metabolizing enzymes (Kotin et al., 1959; Schleder et al., 1970a, 1970b).

Whole-body autoradiography has demonstrated that 3-methylcholanthrene is widely distributed in maternal tissues and was also detected in the fetus following intravenous administration to mice (Takahashi & Yasuhira, 1973; Takahashi, 1978). Similar results were obtained following inhalation, intragastric or

intravenous administration of benzo[a]pyrene to rats and mice (Shendrikova et al., 1973, 1974; Shendrikova & Aleksandrov, 1974; Neubert & Tapken, 1988; Withey et al., 1992).

In a study of 24 women in south India, samples of placenta, maternal and umbilical cord blood and milk were examined for the presence of selected PAHs. The highest levels were detected in umbilical cord blood and milk (0.005–0.41, 0.013–0.6 and 0.002–2.8 ppm for benzo[a]pyrene, dibenz[a,c]anthracene and chrysene, respectively). The authors concluded that the developing fetus and newborn infant are exposed to these PAHs, probably from the maternal diet (Madhavan & Naidu, 1995).

The average benzo[a]pyrene levels in human tissues taken at autopsy were 0.32 µg/100 g tissue dry weight in liver, spleen, kidney, heart and skeletal muscle and 0.2 µg/100 g tissue dry weight in lung (Gräf, 1970; Gräf et al., 1975).

Levels of nine PAHs have been measured in cancer-free liver and fat biopsies. Levels were higher in fat than in liver. The most abundant PAH was pyrene, with concentrations of 0.38 and 1.1 µg/kg wet weight in liver and fat, respectively (Obana et al., 1981).

(c) *Excretion*

Metabolites of PAHs are excreted in urine and faeces. Interactions between different PAHs result in altered elimination in a compound-specific manner. Co-administration of benz[a]anthracene and chrysene to rats doubled the faecal elimination of benz[a]anthracene from 6% to 13%, whereas elimination of chrysene was unchanged (25% of dose) compared with administration of the individual PAHs alone. Benz[a]anthracene co-administration also doubled the faecal elimination of triphenylene from 3% to 6%. In addition, benz[a]anthracene increased the relative availability of chrysene in blood, liver, brain, adipose tissue and mammary tissue but decreased the relative availability of triphenylene in these tissues (Bartosek et al., 1984). These studies were conducted at relatively high doses of PAHs (11.4 mg/rat), and the relevance to human risk assessment is unclear.

Following intravenous administration of [¹⁴C]benzo[a]pyrene (3 µmol/kg bw) to rats, 60% of the dose was excreted in bile in 6 h, with less than 3% in the urine (Chipman et al., 1981). Studies in rats and rabbits have shown evidence of enterohepatic circulation for a number of PAHs (Boroujerdi et al., 1981; Chipman et al., 1981; Chipman, 1982; Weyand & Bevan, 1986). The gastrointestinal microflora have been shown to hydrolyse glucuronic acid conjugates of biliary PAH metabolites (Renwick & Drasar, 1976; Boroujerdi et al., 1981; Chipman et al., 1981; Chipman, 1982). A study of the pharmacokinetics and bioavailability of pyrene in rats also provided evidence of enterohepatic recirculation (Withey et al., 1991). However, a study in smokers and non-smokers reported non-detectable concentrations of PAHs or metabolites in bile, indicating that enterohepatic circulation in humans is unlikely (de Kok & van Maanen, 2000).

2.1.2 Biotransformation

The metabolism of PAHs has been studied in a wide range of *in vitro* systems, including microsomal preparations, cultured cells and explants from a number of tissues and species, including humans. Metabolism has been studied in whole animals for naphthalene, anthracene, phenanthrene, pyrene, benzo[a]anthracene, chrysene, benzo[a]pyrene, dibenz[a,h]anthracene and 3-methylcholanthrene. PAHs are first oxidized to form phase I primary (e.g. epoxides, phenols and dihydrodiols) and secondary (diol epoxides, tetrahydrotetraols and phenol epoxides) metabolites. The phase I metabolites are then conjugated with glutathione, sulfate or glucuronic acid to form phase II metabolites, which are more polar and water-soluble than the parent hydrocarbons and therefore more readily excreted. *In vitro* studies conducted with cultured cells and tissues are of most relevance to metabolism *in vivo*, because both phase I and phase II metabolites are formed. The metabolism of benzo[a]pyrene is described in detail below.

Phase I metabolism introduces more polar chemical groups, such as hydroxy groups, into the molecule. This can make the molecule more electrophilic, resulting in increased reactivity. For example, the epoxide metabolites of benzo[a]pyrene readily react with water to form dihydrodiols. Further oxidation introduces a second epoxide group into the molecule, with the reactivity of the metabolite being dependent on the relative positions of the substituent groups, which influences the electrophilicity. Consecutive phase I metabolism can first increase reactivity and then decrease reactivity. Thus, conversion of a diol epoxide to a tetraol results in deactivation. As discussed below, the diol epoxides may also react with macromolecules.

In general, phase II metabolism is expected to be a detoxification reaction, as the increased water solubility leads to increased elimination. However, it may also result in activation if the alterations in electrophilicity result in formation of a leaving group. This is particularly likely with sulfate conjugates, such as some metabolites of dimethylbenz[a]anthracene.

Cultured microbiota isolated from human faeces representing *in vivo* colon microbial ecology have been shown to metabolize PAHs to hydroxy derivatives. Cultures of stomach and small intestine digests did not produce detectable levels of hydroxy derivatives under similar conditions (van de Wiele et al., 2005).

(a) Adduct formation

Electrophilic PAH metabolites may bind to macromolecules such as protein or DNA, forming adducts. Particular attention has focused on DNA adducts because they have the potential to result in mutations leading to carcinogenicity. The majority of PAH metabolites shown to react with DNA are vicinal diol epoxides, mainly diol epoxides in the "bay region" formed between three adjoining aromatic rings. However, adducts may also form with some non-bay region epoxides, such as that formed from benzo[j]fluoranthene, or other electrophilic metabolites, such as the carbonium ion formed from the sulfate ester of methyl-substituted PAHs such as 9-hydroxymethylanthracene.

The usual sites of adduction to nucleic acids are the extranuclear amino groups of guanine and adenine. Although many of the PAH–DNA adducts from human cells and tissues have not been fully characterized, the available evidence from bronchial epithelium, colon, skin and cultured mammary cells suggests that the adducts formed are very similar to those from the corresponding rodent tissues. The major adduct is formed on the N2 position of guanine. Diol epoxides are thought to react frequently with the N7 position of guanine, but these adducts are labile and normally spontaneously released.

Interaction with DNA can result in damage such as strand nicking, which may also be of significance in carcinogenesis. Although data are not available for all PAH–DNA adducts, the current evidence indicates that they should all be considered as potentially damaging. Following different routes of exposure, adduct formation was reported to occur both at the site of contact and systemically, but it does not readily correlate with peak or total systemic benzo[a]pyrene exposure (Godschalk et al., 2000).

More than a 70-fold interindividual variation in formation of PAH–DNA adducts has been reported when human tissues were exposed to the same concentration of PAHs (Autrup, 1986). This could be due to differences in uptake and biotransformation and/or in the repair of PAH–DNA adducts.

(b) Benzo[a]pyrene as an example of PAH metabolism

Benzo[a]pyrene can undergo many simultaneous and sequential metabolic transformations. The initial stage is cytochrome P450 (CYP) dependent formation of several epoxides, which may undergo spontaneous rearrangement to phenols, hydrolysis by epoxide hydrolase to dihydrodiols or conjugation with glutathione, either non-enzymatically or catalysed by glutathione-S-transferase. The primary metabolites may similarly undergo further oxidation, hydrolysis and/or formation of glutathione, sulfate or glucuronide conjugates.

PAHs are metabolized stereoselectively. Thus, benzo[a]pyrene-7,8-diol-9,10-epoxide has the potential to generate four isomers, as there are two enantiomers for each diastereoisomer. In rat liver microsomal preparations, the (+) isomer accounts for about 90% of the 7,8-epoxide formed, which is stereospecifically metabolized to the (–)7,8-dihydrodiol and then to the (+)anti-7,8-diol-9,10-epoxide. This predominant isomer is also the isomer with the highest tumour-inducing activity and that is predominantly covalently bound to DNA following benzo[a]pyrene exposure in various mammalian cells and organs.

The profile of benzo[a]pyrene metabolites generated in a range of human tissues is qualitatively similar to those in animal tissues, and the same metabolites have been identified. Species and tissue differences in susceptibility are therefore likely to be due to quantitative differences in metabolism. However, for some PAHs such as benz[a]anthracene, there is evidence for species-dependent bioactivation.

(c) *Enzymes of PAH metabolism*

A range of isozymes of the CYP1, 2 and 3 families are capable of metabolizing PAHs; the importance of each CYP is dependent on its catalytic properties (e.g. Michaelis-Menten constant K_m and maximum rate of metabolism V_{max}), its mode of regulation and the tissue specificity of its expression.

CYP1A1 can metabolize a wide variety of PAH molecules. It is present in many tissues at constitutively low levels. Induction of CYP1A1 is controlled by the aryl hydrocarbon receptor (AhR), which can be activated by PAHs. Thus, PAHs can induce their own metabolism. Induction can lead to high levels of CYP1A1 in tissues such as the placenta, lung and peripheral blood cells, but hepatic levels remain low, particularly in humans. CYP1A2 has a lower capacity than CYP1A1 to hydroxylate benzo[a]pyrene, but is very active in forming the 7,8-dihydrodiol and diol epoxides. It is also controlled by the AhR. Because constitutive hepatic levels of CYP1A2 are much higher than levels of CYP1A1, CYP1A2 may play a greater role in activation of benzo[a]pyrene. It has also been shown to be inducible by various PAHs.

Induction of CYP1A1 may be involved in detoxification of PAHs, as well as activation. Benzo[a]pyrene was administered in the diet to *Cyp1a1*^{-/-} knockout and *Cyp1a1*^{+/*} wild-type mice at doses equivalent to about 1.25, 12.5 and 125 mg/kg bw per day for 18 days. The concentrations of adducts were significantly higher in the liver, spleen and bone marrow of the knockout mice than in the wild-type mice at 12.5 and 125 mg/kg bw per day. In the small intestine, the levels were higher in the knockout mice at 12.5 mg/kg bw per day but not at 125 mg/kg bw per day (Uno et al., 2004).

The CYP1B subfamily is also capable of metabolizing PAHs, and its expression is controlled by the AhR. CYP1B1 is present in a wide range of mammalian extrahepatic tissues and exhibits high enzymatic activity towards a wide range of PAHs. It has been shown to activate dibenzo[a,h]pyrene by generation of a diol epoxide in the "fjord region" between four adjoining aromatic rings (Buters et al., 2002).

CYP2B isozymes are constitutively low in humans but may play a significant role in PAH metabolism following induction by phenobarbital-type inducers.

Some CYP2C isozymes are expressed at relatively high levels in human liver and may play a prominent role in metabolism of benzo[a]pyrene in phenobarbital-induced liver.

Some members of the CYP3A subfamily, especially CYP3A4, are particularly abundant in human liver and also metabolize PAHs.

Other enzymes such as prostaglandin H synthase (COX) and lipoxygenases may catalyse the oxidation steps in some circumstances, such as when levels of CYP are low or in chronic irritation or inflammation.

More recent studies indicate that the diol metabolites of a number of PAHs may undergo oxidation catalysed by aldo-keto reductases to redox-active

o-quinones. Redox cycling of the quinones leads to formation of reactive oxygen species, which may contribute to the toxicity of PAHs (Palackal et al., 2001, 2002; Penning, 2004).

Phase II metabolism is catalysed by enzymes such as uridine diphosphate-glucuronosyltransferases (including UDPGT1A6 and UDPGT2B7), glutathione-S-transferases (GSTA1-1, GSTM1-1 and GSTP1-1), sulfotransferases and methyltransferases.

Many of the data on PAH metabolism have been generated with single PAH compounds in *in vitro* systems, such as subcellular fractions or cell lines. Information on formation of specific metabolites *in vivo* is generally lacking. There is also a lack of information on modification of metabolism of individual PAHs due to co-exposures to other PAHs in food and the environment. There is some evidence that at high dose levels, some PAHs can affect the excretion of other PAHs, but the significance of this for humans at dietary exposure levels is not known.

The enzymes involved in activation and detoxification of PAHs are present in the tissues of the gastrointestinal tract, although generally at lower levels than in the liver. The small intestine contributes to first-pass metabolism of ingested and absorbed PAHs. Ingestion of PAHs has been shown to cause a rapid and marked induction of CYP1A1. Colon tissue expresses COX, which is inducible by PAHs. Human COX-1 and COX-2 have been reported to activate benzo[a]pyrene-7,8-diol and may be relevant for a possible role of dietary PAHs in colorectal carcinogenesis (Ramesh et al., 2004).

Many of the enzymes involved in the metabolism of PAHs have a polymorphic distribution. Individual polymorphisms are common, but may have little functional significance for a chemical that is metabolized by a number of alternative enzymes and pathways. Enzyme activity depends not only on genotype, but also on exposure to other chemicals and environmental factors that can either increase expression of the gene or inhibit the enzyme activity by binding to the enzyme's active site. Many of the compounds present in fruits and vegetables that exhibit chemopreventive activity induce enzymes involved in the detoxification of PAHs. Dietary habits characterized by frequent consumption of fresh fruit and vegetables and high intake of antioxidants have been reported to be strongly associated with reduced levels of PAH-DNA adducts (Palli et al., 2000).

2.1.3 Effects on enzymes and other parameters

(a) Receptor binding

PAHs bind to the AhR, but with lower affinity than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other dioxin-like compounds. However, PAHs may have effects differing from those of TCDD. Binding to the AhR results in up-regulation of transcription and subsequent increase in messenger RNA (mRNA) and protein levels of a large number of genes, referred to as the aryl hydrocarbon (Ah) gene battery, including genes coding for various CYPs, glutathione transferases and glucuronosyltransferases, oncogenes (*c-fos*, *c-jun*, *c-erb-A*, *bcl-2*, *Bax*), growth factors and receptors. The AhR may also interact with other signalling pathways,

transcription factors, etc. It is also involved in the down-regulation of various genes that play key roles in cell growth and differentiation. Down-regulation of CYP2C11 by PAHs correlated with AhR binding. Down-regulation of liver methionine adenosyltransferase gene expression by PAHs has also been reported, which could lead to impaired cellular defence processes, e.g. by decreased glutathione synthesis. However, this effect may not be mediated solely by the AhR, as a similar effect was not observed with TCDD (Carretero et al., 2001).

The AhR has been detected in most cells and tissues, including the gastrointestinal tract. Large interspecies and interstrain differences in the concentration of AhR have been reported. In humans, the receptor has been found in, for example, liver, lung, colon and placenta, with the highest level in the lung. A 4-fold interindividual variation in expression has been reported, and genetic polymorphisms have been reported in the human AhR.

AhR interaction is involved in many of the toxic responses of PAHs, including immunosuppression, carcinogenicity and reproductive effects.

However, studies in AhR-null mice have shown the existence of AhR-independent mechanisms for metabolic activation of benzo[a]pyrene. Treatment with a single intraperitoneal dose of benzo[a]pyrene (100 $\mu\text{mol/kg}$ bw) resulted in marked induction of CYP1A1 and CYP1A2 in wild-type mice, but not in AhR-null mice. Multiple benzo[a]pyrene-DNA adducts were present in the livers of both wild-type and AhR-null mice. The total numbers of adducts did not differ significantly between the two strains. However, levels of five adducts were significantly decreased and the level of one adduct was significantly increased in AhR-null mice, compared with wild-type mice. Although the adducts were not identified, the major adduct was reported to exhibit chromatographic properties similar to those of the adduct derived from benzo[a]pyrene 7,8-dihydrodiol-9,10-epoxide, and the levels did not differ significantly between the wild-type and AhR-null mice (Kondraganti et al., 2003).

(b) Receptor-mediated activity

There are numerous short-term bioassays of AhR-mediated activity, based on 1) induction of CYP1A-dependent 7-ethoxyresorufin O-deethylase (EROD) activity; 2) binding affinity of the ligand to the receptor; 3) binding of the ligand:AhR to the consensus sequence measured by the gel retardation of AhR binding assay; and 4) AhR-mediated reporter gene expression. In analysis of complex PAH mixtures, there is significant discrepancy between the different assays, demonstrating that the *in vitro* bioassay alone is not a good predictor of the ability of a chemical to activate AhR.

The AhR-inducing potency of many PAHs has been estimated in a chemical-activated luciferase expression assay. Induction equivalency factors (IEFs) relative to benzo[a]pyrene (IEF = 1) ranged from 0.001 for fluoranthene to 67.76 for benzo[k]fluoranthene. TCDD was about 100 000 times more potent than benzo[a]pyrene in this assay (Machala et al., 2001).

However, the metabolism of PAHs could play an important role in the determination of AhR-mediated activity, as the hydroxylated metabolites may have a very different affinity for the receptor than the parent compound. Transient induction of AhR-mediated activities is assumed to be a result of the disappearance of the inducing PAH following metabolism by the induced CYP1A1 activity. Non-planar PAH compounds, such as 7,8-dihydroxy-7,8-dihydro-benzo[a]pyrene, also induce CYP1A1 activity, but with a delayed time course.

Even in cells expressing high levels of AhR, no activation and/or translocation of the activated PAH:AhR complex to the nucleus were observed at low concentrations of the PAH, suggesting that a threshold may exist for the AhR-mediated responses.

The steric resemblance of PAHs to steroid molecules has led to the postulation that they would be able to bind to steroid hormone receptors. In vitro and in vivo studies have shown that PAHs have estrogenic and anti-estrogenic activity. Phenolic metabolites were responsible for the effects, and the potencies of 3-hydroxy- and 9-hydroxy-benzo[a]pyrene were equivalent to that of estradiol (Charles et al., 2000).

Metabolism of PAHs by cultured human colon microflora produced metabolites that showed estrogenic activity in a yeast assay (van de Wiele et al., 2005).

(c) Effects on other enzymes

In rats, oral administration of anthracene or phenanthrene at doses of 100 mg/kg bw per day for 4 days resulted in increased carboxylesterase activity in the intestinal mucosa. Following administration of benzo[a]pyrene at 50 and 150 mg/kg bw per day or benz[a]anthracene at 150 mg/kg bw per day, carboxylesterase activity was suppressed in the intestinal mucosa and moderately induced in renal microsomal fractions (Nousiainen et al., 1984).

Oral administration of benzo[a]pyrene, benz[a]anthracene, anthracene, chrysene or phenanthrene to Wistar rats at 100 mg/kg bw per day for 4 days resulted in induction of cytosolic aldehyde dehydrogenase activity. Benzo[a]pyrene and benz[a]anthracene were more effective than the other substances, increasing liver weights by 27% and 19%, respectively (Törrönen et al., 1981).

2.2 Toxicological studies

2.2.1 Acute toxicity

Relatively few acute toxicity studies are available. IPCS (1998) listed individual oral median lethal dose (LD₅₀) values in mice and rats for anthracene, benzo[a]pyrene, fluoranthene and phenanthrene varying from 700 to 18 000 mg/kg bw. For naphthalene, oral lethality data are more numerous, with LD₅₀ values in rats ranging from 490 to 9430 mg/kg bw and in mice from 354 to 710 mg/kg bw. Intraperitoneal LD₅₀ values have been determined for chrysene and pyrene, but no lethality studies are available for all other PAHs. Taken together, IPCS (1998)

concluded that the lethality data indicate that PAHs possess a moderate to low acute toxicity. Available LD₅₀ values are summarized in Table 2.

Table 2. Results of studies of the acute toxicity of PAHs

Compound	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference
Anthracene	Mouse		Oral	18 000	Montizaan et al. (1989)
	Mouse		Intraperitoneal	>430	Salamone (1981)
Benzo[a]pyrene	Mouse		Oral	>1600	Awogi & Sato (1989)
	Mouse		Intraperitoneal	~250	Salamone (1981)
	Mouse		Intraperitoneal	>1600	Awogi & Sato (1989)
	Rat		Subcutaneous	50	Montizaan et al. (1989)
Chrysene	Mouse		Intraperitoneal	>320	Simmon et al. (1979)
Fluoranthene	Rat		Oral	2000	Smyth et al. (1962)
	Rabbit		Dermal	3180	Smyth et al. (1962)
	Mouse		Intravenous	100	Montizaan et al. (1989)
Naphthalene	Rat		Oral	1250	Sax & Lewis (1984)
	Rat	M	Oral	2200	Gaines (1969)
	Rat	F	Oral	2400	Gaines (1969)
	Rat		Oral	9430	US EPA (1978)
	Rat		Oral	1110	Montizaan et al. (1989)
	Rat		Oral	490	Montizaan et al. (1989)
	Rat		Oral	1800	Montizaan et al. (1989)
	Rat	M	Dermal	>2500	Gaines (1969)
	Rat	F	Dermal	>2500	Gaines (1969)
	Rat		Intraperitoneal	~1000	Bolonova (1967)
	Rat	M	Intraperitoneal	~1600	Plopper et al. (1992)
	Rat		Inhalation	>0.5 mg/l (8 h)	US EPA (1978)
	Mouse	F	Oral	354	Plasterer et al. (1985)
	Mouse	M	Oral	533	Shopp et al. (1984)
	Mouse	F	Oral	710	Shopp et al. (1984)
	Mouse		Subcutaneous	5100	Sandmeyer (1981); Shopp et al. (1984)
	Mouse		Subcutaneous	969	Sax & Lewis (1984)

Table 2. (contd)

Compound	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference
	Mouse		Intraperitoneal	150	Sax & Lewis (1984)
	Mouse		Intraperitoneal	380	Warren et al. (1982)
	Mouse	M	Intraperitoneal	~400	Plopper et al. (1992)
	Mouse		Intravenous	100	Sax & Lewis (1984)
	Hamster	M	Intraperitoneal	~800	Plopper et al. (1992)
	Guinea-pig		Oral	1200	Sax & Lewis (1984)
Phenanthrene	Mouse		Oral	700	Montizaan et al. (1989)
	Mouse		Oral	1000	Montizaan et al. (1989)
	Mouse		Intraperitoneal	700	Simmon et al. (1979)
	Mouse		Intravenous	56	Montizaan et al. (1989)
Pyrene	Mouse		Intraperitoneal	514 (7 days)	Salamone (1981)
	Mouse		Intraperitoneal	678 (4 days)	Salamone (1981)

Adapted from IPCS (1998)

M, male; F, female

Knuckles et al. (2001) performed an acute oral toxicity study with benzo[a]pyrene in which they administered F-344 rats single doses of 0, 100, 600 or 1000 mg/kg bw by gavage as a solution in peanut oil. After 14 days, the animals were killed. Toxicological end-points examined included haematology, blood biochemistry, body weights, tissue weights and tissue histopathology on selected tissues. Effects observed were limited to increased liver weights (at all dose levels), decreased white blood cell counts (two highest dose levels, males only) and slightly increased mean cell haemoglobin concentration (all dose levels, males only). No histological effects were found (Knuckles et al., 2001).

2.2.2 Short-term studies of toxicity

Only few limited subacute toxicity data are available. Oral studies in which at least a minimum set of standard toxicity parameters was measured have not been performed for PAHs. A number of subchronic oral toxicity studies with individual PAHs are available. Table 3 provides basic information on these studies.

Table 3. Summary of subchronic oral toxicity studies with PAH

Compound	Species, strain	Duration (route)	Effects/NOAEL	Reference
Acenaphthene	CD-1 mouse	90 days (gavage)	Liver weight changes accompanied by cellular hypertrophy, increased serum cholesterol. NOAEL 175 mg/kg bw per day (LOAEL 350 mg/kg bw per day)	US EPA (1989a)
Anthracene	CD-1 mouse	90 days (gavage)	No toxic effects observed. NOAEL >1000 mg/kg bw per day	US EPA (1989b)
Benzo[a]pyrene	Wistar rat	90 days (diet)	Increased liver weights, decreased thymus weights, increased forestomach basal cell hyperplasia, slight thymus atrophy. NOAEL 3 mg/kg bw per day (LOAEL 10 mg/kg bw per day)	Kroese et al. (2001)
Benzo[a]pyrene	F-344 rat	90 days (diet)	Increased liver weights, decreased red blood cell parameters, renal tubular casts in males only. NOAEL 5 mg/kg bw per day (LOAEL 50 mg/kg bw per day)	Knuckles et al. (2001)
Fluoranthene	CD-1 mouse	13 weeks (gavage)	Increased liver weight accompanied by microscopic liver lesions, increased ALT, nephropathy. NOAEL 125 mg/kg bw per day (LOAEL 250 mg/kg bw per day)	US EPA (1988)
Fluorene	CD-1 mouse	13 weeks (gavage)	Clinical signs suggestive of neurotoxicity, decreased red blood cell parameters, increased serum bilirubin, increased liver and spleen weights accompanied by increased haemosiderin deposition in both organs. NOAEL 125 mg/kg bw per day (LOAEL 250 mg/kg bw per day)	US EPA (1989c)

Table 3. (contd)

Compound	Species, strain	Duration	Effects/NOAEL	Reference
Naphthalene	CD-1 mouse	90 days (gavage)	Decreased organ weights in females (brain, spleen, liver). Significance unclear since no histopathology was done. NOAEL 53 mg/kg bw per day (LOAEL 267 mg/kg bw per day)	Shopp et al. (1984)
Naphthalene	B6C3F1 mouse	13 weeks (gavage)	Possible effect on growth, only other effect: transient increase in clinical signs. NOAEL 100 mg/kg bw per day (LOAEL 200 mg/kg bw per day)	BCL (1980a)
Naphthalene	F-344 rat	13 weeks (gavage)	Low incidence of histological changes in kidney and thymus, decreased growth. NOAEL 100 mg/kg bw per day (LOAEL 200 mg/kg bw per day)	BCL (1980b)
Naphthalene	Rat (strain unknown)	14 weeks (application not known)	Liver parenchyma cell oedema and congestion, renal inflammation. LOAEL 150–220 mg/kg bw per day (only dose level)	Kawai (1979)
Pyrene	CD-1 mouse	13 weeks (gavage)	Nephropathy and decreased kidney weights. NOAEL 75 mg/kg bw per day (LOAEL 125 mg/kg bw per day)	US EPA (1989d)

ALT, alanine transferase; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level

(i) *Mice*

The only available subacute study is for naphthalene, which was studied by Shopp et al. (1984) in mice at oral dose levels of 27, 53 and 267 mg/kg bw per day for 2 weeks. Toxic effects — i.e. increased mortality, body weight depression and decreased weights of thymus and spleen — occurred only at the top dose. An immunotoxicity screen showed no effect.

Administration of acenaphthene by gavage at doses of 350 and 700 mg/kg bw per day to mice for 90 days resulted in increased liver weight and cellular hypertrophy. No effects were seen at 175 mg/kg bw per day (US EPA, 1989a).

No treatment-related effects were observed in CD-1 (ICR) BR mice following gavage doses of anthracene up to 1000 mg/kg bw per day for at least 90 days (US EPA, 1989b).

Oral administration of benzo[a]pyrene at 120 mg/kg bw per day for 1–4 weeks resulted in death due to myelotoxicity in poor-affinity AhR DBA/2N mice, whereas high-affinity C57BL/6N mice survived with no myelotoxicity for at least 6 months under these conditions (Legrauerend et al., 1983).

Non-responsive strains of mice (C57BL/6, C3H/HeN and Balb/cAnN) had increased relative liver weights after they were fed for 180 days on a diet providing benzo[a]pyrene at 120 mg/kg bw per day (Robinson et al., 1975).

Dose-dependent nephropathy and liver toxicity were observed in CD-1 mice given fluoranthene at 250 or 500 mg/kg bw per day for 13 weeks. No adverse effects were observed at 125 mg/kg bw per day (US EPA, 1988).

Gavage administration of fluorene to CD-1 mice at 250 or 500 mg/kg bw per day for 13 weeks resulted in haematological changes, increased relative and absolute weights of liver, kidney and spleen and increased haemosiderin in the spleen and Kupffer cells of the liver. No effects were observed at 125 mg/kg bw per day (US EPA, 1989c).

In a 90-day study in mice, oral administration of naphthalene at 5.3, 53 or 133 mg/kg bw per day resulted in decreased body weight and relative spleen weight at the highest dose. Blood urea nitrogen levels were decreased in females at all doses, but the clinical significance of this was considered to be unclear, and the no-observed-adverse-effect level (NOAEL) was reported as 53 mg/kg bw per day (Shopp et al., 1984).

Administration of pyrene by gavage to CD-1 mice at 75, 125 or 250 mg/kg bw per day for 13 weeks resulted in dose-dependent nephropathy. Renal lesions in all groups were described as minimal or mild. Relative and absolute kidney weights were decreased at the two higher doses, and the NOAEL was reported to be 75 mg/kg bw per day (US EPA, 1989d).

(ii) Rats

In Fischer 344 rats administered naphthalene by gavage at 0, 25, 50, 100, 200 or 400 mg/kg bw 5 days a week for 13 weeks, decreased body weight and microscopic kidney lesions were observed in the two highest dose groups. The NOAEL was reported to be 100 mg/kg bw per day, adjusted to 71 mg/kg bw per day allowing for the dosing regimen (BCL, 1980b).

In rats that ingested naphthalene at 150 mg/kg bw per day for 3 weeks and then 200–220 mg/kg bw per day for a further 11 weeks, reduced weight gain and food intake, enlarged livers and microscopic liver and kidney lesions were observed (Kawai, 1979).

In Wistar rats treated with benzo[a]pyrene at 0, 3, 10 or 30 mg/kg bw, 5 days a week for 3 months, dose-related increases in liver weight in males and in basal

cell hyperplasia in both sexes occurred at the two highest doses. Effects were also seen in the thymus at the highest dose (Kroese et al., 2001).

(iii) *Dogs*

Administration of single oral doses of 3 or 9 g naphthalene or a total dose of 10.5 g naphthalene over 7 days to groups of three dogs resulted in anaemia and neurophysiological symptoms (Zuelzer & Apt, 1949).

2.2.3 Long-term studies of toxicity and carcinogenicity

The available long-term studies have focused primarily on the potential of PAHs to induce neoplastic effects. Significant toxic effects have been observed only at dose levels at which carcinogenic effects also occurred.

A large number of studies have assessed the carcinogenicity of PAHs. Studies involving dermal, subcutaneous or inhalation exposure are of less relevance to the evaluation of dietary exposure and are not discussed in detail in this monograph. However, they contribute to the classification of evidence for carcinogenicity in animals and are included in Table 4. Studies involving oral administration are summarized in more detail in Table 5.

In most studies, the site of tumour development was related to the route of administration, e.g. gastric tumours following oral administration, skin tumours following dermal application. However, tumours at other sites are also observed. Based on observations in animal bioassays using the same route of exposure, dibenz[*a,h*]anthracene, dibenzo[*a,h*]pyrene, dibenzo[*a,l*]pyrene, benzo[*a*]pyrene, benzo[*b*]fluoranthene and 5-methylchrysene seem to be the most potent carcinogenic PAHs. IPCS (1998) concluded that anthracene, benzo[*gh*]fluoranthene, benzo[*gh*]perylene, fluorene, 1-methylphenanthrene, naphthalene, perylene and triphenylene should not be considered carcinogenic.

Based on all information, it can be concluded that most PAHs are carcinogenic in experimental animals. A number of individual PAHs, however, appear as non-carcinogenic or only weakly carcinogenic: anthracene, acenaphthalene, benzo[*e*]pyrene, fluorene, fluoranthene, perylene, phenanthrene and pyrene. For naphthalene, the 2002 evaluation by the International Agency for Research on Cancer (IARC) led to classification in Group 2B, based on *inadequate evidence* for carcinogenicity in humans and *sufficient evidence* in experimental animals. This conclusion deviates from the conclusion drawn by IPCS in 1998, primarily because of the observation of olfactory and nasal tumours in an inhalation study (NTP, 2000). However, particularly as naphthalene is probably not genotoxic (see section 2.2.4), the relevance to dietary exposure is doubtful.

One specific PAH, the high carcinogenic potential of which may lead it to contribute strongly to the total effect at least in some PAH mixtures, is dibenzo[*a,l*]pyrene. As Luch & Jacob (2004) and Jacob & Seidel (2004) point out, bioassay data (non-oral) indicate that this compound is much more potent than benzo[*a*]pyrene (by a factor of 100), and they recommend that the dibenzopyrenes

Table 4. Overview of PAH carcinogenicity by route of administration

Common name	Oral	i.p.	i.p./s.c. newborn	Resp.	Skin init.	Skin compl.	s.c. or i.m.	IARC ^a
Acenaphthene								
Acenaphthylene								
Anthanthrene				+ r	0 m	0 m		L
Anthracene	0 r	0 r	+ m	0 r		0 m	0 m, r	I
Benz[a]anthracene	+ m		+ m	(+) m	(+) m		+ m, 0 r	S
Benzo[a]fluorene					0 m	0 m	0 m	I
Benzo[b]fluorene					0 m			I
Benzo[b]fluoranthene	+ m		+ m	+ r	+ m	+ m	+ m	S
Benzo[ghi]fluoranthene			+ m	+ r		0 m		
Benzo[j]fluoranthene			+ m	+ r	+ m	+ m		S
Benzo[k]fluoranthene			+ m	+ r	(+) m	0 m	+ m	S
Benzo[ghi]perylene						(+) m	0 m	I
Benzo[c]phenanthrene					+ m	(+) m	0 m, (+) r	I
Benzo[a]pyrene	+ m, r, h	+ m, r	+ m	+ m	+ r, h	+ m	+ m, r	S
Benzo[e]pyrene	0 m			0 r		0 m		I

Table 4. (contd)

Common name	Oral	i.p.	i.p./s.c. newborn	Resp.	Skin init.	Skin compl.	s.c. or i.m.	IARC ^a
Chrysene			(+) m	(+) r	(+) m	(+) m	+ m, r	L
Coronene			+ m			(+) m		I
Cyclopenta[cd]pyrene			+ m		+ m	+ m		L
Dibenz[a,h]anthracene	+ m		+ m	+ r	+ m	+ m	+ m, r	S
Dibenzo[a,e]pyrene					+ m	+ m	+ m	S
Dibenzo[a,h]pyrene			+ m		+ m	+ m	+ m	S
Dibenzo[a,i]pyrene					+ m	+ m	+ m	S
Dibenzo[a,j]pyrene					+ m	+ m	+ m	S
Fluoranthene			+ m		0 m	0 m		I
Fluorene		0 r					0 m	I
Indeno[1,2,3-cd]pyrene			0 m	+ r	+ m	(+) m	+ r	S
5-Methylchrysene					+ m	+ m		S
1-Methylphenanthrene					0 m			I
Naphthalene	? r	? m		+ m, r			? r	S ^b
Perylene					(+) m			I
Phenanthrene	0 r		0 m	0 r	(+) m		0 m	I

Table 4. (contd)

Common name	Oral	i.p.	i.p./s.c. newborn	Resp.	Skin init.	Skin compl.	s.c. or i.m.	IARC ^a
Pyrene	0 m			(+) h	(+) m	(+) m	0 m	I
Triphenylene						0 m		I

From EC (2002)

0, no effect; (+), weak effect; +, clear effect; ?, study too limited for evaluation; m, mouse; r, rat; h, hamster; i.p., intraperitoneal; s.c., subcutaneous; i.m., intramuscular; resp., respiratory instillation or inhalation; init., initiation; compl., complete carcinogen protocol

^a International Agency for Research on Cancer (IARC) conclusion with respect to carcinogenicity in animals: I, inadequate; L, limited; S, sufficient.

^b Evaluation by IARC (2002).

Table 5. Overview of oral PAH carcinogenicity data

PAH Species, strain	Number per group	Dosage	Duration at death/sacrifice	Incidence and tumour type	Result stat/val	Reference
<i>Anthracene</i>						
Rat	31	6 mg/animal per day, 7×/week (diet)	33 months	22/31 alive after 1 year; no tumours after 33 months	ld	Schmahl & Reuter, cited by Gerarde (1960)
Rat BD I/ BD III	28	5–15 mg/animal per day, 6×/week, 78 weeks (diet)	700 days	2/28 malignant tumours	ld	Schmahl (1955)
<i>Benz[a]anthra- cene</i>						
Mouse C57BL	8–19	0.5 mg/animal, 1×, 8× or 16× (highest dose)	16 months	0/13, 1/19 and 1/8 with papillomas, no carcinomas (forestomach)	q, ln	Bock & King (1959)
Mouse, m B6Afl/J, newborn	20 or 40	1.5 mg/animal, 3×/week, 5 weeks	≤547–600 days	100% hepatomas, 95% pulmonary adenomas; vehicle only: 10% hepatomas, 35% pulmonary adenomas	val	Klein (1963)
Mouse, m B6Afl/J, newborn	20	1.5 mg/animal, 1×/day, 2 days	≤568 days	80% hepatomas, 85% lung adenomas (inadequately reported)	val	Klein (1963)
Rat, f SD	10	200 mg/rat, 1×	60 days	No tumours in treated animals; control: 8/164 after 310 days	ln, lc	Huggins & Yang (1962)

Table 5. (contd)

PAH Species, strain	Number per group	Dosage	Duration at death/sacrifice	Incidence and tumour type	Result stat/val	Reference
<i>Benzo[a]pyrene</i>						
Mouse, f A/HeJ	15	3 mg/animal in sesame oil, 2×	30 weeks	Pulmonary tumours: 16.6; control: 0.3	Yes, val	Wattenberg & Leong (1970)
Mouse, f A/J	15	2 mg/animal, 3× every 2 weeks	26 weeks	100% forestomach tumours, 100% pulmonary adenomas; no control	Yes, val	Sporn et al. (1986)
Mouse, m/f CFW	25–73	0.004–1 mg/animal per day (diet)	140–200 days	Dose-dependent gastric tumours (0–90%); control: no tumours	No, val	Neal & Rigdon (1967)
Mouse, m/f CFW	9–26	1–20 mg/animal per day (diet), ≤1–30 days	150–300 days	Dose-dependent gastric tumours (0–100%); control: no tumours	No, val	Neal & Rigdon (1967)
Mouse, m/f White Swiss	60–175	0.25 and 1 mg/g food	≤34 weeks	33% and 61% stomach tumours; 53% and 20% lung tumours; controls: 1% and 21%	No, val	Rigdon & Neal (1966)
Mouse, f B6C3F1	48	0, 5, 25, 100 mg/kg diet, equivalent to 0, 0.7, 3.6, 14 mg/kg bw per day	2 years	Forestomach papillomas and carcinomas: 1/48, 4/47, 36/47, 46/47 Oesophageal papillomas and carcinomas: 0/48, 0/48, 2/45, 27/46 Tongue papillomas and carcinomas: 0/48, 0/48, 2/46, 23/48	Yes, val	Culp et al. (1998)

Table 5. (contd)

PAH Species, strain	Number per group	Dosage	Duration at death/sacrifice	Incidence and tumour type	Result stat/val	Reference
Rat, f SD	9	100 mg/kg, 1×	60 days	8/9 mammary tumours; control: 8/164 in 310 days	No, ln, lc	Huggins & Yang (1962)
Rat, f LEW/Mai	20	625 mg/animal, 1×/week, 8×, 50 mg/animal, 1×	90 weeks	67–77% mammary tumours; control: 30%	Yes, val	McCormick et al. (1981)
Rat, m/f Wistar	52	0, 3, 10, 30 mg/kg bw, 5 days/week in soya oil	104 weeks	Forestomach papillomas and carcinomas: f – 1/52, 6/51, 30/51, 50/52, m – 0/52, 8/52, 43/52, 52/52 Liver papillomas and carcinomas: f – 0/52, 2/52, 39/52, 51/52, m – 0/52, 4/52, 38/52, 49/52. Also tumours at other sites	Yes, val	Kroese et al. (2001)
Hamster, m/f Syrian <i>Dibenz[a,h]- anthracene</i>	13	2.5 mg/animal (diet), 4 days/week	≤14 months	9/13 forestomach cancer, 2/13 papillomas	No, val	Chu & Malmgren (1965)
Mouse, m Swiss	–	1.5 mg/animal in PEG-400, 1×, initiation experiment	30 weeks	21% forestomach papillomas; promoter only: 14%	Q, no, lc	Berenblum & Haran (1955)
Mouse, m/f DBA/2	21/21 control: 25/10	0.8 mg/animal per day in olive oil	8–9 months	14/14 m and 13/13 f with pulmonary adenomas; 14/14 m and 10/13 f with alveologenic carcinomas; control: 1 tumour	No, val	Snell & Stewart (1962)

Table 5. (contd)

PAH Species, strain	Number per group	Dosage	Duration at death/sacrifice	Incidence and tumour type	Result stat/val	Reference
<i>Fluorene</i>						
Rat, f buffalo	20	0.05% diet; 4.3 mg/rat per day = 796 mg/rat total over 6 months	10.7 months	2/11 carcinomas (renal pelvis, ureter); control: 4/16 with carcinomas	q, no, ld	Morris et al. (1960)
Rat, f buffalo	18	0.05% diet; 4.6 mg/rat per day = 2553 mg/rat total over 18 months	≤20.1 months	7/18 tumours; control: 4/18 or 15/18 tumours	q, no, val	Morris et al. (1960)
<i>Naphthalene</i>						
Rat BD I/BD III	28	10–20 mg/animal per day, 6×/week, 70 weeks (diet)	Life	No tumours	No, ld	Schmahl (1955)
<i>Phenanthrene</i>						
Rat, f SD	10	200 mg/rat, 1×, experiment on mammary tumours	60 days	No tumours at 60 days; controls: 8/164 after 310 days	No, ln	Huggins & Yang (1962)

Adapted from EC (2002)

lc, limited documentation; ld, limited design; ln, limited number of animals; q, questionable; stat, statistical evaluation (yes or no); val, valid; f, female; m, male

therefore be included in the routine chemical analyses for environmental PAH mixtures. It is not known to what extent dibenzo[a,l]pyrene occurs in food.

The PAH most extensively examined is benzo[a]pyrene, which was studied by all current methods in as many as seven species. Specifically for the oral route, benzo[a]pyrene is also the most studied PAH. In oral studies in rats and mice, the compound induced tumours of the gastrointestinal tract, liver, lungs, mammary glands and several other organs/tissues. IPCS (1998) lists oral studies with benzo[a]pyrene in mice, rats and hamsters. Each of these studies had limitations as to their design and scope. Since the IPCS review, two important new oral studies have been published, i.e. a rat study with benzo[a]pyrene by Kroese et al. (2001) and a study in mice by Culp et al. (1998) in which the tumorigenic response of benzo[a]pyrene was compared with that of two coal tar mixtures. Since these two studies are clearly superior in the dose-response information they provide, their results are summarized here in more detail.

Culp et al. (1998) administered either benzo[a]pyrene or two coal tar mixtures in the diets of a large number of groups of 48 female B6C3F1 mice for 2 years. The pattern of increased tumour incidences they observed is indicated in Table 6. The most significant finding was that benzo[a]pyrene alone induced only tumours of the alimentary tract (tongue, oesophagus, forestomach), whereas the coal tar mixtures also induced liver and lung tumours. Thus, coal tar components other than benzo[a]pyrene apparently exert a qualitatively different tumorigenic action, at least in mice. In an editorial comment, Goldstein (2001) cited evidence suggesting that the little studied PAH 7H-benzo[c]fluorene importantly contributes to the lung tumour formation seen in mice after feeding of coal tar mixtures. As to the absence of lung tumours after feeding benzo[a]pyrene to mice, this was found even in two highly sensitive transgenic mouse strains ($Xpa^{-/-}$, $Xpa^{-/-}/p53^{+/+}$) after feeding of benzo[a]pyrene at 75 mg/kg in the diet for 13 weeks. In this study, DNA adducts and cell proliferation were present in lung tissue of these mice, but no *lacZ* mutations (Hoogervorst et al., 2003).

Based on the results of Culp et al. (1998), both Kroese et al. (2001) and Schneider et al. (2002) developed quantitative cancer risk assessments for PAH mixtures. See section 9 for a discussion of these risk estimates.

Kroese et al. (2001) administered oral doses of benzo[a]pyrene at 0, 3, 10 or 30 mg/kg bw per day by gavage to groups of 104 Wistar rats (males, females) on 5 days per week for 2 years. A large variety of tumours was found, most prominent being those in the liver and forestomach. The tumour findings are summarized in Table 7. With respect to morbidity and mortality, the most important site of tumour formation was the liver. The authors developed a quantitative cancer risk assessment for benzo[a]pyrene, and PAHs in general, based on individual tumour types and for all treatment-related tumours combined. See section 9 for a discussion of these risk estimates.

Oral studies with PAHs other than benzo[a]pyrene are few in number and limited in design. These studies are not of use in the quantitative cancer risk assessment for PAHs in food.

[illegible]

Table 6. (contd)

Tumour type	Benzo[a]pyrene concentration in diet (mg/kg) ^a				Coal tar mixture 1 concentration in diet (%) ^b							Coal tar mixture 2 concentration in diet (%) ^c		
	0	5	25	100	0	0.01	0.03	0.1	0.3	0.6	1.0	0.03	0.1	0.3
Larynx papillomas and/or carcinomas	0/35	0/35	3/34	5/38	–	–	–	–	–	–	–	–	–	–
Haemangio-sarcomas ^e	1/48	2/48	3/47	0/48	1/48	0/48	1/48	1/48	11/48*	17/48*	1/45	1/48	4/48	17/48*
Histiocytic sarcomas	2/48	2/48	1/47	0/48	1/48	0/48	0/48	1/48	7/48	5/48	0/45	3/48	2/48	11/48*
Sarcomas ^f	1/48	2/47	7/47	0/48	1/48	4/48	3/48	2/48	7/48	1/48	2/45	0/48	4/48	5/48
Total tumour-bearing animals ^g	–	–	–	–	5/48	12/48	14/48	12/48	40/48	42/48	43/48	17/48	23/48	44/48

From Culp et al. (1998)

* Significantly different from control ($P < 0.05$)

^a Benzo[a]pyrene intake levels in dose groups were 0, 0.6, 3 and 12 mg/kg bw day, respectively (calculated daily intake, corrected for reduced food consumption).

^b Concentrations of benzo[a]pyrene in the diet in these groups were 0, 0.22, 0.66, 2.2, 6.6, 13.4 and 22.0 mg/kg, respectively (determined by high-performance liquid chromatography [HPLC]).

^c Concentrations of benzo[a]pyrene in the diet in these groups were 0, 1.1, 3.7 and 11.1 mg/kg, respectively (determined by HPLC).

^d – no increase reported (actual tumour incidence not given in Culp et al., 1998).

^e Organs involved included skin, liver, mesentery, mesenteric lymph nodes, heart, spleen, urinary bladder, uterus, thoracic cavity, ovary and skeletal muscle.

^f Organs involved included mesentery, forestomach, skin and kidney.

^g Data on total tumour-bearing animals were not included in Culp et al. (1998), but are included for the coal tar-treated groups in Schneider et al. (2002).

Table 7. Incidence of neoplasms in male and female Wistar rats gavage-dosed with benzo[a]pyrene

Site	Benzo[a]pyrene dose (mg/kg bw)							
	0		3		10		30	
	Males	Females	Males	Females	Males	Females	Males	Females
Oral cavity (papillomas, carcinomas and adenomas)	1/24 ^a	1/19	0/24	0/21	7/37	1/9	25/38	23/31
Oesophagus (sarcomas)	— ^b	0/52	—	1/52	—	9/52	—	0/52
Forestomach (papillomas, carcinomas)	0/52	1/52	8/52	6/51	43/52	30/51	52/52	50/52
Duodenum (adenocarcinomas)	0/51	0/49	0/50	0/48	0/51	0/50	1/49	2/51
Jejunum (adenocarcinomas)	0/51	0/50	0/50	0/48	1/51	0/50	8/49	2/51
Liver (adenomas, carcinomas, cholangiomas, cholangiocarcinomas)	0/52	0/52	4/52	2/52	38/52	39/52	49/52	51/52
Kidney (adenomas, carcinomas)	0/52	—	0/52	—	9/52	—	11/52	—
Auditory canal (papillomas, adenomas, carcinomas)	0/1	0/0	0/0	0/1	3/7	0/0	24/33	15/20
Skin and mammary (adenomas, carcinomas, acanthomas, epitheliomas, sarcomas, histiocytomas)	4/52	8/52	6/52	15/52	11/52	11/51	32/51	3/52
Pituitary (adenomas, carcinomas)	32/51	29/46	31/52	24/52	23/51	12/47	1/50	8/52
Total tumour-bearing animals	6/52	8/52	16/52	20/52	51/52	47/52	52/52	51/52

From Kroese et al. (2001)

^a Some tissues were examined histologically only when abnormalities were observed upon macroscopic examination.

^b — no increase reported (actual tumour incidence not given in Kroese et al., 2001).

Induction of preneoplastic lesions has been investigated as an indicator of carcinogenicity in a number of short-term studies. PAHs have produced increases in aberrant crypt foci in the colon of rat and mouse, nuclear anomalies in mouse small and large intestine and altered liver foci (EC, 2002).

The significance of the results of studies showing lesions in the intestine is unclear, as intestinal tumours were not observed in well conducted oral carcinogenicity studies in rats or mice.

2.2.4 Genotoxicity

A large body of data is available and is summarized in Table 8, which shows the overall conclusions from EC (2002).

As can be seen in Table 8, 15 of the individual PAHs show a clear genotoxic action in standard assays *in vitro* and *in vivo*. These were benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[ghi]perylene, benzo[j]fluoranthene, benzo[k]fluoranthene, chrysene, cyclopenta[cd]pyrene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,l]pyrene, dibenzo[a,i]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene. Benz[a]anthracene, benzo[a]pyrene and chrysene gave evidence of genotoxicity in germ cells; few other PAHs have been tested in germ cells.

Anthracene, benzo[a]fluorene, naphthalene and pyrene produced negative, or mainly negative, results and were considered not, or probably not, genotoxic. Four individual PAHs (anthracene, benzo[a]fluorene, naphthalene, pyrene) seem to lack genotoxicity activity. The data for the remaining PAHs were limited or equivocal, and further studies are required to clarify their *in vivo* genotoxic potential.

The interaction of PAHs with DNA has been examined extensively. The formation of DNA adducts by different PAHs was reported in the early 1960s. Since that time, many studies have been carried out. The active moieties that bind to DNA are the bay region diol epoxides (e.g. benzo[a]pyrene-7,8-diol-9,10-epoxide for benzo[a]pyrene), which predominantly bind to the extranuclear amino groups of guanine and adenine. The major adduct is formed on the N2 position of desoxyguanosine, leading to stable adducts. Binding of the diol epoxide to the N7 position of desoxyguanosine destabilizes the *N*-glycosidal bond, which results in depurination (formation of an apurinic site) (Seidel & Angerer, 2004).

The DNA adduct is the precursor lesion for mutation: mutations will occur at DNA adduct sites or close by them, through replication errors during DNA synthesis. The mechanism behind these mutations can be complex. Frameshift, deletion and base substitution mutations can occur. In addition to base-pair substitutions, PAHs may form bulky DNA adducts, resulting in frameshift mutations, deletions, S-phase arrest, strand breakage and a variety of chromosomal aberrations. Transversion of nuclear bases from guanine to thymine has been observed after exposure to benzo[a]pyrene-7,8-diol-9,10-epoxide in bacteria and mammalian cells and also in transgenic mice *in vivo* after treatment with benzo[a]pyrene. These transversions have also been demonstrated in *Ha-ras* oncogenes in mouse skin and lung tumours induced by benzo[a]pyrene. A similar

Table 8. Synopsis of genotoxicity test results with PAHs

Common name	Gene mutation in bacteria	DNA damage/repair in vitro	Genotoxicity in lower eukaryotes	Gene mutation in mammalian cells in vitro	In vitro cytogenetics in mammalian cells	DNA binding in mammalian cells in vitro	DNA binding in vivo	Genotoxicity/mutagenicity in vivo	Overall evaluation
Acenaphthene	+ ^{a,b}	- ^d				- ^c			I
Acenaphthylene	- ^a + ^b								I
Anthanthrene	+ ^{a,b}	+ ^{d,e}							L
Anthracene	Mainly - ^a	- ^d	- ^{f,g,j,m} ± ^k - ^l + ^l	mainly - ^{n,o,p,q}	- ^{r,s} - ^t		-	- ^{v,x}	N
Benz[a]-anthracene	+ ^{a,b}	+ ^{d,e}	+ ^k - ^j + ^{k,l,m} - ^l	mainly + ^{n,o}	± ^{s,t}	+ ^c	+	+ ^{v,w}	G
Benzo[a]-fluorene	- ^a	- ^d		- ^o					Probably N
Benzo[b]-fluorene	+/- ^a + ^b			- ^o					I
Benzo[b]-fluoranthene	+ ^a	+ ^d		- ⁿ (+) ^o		+ ^c	+	+ ^v	G

Table 8. (contd)

Common name	Gene mutation in bacteria	DNA damage/repair in vitro	Genotoxicity in lower eukaryotes	Gene mutation in mammalian cells in vitro	In vitro cytogenetics in mammalian cells	DNA binding in mammalian cells in vitro	DNA binding in vivo	Genotoxicity/mutagenicity in vivo	Overall evaluation
Benzo[ghi]-fluoranthene	+ ^{a,b}	+ ^d		— ^o					L
Benzo[j]-fluoranthene	+ ^{a,b}	+ ^d		(+) ^o		+ ^c	+		G
Benzo[k]-fluoranthene	+ ^a			(+) ^o		+ ^c	+		G
Benzo[ghi]-perylene	+ ^{a,b}	+ ^d		(+) ^o		+			G
Benzo[c]-phenanthrene	+ ^a	+ ^d	+ ^o			(+)			L
Benzo[a]pyrene	+ ^{a,b} — ⁱ	+ ^{d,e}	+ ^{g,i,m} — ^{f,j,k}	+ ^{r,s,t}	+ ^c	+	+	+ ^{u,v,x,y} — ^w	G
Benzo[e]pyrene	+/ ^a — ⁱ	(+) ^a — ^d	— ^m	+ ^o — ^{n,p}	— ^{r,s} — ^t		±	+/ ^v — ^v	E
Chrysene	+ ^{a,b} — ⁱ	+ ^d — ^e	— ^{f,j,l}	+ ^d — ^{n,q}	(+) ^s	+ ^c	+	+ ^{v,w}	G
Coronene	+ ^a — ^b	— ^d		— ^o					I

Table 8. (contd)

Common name	Gene mutation in bacteria	DNA damage/repair in vitro	Genotoxicity in lower eukaryotes	Gene mutation in mammalian cells in vitro	In vitro cytogenetics in mammalian cells	DNA binding in mammalian cells in vitro	DNA binding in vivo	Genotoxicity/mutagenicity in vivo	Overall evaluation
Cyclopenta[<i>cd</i>]-pyrene	+ ^{a,b}			+ ^{n,o}	+ ^s	+ ^c	+		G
Dibenz[<i>a,h</i>]-anthracene	+ ^{a,b}	+ ^{d,e}	– ^{f,l}	+ ^{n,o}	+ ^t	+ ^c	+	+ ^v	G
Dibenzo[<i>a,e</i>]-pyrene	+ ^{a,b}			+ ^o			+		G
Dibenzo[<i>a,h</i>]-pyrene	+ ^a – ^b	+ ^d		+ ^o	+ ^s		+		G
Dibenzo[<i>a,i</i>]-pyrene	+ ^{a,b}	+ ^d – ^e		(+) ^o			+	+ ^v	G
Dibenzo[<i>a,f</i>]-pyrene	+ ^{a,b}	+ ^d		+ ^o		+ ^c	+		G
Fluoranthene	mainly + ^{a,b}	mainly – ^e		mainly – ^o + ⁿ	+ ^s		+	– ^{u,v}	E
Fluorene	– ^{a,b}	– ^{d,e}	– ^m	+ / – ^o	+ ^r	+ ^c			I
Indeno[1,2,3- <i>cd</i>]pyrene	+ ^a	+ ^d		(+) ^o			+		G
5-Methylchrysene	+ ^a			(+) ^o			+		G

Table 8. (contd)

Common name	Gene mutation in bacteria	DNA damage/repair in vitro	Genotoxicity in lower eukaryotes	Gene mutation in mammalian cells in vitro	In vitro cytogenetics in mammalian cells	DNA binding in mammalian cells in vitro	DNA binding in vivo	Genotoxicity/mutagenicity in vivo	Overall evaluation
1-Methyl-phenanthrene	+ ^{a,b}	+ ^e		+ ^{n,o}		+ ^c			L
Naphthalene	- ^{a,b}	- ^d	+ ⁱ		+ ^r			- ^v	Probably N
Perylene									L
Phenanthrene	+/- ^a	+/- ^d	- ^{f,j,l}	+/- ^o - ^{n,p}	- ^{s,t} mainly - ^r			-/+ ^v	E
Pyrene	+/- ^a + ^b - ⁱ	- ^d mainly - ^c	- ^{g,h,j,k,l}	- ⁿ mainly - ^o + ^p	- ^t				N
Triphenylene	+ ^a	+ ^d		+ ^o		+ ^c			L

From IPCS (1998), as given in EC (2002)

Genotoxicity results: +, positive, -, negative, +/-, contrasting results reported in independent studies, ±, equivocal

a, reverse mutation (Ames test, several strains); b, forward mutation (strain TM677); c, DNA binding in mammalian cells in vitro; d, DNA damage/repair in bacteria; e, DNA damage/repair in mammalian cells; f, mitotic gene conversion in yeast; g, mitotic recombination in yeast; h, forward mutation in yeast; i, host-mediated assay (bacteria); j, host-mediated assay (yeast); k, sex-linked recessive lethals (*Drosophila*); l, somatic mutation and recombination (*Drosophila*); m, DNA repair (*Drosophila*); n, HPRT system; o, thymidine kinase system; p, ouabain resistance; q, diphtheria toxic resistance; r, chromosomal aberrations; s, sister chromatid exchanges; t, micronucleus test; u, DNA damage/repair in vivo (various tissues); v, cytogenetic effects in somatic cells; w, cytogenetic effects in germ cells; x, sperm abnormalities; y, dominant lethals.

Overall evaluation: E, equivocal; G, genotoxic; I, inadequate database for evaluation; L, limited evidence of genotoxicity; N, not genotoxic

prevalence of guanine to thymine transversions is seen in *p53* mutations in lung cancers of smokers, indicating that this observation may also be relevant to the carcinogenicity of PAHs in humans (Garner, 1998; EC, 2002).

An important issue for cancer risk assessment is the correlation between the level of adduct formation and actual tumour formation. This topic is discussed in a recent review by Luch & Glatt (2004). The formation of DNA adducts by electrophilic metabolites is generally regarded as one of the earliest steps in PAH carcinogenesis. PAHs have been shown to be able to bind to DNA sequences within or closely linked to *p53*-suppressor genes and *ras*-proto-oncogenes, two types of genes known to be involved in tumour formation in humans. As Luch & Glatt (2004) point out, however, determination of DNA adducts in whole tissues provides only a rough indication of cancer risk. The adduct level measured at a certain point in time is the result of the complex integration of external exposure to the chemical, the balance between activating and detoxifying metabolism, the rate of covalent binding and its enzymatic repair and the diluting effect of subsequent DNA synthesis and cell death. The bottom line is that with increasing levels of DNA adducts in any tissue, the statistical probability of the induction of actually tumorigenic DNA lesions will also increase.

As part of their chronic rat study, Kroese et al. (2001) determined DNA adducts by ^{32}P -postlabelling in a large number of tissues and found that they were equally present in tissues in which tumours later developed (liver, forestomach) and in those in which no tumours were observed (most importantly the lungs). The authors concluded that other factors additional to DNA adduct formation apparently are critical to tumour development by benzo[a]pyrene, and they postulated that local cell proliferation might be this critical additional factor. A similar conclusion was reached by Goldstein et al. (1998), who studied adduct formation as part of the 2-year mouse coal tar feeding study by Culp et al. (1998). They found increased adduct levels in lungs both in groups given benzo[a]pyrene alone and in those given coal tar, but lung tumours developed only in the coal tar group. The level of lung adducts in the coal tar group, however, markedly exceeded that in the benzo[a]pyrene group. In subsequent research in rats, Weyand et al. (2002) found adducts in lungs after feeding of coal tar, i.e. both benzo[a]pyrene-derived and benzo[c]fluorene-derived adducts. The levels of the benzo[c]fluorene-derived adducts, however, were much higher, a finding that indicates that this specific PAH might be responsible for lung tumour formation seen after oral administration of coal tar.

As mentioned above, adduct formation follows the prior conversion of the lipophilic parent PAH to nucleophilic reactive metabolites by xenobiotic-metabolizing enzymes, notably CYP-dependent mono-oxygenases. Genetic differences between humans with regard to enzymatic formation as well as enzymatic degradation of these active metabolites have been shown to correlate with adduct formation (Luch & Glatt, 2004).

Studies have been conducted on the mutagenicity of environmental samples containing complex mixtures of PAHs, such as air particulate, combustion emissions and water sediment extracts. In only a few cases has the mutagenicity of

crude PAH fractions been correlated to the content of genotoxic PAHs. Both increased and decreased genotoxicity compared with benzo[a]pyrene (or benzo[a]pyrene-equivalents) have been reported.

Similarly, studies of defined PAH mixtures have shown combined effects that were equivalent to, less than or more than the response that would be predicted from simple additivity of the components. In a recent study, the combined effects of up to 12 PAHs in the SOS chromotest were studied. At concentrations above 1 µg/ml, the observed responses were much lower than predicted by assumption of additivity. At lower concentrations, the response was additive or slightly less than additive. The author suggested that antagonism at higher concentrations may result from competitive inhibition of the enzymes involved in metabolic activation (White, 2002).

2.2.5 Reproductive toxicity

Some PAHs (and/or their metabolites) have been shown to cross the placenta; because of their lipophilicity, it is assumed that most PAHs are likely to pass into the embryo and fetus. Furthermore, benzo[a]pyrene, chrysene, dibenz[a,h]-anthracene, fluoranthene, perylene and phenanthrene have been shown to induce CYP enzymes in rat placenta, and it is anticipated that induction would increase toxicity in the fetus. PAH-DNA adducts are found in human placenta and in fetal tissues, indicating that PAHs are transferred to and activated by the human fetus.

(a) Reproductive effects

Most investigations have been conducted with benzo[a]pyrene. Studies involving intraperitoneal injection have shown that benzo[a]pyrene can impair female fertility by destruction of oocytes. This effect is AhR-mediated, involving increased expression of the *Bax* gene in oocytes. However, in some studies, the consequence of strain differences in inducibility of the AhR does not correlate with oocyte destruction, leading IPCS to conclude that the sum of activation, detoxification and repair seems to be decisive (IPCS, 1998).

Dietary administration of benzo[a]pyrene to Swiss mice at doses equivalent to 33, 67 or 133 mg/kg bw per day for varying periods of 20–30 days before mating, during gestation and during parturition did not have any effects on the numbers of offspring or on numbers of sperm in the testicular lumen (Rigdon & Neal, 1965).

Administration of benzo[a]pyrene by oral gavage to CD-1 mice from day 7 to day 16 of pregnancy at doses of 10, 40 or 160 mg/kg bw per day reduced the numbers of pregnant females reaching parturition at 160 mg/kg bw per day and reduced the fertility of F1 offspring in all dose groups when bred with untreated animals. There were significant alterations in gonadal morphology and germ cell development and almost complete sterility of the F1 offspring at 40 mg/kg bw per day (MacKenzie & Angevine, 1981).

Dietary administration of benzo[a]pyrene to male and female rats at a dose equivalent to 50 mg/kg bw resulted in fewer pregnancies and increased stillborn

and resorbed fetuses, but the data were poorly reported (Rigdon & Rennels, 1964).

(b) *Developmental effects*

Developmental toxicity (embryo lethality, reduced fetal weight and malformations) has been reported in response to benz[a]anthracene, benzo[a]pyrene, dibenz[a,h]anthracene and, in the presence of maternal toxicity, naphthalene. The effects reported in oral studies are summarized in Table 9.

2.2.6 Special studies

(a) *Immunotoxicity*

PAHs have been reported to be immunotoxic, mostly causing immunosuppression, which could be associated with an increased susceptibility to development of cancer or infectious diseases. Most of the published studies have used parenteral administration, frequently of model PAHs, such as 7,12-dimethylbenz[a]anthracene and 3-methylcholanthrene, which are not informative for risk assessment of dietary PAHs. It has been suggested that these effects are mediated via the AhR or, based on the results of *in vitro* studies, via modulation of intracellular calcium concentration signalling.

Administration of benzo[a]pyrene at 30 or 90 mg/kg bw per day by gavage to male Wistar rats for 35 days resulted in immunotoxic effects, such as decreased thymus and lymph node weights, decreased absolute and relative B-cell numbers in the spleen, decreased numbers of red and white blood cells and decreased serum immunoglobulins. At 10 mg/kg bw per day, thymus weight and spleen B-cell populations were altered, but there was no overt toxicity. No effects were observed at 3 mg/kg bw per day (De Jong et al., 1999).

Injection of benzo[a]pyrene at 150 mg/kg bw to mice in the second trimester of pregnancy resulted in impaired immunocompetence of the offspring (Urso & Johnson, 1987; Urso et al., 1992, 1994).

Studies in *Cyp1a1*^{-/-} knockout and *Cyp1a1*^{+/+} wild-type mice have indicated that CYP1A1 may protect against immunotoxicity of benzo[a]pyrene. Administration in the diet at doses equal to about 125 mg/kg bw per day to groups of six mice resulted in death of the knockout mice within 22–30 days, with no overt signs of toxicity in the wild-type mice. Groups of knockout or wild-type mice were administered diet providing benzo[a]pyrene (*n* = 12) at doses equivalent to 1.25, 12.5 and 125 mg/kg bw per day or control diet (*n* = 6) for 18 days. In the CYP1A1 knockout mice, there were dose-related decreases in body weight gain, which were significant at all dose levels, and in spleen and thymus weights, which were significant at 12.5 and 125 mg/kg bw per day. The wild-type mice were less susceptible, showing small decreases in spleen and thyroid weights but no effect on body weight gain at 125 mg/kg bw per day and no effects at lower doses. In further studies, benzo[a]pyrene at 125 mg/kg bw per day was found to result in marked hypocellularity in the bone marrow and blood of knockout mice, but not of wild-type mice. The authors concluded that the presence of inducible CYP1A1

Table 9. Summary of oral developmental toxicity studies

PAH	Species (strain)	No. per group	Route	Duration, dose	Effects	Reference
Anthracene	Rat (Sprague-Dawley)		Gavage	GD 19; 60 mg/kg bw	No induction of benzo[a]pyrene hydroxylase in fetal liver compared with controls	Welch et al. (1972)
Benz[a]anthracene	Rat (Sprague-Dawley)		Gavage	GD 19; 60 mg/kg bw	Induction of benzo[a]pyrene hydroxylase in fetal liver	Welch et al. (1972)
Benzo[a]pyrene	Mouse (Swiss)	9	Diet	GD 5 or 10 until delivery; 150 mg/kg bw	No malformations	Rigdon & Neal (1965)
	Mouse (C57BL/N, AK/J and back-crosses)	6–17	Diet	GD 2–10; 120 mg/kg bw	Increased uterine toxicity and malformations in Ah ^d /Ah ^d embryos compared with Ah ^b /Ah ^d embryos in pregnant Ah ^d /Ah ^d mice	Legraverend et al. (1983)
	Mouse (CD-1)		Gavage	GD 7–16; 10, 40, 160 mg/kg bw per day	No maternal or embryofetal toxicity	MacKenzie & Angevine (1981)
	Rat (Sprague-Dawley)		Gavage	GD 19; 60 mg/kg bw	Induction of benzo[a]pyrene hydroxylase in fetal liver	Welch et al. (1972)
Chrysene	Rat (Sprague-Dawley)		Gavage	GD 19; 60 mg/kg bw	Induction of benzo[a]pyrene hydroxylase in fetal liver	Welch et al. (1972)
Dibenz[a,h]-anthracene	Rat (Sprague-Dawley)		Gavage	GD 19; 60 mg/kg bw	Induction of benzo[a]pyrene hydroxylase in fetal liver	Welch et al. (1972)

Table 9. (contd)

PAH	Species (strain)	No. per group	Route	Duration, dose	Effects	Reference
Naphthalene	Mouse (CD-1)	50	Gavage	GD 7–14; 300 mg/kg bw per day	Significant increase in maternal mortality, significant reduction in weight gain	Plasterer et al. (1985)
	Mouse (CD-1)		Gavage	GD 6–13; 300 mg/kg bw per day	Significant reduction in live offspring, no malformations Increased maternal mortality, significant reduction in weight gain Significant reduction in live offspring per litter	Hardin et al. (1981)

GD, gestation day

protected against the immunotoxicity, myelotoxicity and wasting induced by oral administration of benzo[a]pyrene (Uno et al., 2004).

(b) Neurotoxicity

Saunders and colleagues performed two acute oral neurotoxicity studies with benzo[a]pyrene and fluoranthene, respectively. Groups of 8-week-old F-344 rats received single gavage dose levels of the test compound and were either evaluated for motor activity and behaviour for 5 days or submitted to a functional observational test battery (FOB) on eight occasions throughout the 5-day post-dosing period. Both compounds induced marked decreases in locomotor activity and clear dysfunction in the FOB. The NOAELs for these effects were 12.5 mg/kg bw for benzo[a]pyrene (lowest-observed-adverse-effect level [LOAEL] 25 mg/kg bw) and 100 mg/kg bw for fluoranthene (LOAEL 200 mg/kg bw) (Saunders et al., 2001, 2003).

(c) Cardiovascular effects

It has been hypothesized that PAHs from cigarette smoke or combustion products could cause endothelial injury and changes in smooth muscle cells that might contribute to the development of atherosclerosis. However, this is unproven, and there is little available information relating to cardiovascular effects following oral administration of PAHs. The effects of benzo[a]pyrene have been investigated in apolipoprotein E knockout mice, a transgenic model in which diet-independent atherosclerotic lesions develop that resemble human atherosclerosis. Benzo[a]pyrene was administered by gavage at 5 mg/kg bw once a week for 12 ($n = 31$) and 24 ($n = 19$) weeks. High levels of benzo[a]pyrene–DNA binding products were detected in the aorta. There was no influence of benzo[a]pyrene on the location or number of atherosclerotic lesions, but plaques were larger, were more prone to lipid-core development and plaque layering and contained more T-lymphocytes and macrophages in the benzo[a]pyrene-treated animals than in controls. The authors suggested that benzo[a]pyrene did not initiate atherosclerosis in apolipoprotein E knockout mice, but accelerated the progression of atherosclerotic plaques via a local inflammatory response (Curfs et al., 2004).

(d) Cataracts

Naphthalene has been reported to induce development of cataracts in rats, mice and rabbits. Particularly in rabbits, cataracts were observed within a few days of oral administration at 500–1000 mg/kg bw per day. It has been postulated that CYP-dependent bioactivation to naphthoquinone or a free-radical derivative is essential for induction of cataracts. In mice, the effect was reduced by pretreatment with CYP inhibitors and antioxidants and increased by pretreatment with CYP inducers or glutathione depletors (IPCS, 1998; EC, 2002).

2.3 Observations in humans

2.3.1 Introduction

PAHs almost always occur as complex mixtures, and most epidemiological studies have been unable to address separate effects of individual compounds. Unless otherwise stated, in this review the term PAHs refers to the group of 33 compounds listed in Table 1 (see section 1).

2.3.2 Biomarkers of exposure

Several methods have been developed to assess internal exposure. In most studies, metabolites of PAHs were measured in urine; the urinary concentration or excretion is dependent not only on the external exposure, but also on absorption, transformation and excretion, which can show considerable between-subject variability. Adducts of PAHs with proteins such as albumin or haemoglobin, as well as with DNA in white blood cells and other tissues, have been used as an indicator of exposure and reveal individual differences in metabolism and repair rates. Since the earliest carcinogen-induced events in chemical carcinogenesis typically include structural DNA damage, which often occurs as a result of adduct formation, measurement of DNA adducts may be considered, to some extent, an indicator of early effect.

(a) Occupational exposure

1-Hydroxypyrene is a metabolite of pyrene that has been widely used as a urinary biomarker of PAH exposure. Pyrene is present in all PAH mixtures at relatively high concentrations (2–10% of the total PAHs), and in certain environments the pyrene content of the total PAHs is fairly constant. A strong correlation has been found between the pyrene concentrations in air and those of benzo[a]-pyrene, other selected PAHs and total PAHs (Jongeneelen et al., 1990). Pyrene is metabolized predominantly to 1-hydroxypyrene and excreted in urine.

A higher level of 1-hydroxypyrene has been reported in coke oven workers compared with controls in Germany (Strunk et al., 2002), Japan (Zhang et al., 2003), Poland (Siwinska et al., 2004), China (Province of Taiwan) (Lu et al., 2002; Wu et al., 2002, 2003a; Chen et al., 2003) and Turkey (Ates et al., 2004). Several studies have addressed the association between 1-hydroxypyrene and exposure to vehicle exhaust, with a higher 1-hydroxypyrene urinary excretion reported among exposed in the Republic of Korea (Sul et al., 2003), China (Province of Taiwan) (Tsai et al., 2004), Denmark (Hansen et al., 2004), Finland (Kuusimäki et al., 2004) and Turkey (Burgaz et al., 2002), while no differences were observed between exposed and unexposed in one study in Thailand (Ruchirawat et al., 2002). A higher concentration of 1-hydroxypyrene was observed among charcoal workers in Brazil (Kato et al., 2004) and paving workers in Finland (Vaananen et al., 2003) as compared with controls. The urinary level of 1-hydroxypyrene did not differ between workers exposed to carbon black and controls in China (Province of Taiwan) (Tsai et al., 2002), although a positive correlation was reported with PAH air concentration.

Other PAHs or PAH metabolites have also been analysed as biomarkers of PAH exposure. As compared with unexposed workers, an increased excretion of 2-naphthol in urine has been reported for charcoal workers (Kato et al., 2004), paving workers (Vaananen et al., 2003), individuals exposed to vehicle exhaust (Sul et al., 2003; Kuusimäki et al., 2004) and United States Air Force personnel exposed to the jet propulsion fuel JP-8 (Serdar et al., 2003). The two Finnish studies in paving workers (Vaananen et al., 2003) and workers exposed to vehicle exhaust (Kuusimäki et al., 2004) as well as one German study on coke oven workers (Strunk et al., 2002) showed an increased excretion of several phenanthrols. Increased excretion has been reported for naphthalene and benzene among those exposed to JP-8 (Serdar et al., 2003) and for naphthalene, phenanthrene, pyrene and benzo[a]pyrene among coke oven workers in the United States (Waidyanatha et al., 2003). One study in China (Province of Taiwan) (Wu et al., 2002) reported a positive correlation of urinary excretion of *trans*-anti-benzo[a]pyrene-tetraol with 1-hydroxypyrene in urine as well as with environmental exposure to benzene. Another study (Hecht et al., 2003) observed an increased excretion of the analogous compound for phenanthrene (*trans*-anti-phenanthrene-tetraol) as compared with unexposed workers.

DNA adducts with reactive metabolites (mainly diol-epoxides) of benzo[a]pyrene and other PAHs have been identified in humans. There is substantial inter-individual variation in PAH–DNA adduct levels; this is probably due to differences in biotransformation, excretion, DNA adduct removal, etc. (Lee et al., 2002). Two recent studies have analysed the level of aromatic adducts in the nuclear DNA of white blood cells among coke oven workers in Japan (Zhang et al., 2003) and China (Province of Taiwan) (Chen et al., 2003). Both studies reported higher levels of DNA adducts among exposed workers as compared with controls, and the adduct levels were correlated with urinary excretion of 1-hydroxypyrene. Two studies looked at the association of adducts with exposure to vehicle exhaust; one study in Bangladesh (Rahman et al., 2003) reported increasing levels of diol-epoxide–PAH adducts for increasing level of exposure, whereas another study in Thailand (Ruchirawat et al., 2002) observed higher levels of both DNA and albumin adducts among exposed than among non-exposed. A meta-analysis regarding DNA adducts in 13 cohorts dealing with occupational exposure to PAHs (Peluso et al., 2001) included studies reporting white blood cells or lymphocyte DNA adducts measured by ^{32}P -postlabelling among workers and referents, taking into account the potential confounding effect of tobacco smoking. The ratio between the mean adduct levels among exposed and referents, referred to as the frequency ratio, was 2.01 (95% confidence interval 1.53–2.64) for industrial workers and 1.51 (1.07–2.14) for urban workers. DNA adduct levels were significantly correlated with levels of benzo[a]pyrene in air. The relation between DNA adducts and benzo[a]pyrene was found to be linear at low doses, while adduct formation tended to reach saturation at exposure above 20 ng/m^3 .

(b) Environmental exposure

A literature search and meta-analysis (Castano-Vinyals et al., 2004) included 36 papers dealing with non-occupationally exposed populations, with available

information on PAH air pollution and measurement of urinary metabolites of PAHs and/or DNA or protein adducts. In 15 out of 21 pairs of data comparing exposed with non-exposed subjects on the basis of PAH air pollution, the exposed had higher 1-hydroxypyrene excretion. A statistically significant correlation with $r = 0.76$ was found between the log mean concentrations of benzo[a]pyrene in air and log mean concentrations of urinary 1-hydroxypyrene based on 17 pairs. After log transformation, the correlation between benzo[a]pyrene in air and DNA adducts was significant with $r = 0.60$ on the basis of 12 pairs. Nine studies determined albumin adducts by different techniques, with higher levels among exposed individuals in five of them; airborne benzo[a]pyrene was measured in only two studies. According to these results, both 1-hydroxypyrene and DNA adducts can be usefully applied to assess environmental exposure to PAHs at a group level. It must be recalled that the major contributors to PAHs in the urban and suburban atmosphere are diesel and gasoline engines, which mainly contain benzo[ghi]perylene, pyrene, fluoranthene and phenanthrene, so that measuring only benzo[a]pyrene as an index substance may result in underestimation of exposure.

(c) *Dietary exposure*

Biomonitoring of PAHs in non-occupationally exposed populations, including the possible contribution of dietary intake, has been undertaken in two studies. A German study (Scherer et al., 2000) measured benzo[a]pyrene adducts in albumin and haemoglobin, urinary excretion of 1-hydroxypyrene, cotinine in plasma and nicotine in personal samplers; dietary benzo[a]pyrene intake was estimated on the basis of published data. Average 1-hydroxypyrene and benzo[a]pyrene adducts in both albumin and haemoglobin were higher for smokers, but there were no significant differences in PAH biomarkers among non-smokers according to environmental tobacco smoking exposure. The estimated benzo[a]pyrene dietary intake did not correlate with any of the PAH biomarkers. Most likely the inaccuracy of the estimate of the PAH intake is the major reason for this observation. These results are consistent with those from a study in Canada carried out in five healthy volunteers who consumed similar amounts of identical foods for 5 consecutive days (Viau et al., 2002). Despite the identical ingested doses of pyrene, there was substantial interindividual variability in the daily excreted amount of 1-hydroxypyrene (coefficient of variation 50–76%). Urinary excretion of 1-hydroxypyrene seems not to be correlated with ingested dose of pyrene under the normal feeding conditions used in this study.

2.3.3 *Biomarkers of effect*

8-Hydroxy-2'-deoxyguanosine (8-OH-dG) is a product of DNA oxidation, released when chemically damaged DNA undergoes exonuclease repair. Urinary excretion of 8-OH-dG was higher in exposed than in control workers in one Swedish study among engine room personnel exposed to oils and engine exhaust (Nilsson et al., 2004) and in coke oven workers in China (Province of Taiwan) (Wu et al., 2003a). Another study among coke oven workers in Japan (Zhang et al., 2003) did not find a clear association between exposure and 8-OH-dG in

lymphocytes, while there was a positive correlation between lymphocytic 8-OH-dG and aromatic DNA adducts.

A higher urinary mutagenicity measured by means of the Ames assay was observed among charcoal workers compared with controls in Brazil (Kato et al., 2004) and in workers exposed to vehicle exhaust in Denmark (Hansen et al., 2004). Exposure to vehicle exhaust has been associated with genotoxic effects measured in lymphocytes by single-cell gel electrophoresis (or Comet assay) in a study in the Republic of Korea (Sul et al., 2003) and with increased frequency of chromosomal aberrations in one Turkish study (Burgaz et al., 2002). Finally, among coke oven workers in Poland (Siwinska et al., 2004), those exposed to PAHs had increased sister chromatid exchanges and increased numbers of micronuclei compared with unexposed controls.

2.3.4 Epidemiological studies

Humans have been occupationally exposed to PAHs via inhalation in the majority of studies reviewed, and through dermal exposure in a few of them. Although experimental studies have shown several toxicological effects, it is the carcinogenic potential of PAHs that has attracted more interest. There are almost no published studies on health effects of PAHs in humans following oral exposure. Furthermore, there is little information on human exposure to single PAHs; thus, most reports focus on exposure to mixtures of PAH.

(a) Oral exposure

There are no published studies that specifically addressed the relationship between dietary intake of PAH and cancer risk; however, some authors analysed the effect of dietary factors that may involve oral PAH exposure. In some areas of Spain, wine has traditionally been stored in leather bottles sealed with a tar-like substance obtained through boiling and distillation of fir and pine wood. Within a multi-centre study (Lopez-Abente et al., 2001), including 354 incident cases of gastric cancer and 354 controls matched by sex, age and residence, 38 cases and 40 controls from the province of Soria answered a self-administered questionnaire on consumption of wine stored in tar-impregnated leather bottles. Consumption of more than 2 litres of wine per week in a leather bottle was associated with gastric cancer in males, with an odds ratio (OR) of 10.5 (95% confidence interval 1.1–97.8), adjusted by several variables that had been found to be associated with gastric cancer in the multi-centre study. Analysis of tar from an unused leather bottle detected the presence of several PAHs, among which were chrysene, fluoranthene, pyrene, naphthalene and benzo(a)fluoranthene. However, no direct measurements were made of the PAH content in wine of such bottles, so that this study does not provide any direct evidence linking occurrence of gastric cancer to oral exposure to PAHs.

A recent review summarized epidemiological literature on cooking methods of red and processed meat and colorectal cancer (Pisani & Mitton, 2002): six case-control studies investigated the association of cooked meat with cancers of the colon and rectum, while two case-control studies investigated the potential

relation with adenomas. One study reported a statistically significant increase in the risk of cancer of both colon and rectum associated with preference for heavily browned meat, which persisted after adjustment for fat intake (OR = 2.0 and OR = 3.4 for colon and rectum, respectively). Excess risk was associated with preference for well done meat (OR = 3.5) and deep-fried food (OR = 2.1) in two studies conducted in the United States for colon and rectal cancers combined. The three remaining studies did not find any association. Most of these analyses accounted for potential confounders, including smoking and diet-related factors. A recent study not included in the previous review with 349 cases of colorectal cancer and 467 controls was carried out in Hawaii (Le Marchand et al., 2002). Among ever-smokers, a preference for well done red meat and having a rapid phenotype of *N*-acetyltransferase 2 (NAT2) and CYP1A2 were associated with a significant 8.8-fold increase in risk as compared with subjects with low NAT2 and CYP1A2 activities who preferred red meat rare or medium. A population-based case-control study including 360 cases of lung cancer and 360 cases and 574 frequency-matched controls was conducted in Iowa, USA (Alavanja et al., 2001). When comparing the highest to the lowest quintile of consumption, an OR of 3.3 (1.7–7.6) was obtained for red meat: the ORs were similar among lifetime non-smokers, ex-smokers and current smokers. However, no differences were seen for consumption of meat cooked at high temperature versus meats cooked at lower temperatures.

None of these studies was specifically designed to investigate dietary exposure to PAHs and cancer. Furthermore, other potential carcinogens, such as *N*-nitrosocompounds and heterocyclic amines, may occur in cooked well done meat in addition to PAHs. Thus, these results do not provide any direct evidence of a relationship between oral PAH exposure and cancer.

(b) Occupational exposure

Many occupations take place in an environment with heavy loads of PAHs. Among the industrial activities and occupations with high exposure to PAHs must be considered working in coke ovens, coal gasification, refineries, iron and steel foundries, handling asphalt and roofing, aluminium production, impregnation of wood with and handling creosotes, asphalt and pavement work, using mineral oils and work involving exposure to diesel exhausts. Occupational exposure to PAHs has been mainly related to cancers of the respiratory and urinary tract, although increased risks of stomach cancer, skin cancer and leukaemia have also been reported. Details of the most relevant studies may be found in previous reports (IPCS, 1998; EC, 2002), and evaluations of their cancer risk have been extensively reviewed by IARC (1984a, 1984b, 1985).

A recent meta-analysis was conducted on the relationship between PAH exposure and lung cancer (Armstrong et al., 2004). Overall, 39 cohorts were included: 10 were based on workers from the coke industry, 8 from aluminium smelting, 4 from gas production, 4 from carbon anode plants and the remaining 13 from different industries, such as asphalt, tar distillery, chimney sweep, thermo-electric power plants and carbon black industry. Exposure to PAHs was measured as cumulative exposure to benzo[a]pyrene ($\mu\text{g}/\text{m}^3\text{-years}$); 16 cohorts provided

such measure, while in the remaining 23 cohorts the index was calculated by the reviewers based on published exposure estimates. The combined relative risk (RR) for all cohorts was 1.2 (1.11–1.29) for a cumulative exposure of 100 $\mu\text{g}/\text{m}^3$ -years; by industry, the RR ranged from 1.15 to 17.5, all of them statistically significant except those for the tar distillery and carbon anode industries. Only four cohorts included adjustment for tobacco smoking, and the overall RR for these studies was 1.31 (1.16–1.48). Confounding by other occupational carcinogens is possible, but it may be limited in this analysis, since cohorts in which PAHs were judged unlikely to be the predominant exposure were excluded.

(c) Integrated exposure

Two recent studies used DNA adducts as indicators of PAH exposure. The first was a nested case–control study within the cohort of the Physicians Health Study (Tang et al., 2001), including 89 lung cancer cases and 173 controls matched by smoking status. There was a significant increase in DNA adduct measured in white blood cells in cases as compared with controls only among current smokers. The fact that cases and controls were matched by smoking points to potential sources of PAH exposure other than smoking, although it is difficult to explain why the excess risk is limited to current smokers. Another study on breast cancer risk compared PAH–DNA adducts measured in mononuclear cells of 576 cases and 427 population controls (Gammon et al., 2002). The adjusted OR for breast cancer, comparing the highest with the lowest quintile, was 1.49 (1.00–2.21), without any consistent elevation in risk with increasing adduct levels. In this study, adduct levels were not associated with cigarette smoking or with consumption of grilled or smoked foods. DNA adducts are formed by PAHs of any source, and thus any association found between DNA adducts and cancer cannot be attributed exclusively to oral PAH exposure.

(d) Effects other than cancer

Several studies have addressed potential effects of PAH exposure on outcomes other than cancer. Mortality from non-malignant respiratory diseases was analysed in a historical cohort of asphalt workers in eight European countries (Burstyn et al., 2003); humoral immunity was assessed in coke oven workers in China (Province of Taiwan), compared with workers from a rolling steel factory used as a reference group (Wu et al., 2003b); infertility in men and its relationship with PAH–DNA adducts have been assessed in Italy (Gaspari et al., 2003); finally, potential effects on birth outcomes of prenatal exposure to PAHs were investigated in non-smoking women in New York (Perera et al., 2003). None of these studies provided direct evidence of any relationship between dietary exposure to PAH and the above-mentioned health effects.

3. ANALYTICAL METHODS

3.1 Chemistry

3.1.1 Introduction

PAHs constitute a large class of organic compounds containing two or more fused aromatic rings. The term "polycyclic aromatic hydrocarbons" refers to compounds containing only carbon and hydrogen atoms (i.e. unsubstituted parent PAHs and their alkyl-substituted derivatives), whereas the more general term "polycyclic aromatic compounds" also includes the functional derivatives (e.g. nitro- and hydroxy-PAHs) and the heterocyclic analogues, which contain one or more hetero atoms in the aromatic structure (aza-, oxa- and thia-arenes).

PAHs may be formed and released during incomplete combustion or pyrolysis of organic matter, industrial processes and other human activities. PAHs also form directly during processing (drying and smoking) or cooking (grilling/barbecuing, roasting) of foods. PAHs are emitted from a number of environmental sources, such as processing of coal, crude oil, petroleum and natural gas, production of aluminium, iron and steel, heating in power plants and residences (oil, gas, charcoal-fired stoves, wood stoves), combustion of refuse, fires, including wood fires, motor vehicle exhaust and used motor lubricating oil. More than 100 PAHs have been identified in atmospheric particulate matter and in emissions from coal-fired residential furnaces, and about 200 compounds have been found in tobacco smoke (IPCS, 1998).

The nomenclature (common names, CAS registry numbers and abbreviations) of PAHs covered in this monograph are given in Table 1 (see section 1). The structural formulae of PAHs are shown in Figure 1.

3.1.2 Physical and chemical properties

Physical and chemical properties of PAHs relevant to this monograph are detailed in the IPCS Environmental Health Criteria monograph entitled "Selected non-heterocyclic polycyclic aromatic hydrocarbons" (IPCS, 1998). The physical and chemical properties are largely determined by the conjugated alpha-electron systems, which vary fairly regularly with the number of rings and molecular mass, giving rise to a more or less wide range of values for each parameter within the whole class. At room temperature, all PAHs are solids. The general characteristics common to the class are high melting and boiling points, low vapour pressure and very low solubility in water (lipophilic in nature). PAHs are soluble in many organic solvents. Water solubility decreases with increasing molecular mass (the octanol-water partition coefficient, K_{ow} , increases).

PAHs are chemically stable and very poorly degraded by hydrolysis (Howard et al., 1991). In the presence of light, they are susceptible to oxidation and photodegradation. When they react, they undergo two types of reactions: i.e. electrophilic substitution and addition. As the latter destroys the aromatic character of the benzene ring that is affected, PAHs tend to form derivatives by the former reaction; addition is often followed by elimination, resulting in net substitution.

Photodecomposition in the presence of air and sunlight forms a number of oxidative products, including quinones and endoperoxides. PAHs react with nitrogen oxides and nitric acid to form the nitro derivatives of PAHs; they also react with sulfur oxides and sulfuric acid (in solution) to form sulfinic and sulfonic acids. Little information is available on the reactions and fate of PAHs in foods. Since PAHs are chemically stable compounds, they are assumed to be stable in different food matrices.

Figure 1. Structural formulae of PAHs covered in this monograph

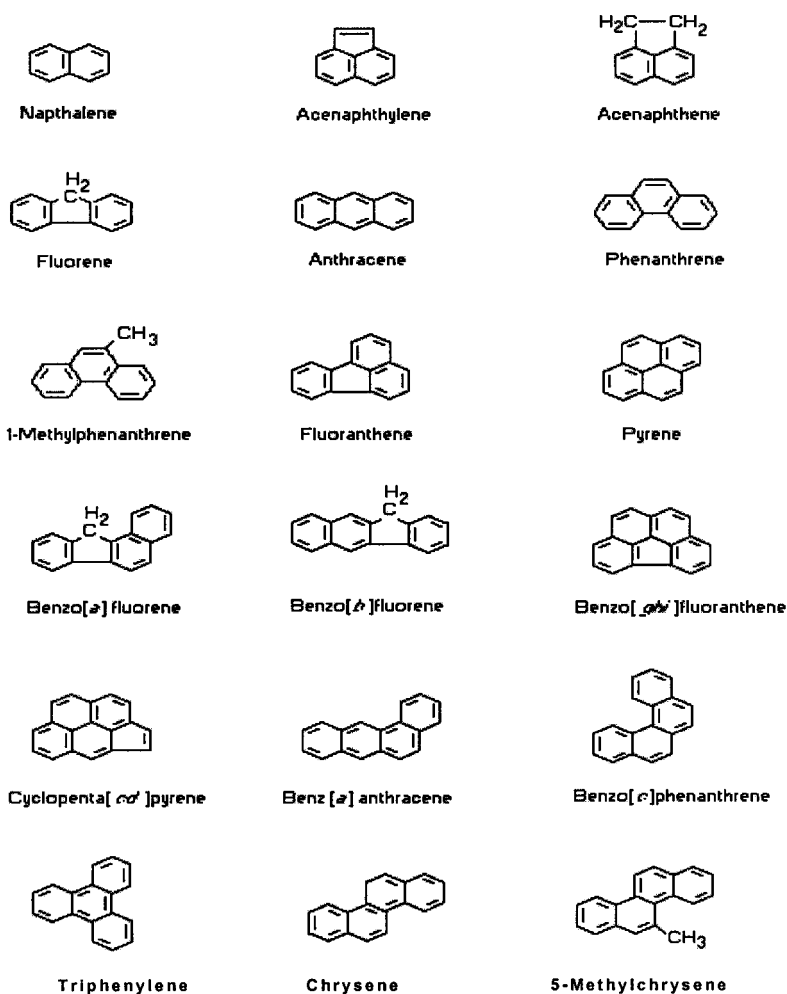
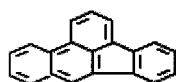
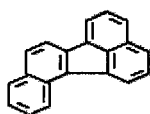
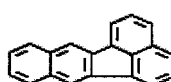
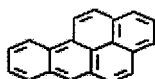
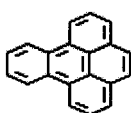
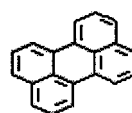
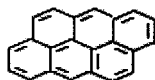
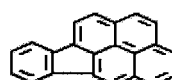
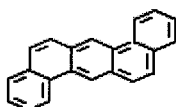
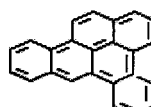
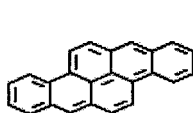
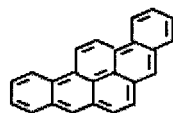
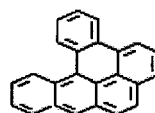


Figure 1. (contd)**Benzo[Δ]fluoranthene****Benzo[γ]fluoranthene****Benzo[Λ]fluoranthene****Benzo[a]pyrene****Benzo[e]pyrene****Perylene****Anthanthrene****Benzo[ghi]perylene****Indeno[1,2,3- cd]pyrene****Dibenz[a,h]anthracene****Coronene****Dibenz[a,e]pyrene****Dibenz[a,h]pyrene****Dibenz[a,i]pyrene****Dibenz[a,j]pyrene**

3.2 Description of analytical methods

3.2.1 Introduction

High-performance liquid chromatography (HPLC) with fluorescence detection has been widely used for the determination of PAHs in foods. Gas chromatography (GC) coupled with mass spectrometry (MS) has also been used. GC with

flame ionization detection (FID) was used by a few researchers. Each method has its relative advantages and disadvantages. Critical reviews have been published on the analytical methods for the determination of PAHs in meat and meat products (Chen et al., 1996; Simko, 2002), edible fats and oils (Moret & Conte, 2000) and marine matrices (de Boer & Law, 2003). HPLC and GC-MS methods for the determination of PAHs in fish have been reviewed (Stolyhwo & Sikorski, 2005). Thin-layer chromatography (TLC), GC, GC-MS and HPLC methods for the determination of PAHs in smoked meat and fish products and smoke flavouring food additives have been exhaustively reviewed (Simko, 2002). The analysis, formation and inhibition of PAHs in foods are reviewed in Chen & Lin (1997). Analytical methods for the determination of PAHs in natural waters are reviewed in Manoli & Samara (1999). The effectiveness of the steps of a general method (which include alkaline treatment of the sample, extraction, cleanup and analysis) for the determination of PAHs by GC-MS in liquid smoke flavourings and smoked foods has been investigated (Guillen et al., 2000).

3.2.2 Screening methods

The literature on PAH screening methods is rather limited. A supercritical fluid extraction system coupled on-line with a fluorimetric detector has been developed for the screening of PAHs in vegetable oils (Zougagh et al., 2004). PAHs are selectively extracted by using silica gel in a thimble and cleaned up by passage through an on-line C18 column. The column is purged to remove carbon dioxide, and the desorbed PAHs are detected by a fluorescence detector. This method allows for the screening of a large number of samples. PAHs in positive samples are further separated by HPLC and quantified using a fluorescence detector.

3.2.3 Quantitative methods

(a) Extraction and cleanup of PAHs in foods

The extraction and cleanup techniques for the determination of PAHs vary with the type of food matrix. PAHs from meat, fish and their products, total diet samples and smoke flavouring food additives are extracted by saponification of the sample with methanolic potassium hydroxide, liquid-liquid extraction into non-polar solvents, partitioning into solvents such as dimethyl formamide or dimethyl sulfoxide-water followed by re-extraction into solvents such as cyclohexane (Dennis et al., 1983; Joe et al., 1984; de Vos et al., 1990; Perfetti et al., 1992; Simko, 2002). Vegetable oils and animal fats are diluted with non-polar solvents, and PAHs are partitioned into acetonitrile, dimethyl formamide or dimethyl sulfoxide (Troche et al., 2000; Barranco et al., 2003; Diletti et al., 2005). PAHs in cereals and plantation products such as coffee, tea and instant coffee are extracted with non-polar solvents either by blending or by ultrasonication (Kaupp & Sklorz, 1996; Garcia-Falcon et al., 2005; Lin et al., 2005). Coffee brew and tea infusion are diluted with methanol and preconcentrated and cleaned up on C18 cartridges (Kayali-Sayadi et al., 1998, 1999). PAHs in leafy vegetables such as lettuce are extracted with toluene (Dugay et al., 2002). Supercritical fluid extraction using liquid carbon dioxide has also been proposed for the selective extraction of low-

molecular-mass PAHs in smoked fish (Ali & Cole, 2002). Supercritical fluid extraction followed by HPLC was found suitable for the determination of PAHs in smoked and broiled fish (Järvenpää et al., 1996). Selective extraction of PAHs from sample extracts with caffeine and formic acid has also been proposed (Simko, 2002). The feasibility of solid-phase extraction and solid-phase micro-extraction techniques for the determination of PAHs by HPLC and GC methods in drinking-water samples has been evaluated, and solid-phase extraction was reported to give better performance (Garcia-Falcon et al., 2004). Solid-phase microextraction using polymethylsiloxane fibres has been found to be useful for the GC determination of PAHs in water samples (King et al., 2004). Solid-phase extraction cleanup conditions, using Sep-Pak C 18 cartridge, for PAHs in water were studied (Kiss et al., 1996).

Sample extracts are cleaned by column chromatography (Florisil, alumina, silica gel and mixed stationary phases). Solid-phase extraction cleanup using columns (C18, Florisil, Oasis LHB, CN bonded silica, Kisel gel) has been widely used. Other cleanup techniques include gel permeation chromatography on columns (SX-3 Bio beads, Sephadex LH 20 and styrene/divinylbenzene copolymer). The efficiency of alumina and donor-acceptor complex chromatography cleanup techniques has been evaluated, and alumina has been found to give better recoveries (Barranco et al., 2004).

(b) GC and GC-MS methods

GC and GC-MS methods for the determination of PAHs in different food matrices are reviewed by Simko (2002). GC-MS methods (Lee et al., 1981; Olufsen & Bjorseth, 1983; Bartle, 1985; Hites, 1989; Kaupp & Sklorz, 1996; Mottier et al., 2000; Ali & Cole, 2002; Dugay et al., 2002; King et al., 2004; Diletti et al., 2005) and GC-FID (Grimmer & Böhnke, 1975, 1979a, 1979b; Larsson, 1982) have been used for the determination of PAHs in various food matrices. MS methods have gained importance for the determination of PAHs, as they provide independent confirmation of compounds. Labelled PAH standards have been used as internal standards in most of the GC-MS methods.

Excellent separation is obtained by the use of commercially available fused silica capillary columns, making it possible to analyse very complex mixtures containing more than 100 PAHs. The most widely used stationary phase is 5% phenyl methyl silicone (DB-5, DB-5 MS, HP-5, HP-5 MS, RTX-5 MS). Polymethylsiloxanes SE-54 (5% phenyl-, 1% vinyl-substituted), SE-52 (5% phenyl-substituted), SE-30, OV-1, OV-101 (unsubstituted), OV-7, OV-17 (50% phenyl-substituted) and Dexsil 300 (carborane-substituted) are also used. Column lengths and inner diameter vary between 25 and 50 m and 0.20 and 0.32 mm, respectively. Chemically bonded and cross-linked phases are increasingly used, as they withstand higher temperatures (little "bleeding" at about 300 °C) and can also be rinsed to restore column performance; also, higher column temperatures are required for determining high-boiling-point compounds. The suitability of GC columns for the analysis of high-molecular-mass PAHs was studied by Bemgard et al. (1993).

On-column injection is preferred, as it allows better sensitivity and reproducibility. GC-FID has been used by some researchers because of its excellent linearity, sensitivity and reliability of response. Extensive sample cleanup is required while using FID to avoid incorrect identification of the analytes. Confirmation of compounds needs to be achieved by analysing the sample on a different GC column or by an independent technique, such as HPLC. On-line coupling of liquid chromatography (LC) and capillary GC quadrupole MS has been used to determine PAHs in vegetable oils (Vreuls et al., 1991).

Nematic liquid crystal phases (Bartle, 1985) have also been used to separate some isomeric compounds that are poorly resolved by siloxane phases, such as chrysene and triphenylene on *N,N'*-bis(*para*-methoxybenzylidene)-*a,a'*-bi-*para*-toluidine (Janini et al., 1975) and *N,N'*-bis(*para*-phenylbenzylidene)-*a,a'*-bi-*para*-toluidine (Janini et al., 1976).

(c) HPLC and LC-MS methods

HPLC methods for the determination of PAHs in meat, meat products and smoke flavouring additives (Simko, 2002) and edible oils and fats (Moret & Conte, 2000) have been reviewed. Reversed-phase HPLC columns (Vydac ODS, C18 Vydac 201 TP, Vydac TP 54, Envirosep-pp-C18, Lichrosphere 100 RP-18, Lichrosorb RP 18, Symmetry 5 C18, Partisil 10 ODS, C18 Spheri 5, Separon SGX C18) with lengths ranging from 15 to 30 cm, inner diameter of 3.0–4.6 mm and 5 or 10 μm particle size are found suitable for their separation. A mobile phase consisting of mixtures of acetonitrile and water/methanol and water, used in the gradient elution, has been found to resolve the analytes (Garcia-Falcon et al., 1999, 2005; Kayali-Sayadi et al., 1999; Troche et al., 2000; Kishikawa et al., 2003; Barranco et al., 2004; Lin et al., 2005; Yusty & Davina, 2005). Tailor-made PAH columns (Supelcosil LC-PAH, Chrompack PAH, Nucleosil 5 C₁₀ PAH, Wakosil-PAHs) have also been developed by different column manufacturers. Baseline separation of isomers, such as the pairs of chrysene–triphenylene and benzo[*b*]fluoranthene–benzo[*k*]fluoranthene, is achieved owing to the selectivity of reversed-phase packing materials (Wise et al., 1993).

Use of wavelength-programmed fluorescence detectors has paved the way for the selective determination of PAHs with adequate detection limits. Enrichment of detection limits of compounds having low fluorescence intensity (naphthalene, acenaphthene and acenaphthylene) has been achieved through a combination of ultraviolet (UV) or photo-diode array detector in conjunction with a fluorescence detector (Dong & Greenberg, 1988; Kicinski et al., 1989; Rivera et al., 1996; Lin et al., 2005). Pyridinium chloride was used as a selective PAH fluorescence quenching agent, which substantially decreases the fluorescence signal of selected PAHs and facilitates their detection in complex chromatograms (Mao & Tucker, 2002). Electrospray ionization LC-MS with silver nitrate as post-column reagent has been used for the analysis of PAHs in water (Takino et al., 2001). HPLC with amperometric detection has been studied for the determination of PAHs in water (Nirmaier et al., 1996). Strategies for the extraction of PAHs from drinking-water are compared in Garcia-Falcon et al. (2004).

(d) *Other methods*

TLC has been used in the past for identifying individual compounds, such as benzo[a]pyrene (IUPAC, 1987). It is an inexpensive, quick analytical technique, but has low separation efficiency. The separation efficiency has been improved by a two-dimensional process (Borneff & Kunte, 1979). Quantification is carried out by spectrophotometric or spectrofluorimetric methods in solution after scrubbing the substance spot and extraction (Fazio, 1990) or in situ by scanning spectrofluorimetry (Borneff & Kunte, 1979). The TLC-spectrofluorimetry method was compared with the HPLC method for the determination of benzo[a]pyrene, which was found in high correlation with both benzo[a]pyrene and sum of carcinogenic PAHs measured by the HPLC technique (Kazerouni et al., 2001). Acetylated cellulose has been used most widely for one-step separation of PAH fractions, and mixed aluminium oxide and acetylated cellulose have been used for two-dimensional development (Daisey, 1983).

A spectrofluorimetric method developed for the determination of benzo[a]pyrene, chrysene and fluorene alone and in mixtures in water was not found adequate for the determination of mixtures of arbitrary composition (Miller, 1999).

Infrared analysis, particularly Fourier transform infrared spectroscopy coupled to GC (Stout & Mamantov, 1989), and capillary supercritical fluid chromatography (Wright & Smith, 1989) have also been used for the analysis of PAHs.

A commercially available enzyme-linked immunosorbent assay kit for carcinogenic PAHs has been evaluated for the determination of PAHs in river water (Barcelo et al., 1998).

(e) *Quality assurance in analysis*

Extraction, separation and detection methods for PAH are evaluated in Chen et al. (1996). Method performance characteristics for selected PAHs in different matrices (fish, meat, oils) were developed by various authors. Fifty-nine laboratories from 36 countries have participated in a recent interlaboratory collaborative study for the determination of organochlorinated compounds and petroleum hydrocarbons, including PAHs, in fish homogenate (IAEA 406). The results indicated that laboratories were underperforming by about 50% for PAH analysis (Villeneuve et al., 2004).

The detection limits of PAHs are method- and matrix-dependent. Detection limits of both HPLC and GC-MS methods are adequate to detect the concentrations normally encountered in foods. The recovery of PAHs in different food matrices varies. The recovery in most matrices, analysed by either GC-MS or HPLC methods, is usually greater than 70%, except for a few compounds (e.g. benzo[ghi]perylene), for which recoveries may be as low as 50%. Solid-phase extraction cleanup on reversed-phase cartridges gives better recoveries for high-molecular-mass PAHs.

Several certified reference materials (CRMs) are used in the method validation studies for PAHs, including 1) coconut oil (BCR, CRM 459: blank

coconut oil for background values, BCR, CRM 458: coconut oil artificially fortified with six selected PAHs); 2) mussel (IAEA 140 & 142, NIST 1974a, 2974, 2977, 2978) and c) dry fish (NRC CARP-2). Current proficiency testing programmes cover a few matrices for selected PAHs only.

(f) General conclusions on analytical methods

HPLC with fluorescence detection has been widely used for the determination of PAHs in several food matrices. Use of UV or photo-diode array detector in conjunction with a fluorescence detector has improved the detection limits of compounds having low fluorescence intensity. Also, a photo-diode array detector provides additional information on peak identity as well as peak purity. GC-MS methods have the added advantage of independent confirmation of compounds through their mass spectra as well as the use of labelled compounds as internal standards. Confirmation of compounds is achieved by some researchers by analysis on a different column when GC is used. Confirmation of compounds analysed by HPLC is achieved through GC-MS analysis by some researchers. Both HPLC and GC-MS methods are adequate for their determination in foods.

PAHs may be lost if samples are stored for prolonged periods prior to analysis due to reaction with components of the food matrix or degradation when exposed to light and high temperatures (leading to volatilization or chemical conversion). In addition, losses of PAHs may occur during homogenization, extraction and cleanup stages. Care must be taken not to expose samples to high temperatures during homogenization, and all analytical operations should be carried out under subdued light.

PAHs may co-elute during GC and HPLC analysis. For example, under the conditions used in GC analysis, chrysene + triphenylene, benzo[*b+h+k*]fluoranthenes and the dibenz[*a,h* + *a,c*]anthracenes may co-elute and give rise to a single peak. When HPLC is used, the separation of benzo[*b*]fluoranthene + perylene, benzo[*k*]fluoranthene + dibenz[*a,c*]anthracene and benzo[*j*]fluoranthene + benzo[*e*]pyrene may be critical.

Lack of CRMs and proficiency testing programmes covering a wide range of PAHs (especially those that are genotoxic and carcinogenic) in a wide range of food matrices, including high-risk food commodities, has been a limitation in the validation of analytical methods. CRMs developed so far cover selected PAHs in limited matrices only. Current proficiency testing programmes seem to be inadequate, as they cover a few matrices for selected PAHs. Most analytical methods developed so far include the 16 United States Environmental Protection Agency priority pollutant PAHs and do not include most dibenzopyrenes and 5-methylchrysene, which are considered genotoxic and carcinogenic. Development of analytical methods including these compounds is required. Occurrence data on PAHs in foods reported in some publications lack in quality control and quality assurance information (limit of detection, limit of quantification, precision, accuracy, spike and recovery, analysis of CRMs), making it difficult to judge the quality of the reported data.

4. SAMPLING PROTOCOLS

Since PAHs are chemically stable compounds, they are assumed to be stable in different food matrices, although PAHs deposited on the surface of crops may undergo degradation, and some PAHs may react with components of the food matrix during prolonged storage. Sampling protocols in the European Union are detailed by the Scientific Cooperation on Food (SCOOP) task force 3.2.12 on the occurrence of PAHs (SCOOP, 2004). Sampling protocols are available for total diet studies in the United Kingdom. Several researchers followed either routine sampling or sampling based on suspicion (oils and fats, industrial pollution, oil spills, etc.).

5. ROUTES OF PAH CONTAMINATION IN FOOD

The occurrence and determination of PAHs have been reviewed (Howard & Fazio, 1980; Stall & Eisenbrand, 1988; Guillen et al., 1997; IPCS, 1998; Phillips, 1999; Camargo & Toledo, 2002c, 2003; Simko, 2002). There are two main routes of entry of PAHs into the food-chain. Food can be contaminated by environmental PAHs that are present in air (by deposition), soil (by transfer) and water (by deposition and transfer). PAHs also form directly during processing (drying and smoking) or cooking (grilling/barbecuing, roasting, frying, etc.) of food. The third, but minor, route of contamination includes the use of contaminated smoke flavouring additives and migration from contaminated packaging materials.

5.1 Environmental contamination

The contamination of food by environmental PAHs depends on a number of physical and chemical properties, such as their relative solubility in water and organic solvents, volatility, chemical reactivity and biotic and abiotic degradability. PAHs are lipophilic compounds with poor water solubility. PAHs with two or three rings are almost entirely in the vapour phase, whereas those with four rings exist in the vapour phase as well as in association with particulate matter. In the atmosphere, PAHs containing five or more aromatic rings are found predominantly in association with particulates, usually on small (<2.5 µm) particles such as fly ash and soot. The association of the heavier PAHs with particulate matter makes atmospheric fallout a principal route of contamination on crops (Edwards, 1983; Nielsen et al., 1996). Consequently, vegetables with large leaves, grazing cattle and pecking poultry, which may ingest particulate matter from contaminated grass and soil, are susceptible to contamination by PAHs adsorbed to particles. The waxy surface of vegetables and fruits can concentrate low-molecular-mass PAHs mainly through surface adsorption. Since PAHs strongly adhere to the organic fraction of soil, they do not penetrate deeply in soils (other than sandy soils); therefore, leaching to groundwater and uptake by root vegetables are low.

When PAH-containing particulates fall out into surface water, they are transported in suspension and finally may end up in the freshwater or marine sediments to which they are strongly bound. Fish and marine invertebrates may naturally contain small amounts of PAHs absorbed from the environment, as seawater is

contaminated with PAHs due to oil spills and atmospheric pollution (Jones et al., 2001; Storelli & Marcotrigiano, 2001; EC, 2002; Mostafa, 2002; O'Connor, 2002). Fish, in contrast to bivalves, have the ability to further metabolize PAHs to water-soluble compounds (Mottier et al., 2000; Stolyhwo & Sikorski, 2005); hence, the levels of PAHs in fish are lower than levels in molluscs. Filter-feeding bivalves such as mussels and oysters may accumulate particles contaminated by high-molecular-mass PAHs because they filter large volumes of water and are not capable of efficiently metabolizing all PAHs. The contamination of sediment-dwelling organisms may potentially cause contamination higher up in the aquatic food-chain. However, because most of these organisms have a high biotransformation potential for PAHs, significant biomagnification has not been reported in aquatic systems. Background levels of some PAHs in fish and molluscs from unpolluted as well as polluted waters (from industrial pollution, oil spills, etc.) are reported by IPCS (1998).

In the neighbourhood of industrial areas or along highways, the contamination of crops (vegetables, especially leafy vegetables, fruits and other agricultural produce) by PAHs can be 10-fold higher than in rural areas. In areas remote from urban or industrial activities, the levels of PAHs found in unprocessed foods reflect the background contamination, which originates from long-distance airborne transportation of contaminated particles and natural emissions from volcanoes and forest fires. Background levels of PAHs in some vegetation grown along the roadside, in the vicinity of industrial areas as well as in deeper locations (unpolluted areas) are summarized in IPCS (1998).

5.2 Contamination during processing and cooking of food

Processing of foods (such as drying and smoking) and cooking (grilling/barbecuing and roasting) are major sources of PAHs (Guillen et al., 1997; Phillips, 1999). The amount of PAHs formed during roasting, baking and frying depends markedly on the cooking conditions (IPCS, 1998; EC, 2002).

5.2.1 Grilling

Although not precisely known, it is likely that there are several mechanisms of formation of PAHs, such as melted fat that undergoes pyrolysis when dripping onto the heat source (hot coals) during grilling and pyrolysis of the food due to high temperatures (above 200 °C) (Lijinsky & Shubik, 1965a, 1965b; Larsson et al., 1983; IPCS, 1998; EC, 2002). PAH formation during charcoal grilling was shown to be dependent upon the fat content of the meat, distance of the food from the heat source, the duration of cooking and the temperature used. Mean PAH levels in charcoal barbecued meat cooked at 15 cm from the heat source were 3- to 10-fold higher than levels in meat cooked at 40 cm from the heat source (Noll & Toledo, 1995). In another study, the highest concentration of benzo[a]pyrene (130 µg/kg) in cooked meat was found in fatty beef, and the concentration appeared to be proportional to the fat content (Doremire et al., 1979). A heavily barbecued lamb sausage contained 14 µg/kg of the sum of six PAHs considered by IARC to be carcinogenic (Mottier et al., 2000). A study in duck breast steaks undergoing

various processing and cooking treatments for 0.5–1.5 h showed that charcoal grilling samples without skin resulted in the highest concentration of total PAHs (320 µg/kg) in the meat, followed by charcoal grilling with skin (300 µg/kg), smoking (210 µg/kg), roasting (130 µg/kg) and steaming (8.6 µg/kg). In addition, the highest amounts of total and carcinogenic PAHs were observed after smoking of duck breast samples for 3 h (Chen & Lin, 1997).

The presence of PAHs was studied in several samples of meat and fish that were grilled on two geometrically different gas barbecues. In contrast to the horizontal barbecue, the vertical barbecue prevented fat from dripping onto the heat source, and the PAH levels were 10–30 times lower in the vertical barbecue system than in the horizontal one (Saint-Aubert et al., 1992).

5.2.2 Smoking

Smoking technology uses mainly various sensory active components contained in smoke for aromatization of meat, fish and their products. Smoke is generated by thermal pyrolysis of wood under the conditions of limited oxygen. The concentration of PAHs in smoke formed during pyrolysis increases linearly with the smoke temperature within the interval 400–1000 °C (EC, 2002). Direct exposure of meat products to smoke brings about higher concentrations of PAHs as compared with indirect methods, when PAHs are partially eliminated by condensation in tars. Also, hot smoking, used for treating a major part of meat production, brings about higher concentrations of PAHs than cold smoking, used for fermented, thermally non-processed meat products. Heavy or “wild” smoking increases the PAH concentration to high levels. In some types of products, it is possible to decrease the concentration of PAHs by cooking. The highest concentration of PAHs in smoked products is found immediately after finishing the smoking and then decreases due to light decomposition and interaction with compounds present in the product. However, PAHs also penetrate into smoked products, where they are protected from light and oxygen; after some time, the concentration stabilizes at a certain constant level (Simko, 2002).

Data reported in the literature on PAHs in smoked foods are highly variable. The main reason for such discrepancies is the differences in the procedures used for smoking. Such variables include the type and composition of wood, type of generator (internal or external), oxygen accessibility, temperature of smoke generation and smoking time. The PAH content in smoked fishery products from modern smoking kilns with external smoke generation and procedures that remove high-boiling compounds such as PAHs and particles potentially containing PAHs has been compared with the PAH content in products from traditional smoking kilns where the smoke is generated in direct contact with the product. The average benzo[a]pyrene concentration determined was 1.2 µg/kg for the traditional kilns and 0.1 µg/kg for the modern kilns (Karl & Leinemann, 1996).

5.2.3 Drying and roasting

Contamination of cereals, oil seeds/oils and plantation products (coffee, tea) by PAHs usually occurs during technological processes such as direct fire drying,

where combustion gases may come into contact with the products (Speer et al., 1990; IPCS, 1998; EC, 2002). Vegetable oils and fats are a significant source of PAHs in the diet, either directly, as in the case of some vegetable oils used for seasoning and margarine used for cooking, or indirectly, by their incorporation into other foods, such as cereal-based products, biscuits and cakes (Dennis et al., 1991; Swetman et al., 1999).

Also, the practice of firing the field after harvesting may be a suspect operation for PAH contamination (EC, 2002). Analyses of rapeseed samples, purified in the laboratory by rinsing seeds with organic solvent in an ultrasonic bath, revealed that solid particles, which contaminate rapeseeds during harvesting, transport and storage, contributed to PAH contamination to the extent of 36% (light PAHs) to 64% (heavy PAHs) on average. Roasting and drying of coffee beans and tea leaves may result in PAH contamination (Stall & Eisenbrand, 1988). A Finnish study showed that roasted ground coffee and dried tea leaves contained high levels of PAHs, namely 100–200 µg/kg and 480–1400 µg/kg, respectively. The concentration of PAHs in the coffee brew was only a few nanograms per litre (Kayali-Sayadi et al., 1999).

5.3 Other minor routes of contamination

A minor route of PAH contamination may include the use of smoke flavouring additives, which may contain PAHs (Simko, 2002). PAHs may also migrate from recycled polyethylene film used for packaging into oils through diffusion. A different form of contamination may occur through contact with mineral oil residues (rich in naturally occurring alkylated PAHs) that have been generated by geochemical processes. For example, the practice of storing and transporting oil seeds in jute bags treated with mineral oils (before spinning of jute fibres) can cause the migration of PAHs into the food. Traces of lubricating oils used in the maintenance of extraction plants can be occasionally found in vegetable oils (Moret & Conte, 2000).

6. LEVELS AND PATTERNS OF CONTAMINATION OF FOOD COMMODITIES

6.1 Surveillance data on PAHs in foods

Data on the occurrence of PAHs in foods are not available in the Global Environment Monitoring System Food Contamination Monitoring and Assessment Programme (GEMS/Food) format. However, SCOOP task force 3.2.12 has provided comprehensive occurrence data in the European Union on a total of 8861 samples in 45 food groups collected during the period from 1992 until 2003. Five food groups accounted for more than 80% of the samples. Twenty-two parent PAHs and nine substituted PAHs were included for collection of occurrence data (SCOOP, 2004). Data on the occurrence of PAHs in selected foods were also reported by the Scientific Committee on Foods (EC, 2002). PAH levels in various food groups/total diet are reported from the United Kingdom (COT, 2002), the Netherlands (de Vos et al., 1990) and Italy (Lodovici et al., 1995). A food database

for nitosamines, heterocyclic amines and PAHs from 23 different countries was developed, which contains 317 food items with information on PAH levels (Jakszyn et al., 2004).

In the past, benzo[a]pyrene was the most common PAH determined in foods and was used as an indicator of the presence of PAHs (Tilgner, 1968); the earliest measurement dates to 1954 (Lo & Sandi, 1978; Howard & Fazio, 1980). Benzo[a]pyrene, dibenz[a,h]anthracene and total PAH levels in several food groups are summarized by the European Prospective Investigation of Cancer (EPIC, 2002).

Data from the IPCS (1998) and EC (2002) reports and available data from the literature are summarized in this monograph. The levels of individual PAHs in major food groups — namely, meat and meat products, fish and seafood, cereal and cereal products, oils and fats, dairy products, fruits and vegetables and beverages — are summarized in Tables 10–17 below.

6.1.1 Meat and meat products

The concentrations of individual PAHs found in meat are shown in Table 10. In meat, poultry and fish in Canada, benzo[k]fluoranthene was detected at concentrations up to 0.30 µg/kg and benzo[a]pyrene up to 1.1 µg/kg (Environment Canada, 1994). Benzo[a]pyrene was found in some German meat products in 1994 at concentrations generally below 1 µg/kg. The highest concentration, 9.2 µg/kg, was found in a Black Forest ham (State Chemical Analysis Institute, 1995). The concentration of benzo[a]pyrene ranged from 0.03 to 100 µg/kg in meat products and from 0.04 to 337 µg/kg in smoke flavouring additives (Simko, 2002). PAH levels in grilled meat products were reviewed by Fretheim (1983).

Results of surveillance data on 12 different PAHs in food samples from Kuwait revealed that non-carcinogenic PAHs were detected in considerable amounts in commodities such as liver and kidney of sheep and goat as well as hen eggs. Among the carcinogenic PAHs detected, chrysene was present in the highest levels. The average content of benzo[a]pyrene in 190 samples of hen eggs was reported to be 7.49 µg/kg (Husain et al., 1997). In a study by Gomaa et al. (1993), the total PAH concentration in smoked meat products ranged from 2.6 µg/kg in a cooked ham sample to 29.8 µg/kg in grilled pork chops. The levels of carcinogenic PAHs ranged from non-detectable in several meat products to 7.4 µg/kg in grilled pork chops.

Smoked and barbecued food in particular can contain PAHs (McGill et al., 1982; Joe et al., 1984; de Vos et al., 1990; Menichini et al., 1991). A study of PAHs in charbroiled and fried hamburgers from four sources indicated that charbroiled products contained higher levels of PAHs compared with fried hamburgers (Lawrence & Weber, 1984a). Benzo[a]pyrene levels in 200 selected food items were about 4 µg/kg in grilled/barbecued steaks, hamburgers and chicken with skin. Benzo[a]pyrene levels were lower in meats grilled/barbecued to medium done and in all broiled or pan-fried meat samples regardless of doneness level (Kazerouni et al., 2001). Benzo[a]pyrene levels in Spanish commercial smoked products (5 samples of meat products, 3 samples of cheese and 22 samples of

Table 10. (contd)

Compound	PAH concentration (µg/kg)											
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]
Perylene											ND-3	ND
Phenanthrene		3.0									22-64	10-16
Pyrene										0.55	38-63	5-7

Table 10. PAH concentrations in meat and meat products (raw, grilled, barbecued meat products, smoked meat and poultry, eggs, smoke flavourings and seasonings) (contd)

Compound	PAH concentration (µg/kg)											
	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[22]	[23]	
Acenaphthene											0.05	
Acenaphthylene	ND–500										0.33	
Anthanthrene	ND–66.5											
Anthracene	0.5–133		ND–6.4	29.7		ND–0.5			0.1–6.8		0.18	
Benz[a]anthracene	0.2–144	0.3–2.1	ND– 4.0	4.46	0.03	ND–31.8	0.13–2.22	0.50–0.53	ND–3.7	ND–0.61	0.41	
Benzo[a]fluorene ^a	ND–174											
Benzo[b]fluorene	ND–71.9											
Benzo[b]fluoranthene ^b	ND–92.3	0.1–0.9	ND–1.1	3.49	0.435	ND–1.8	0.01–0.66	0.40–1.20	ND–1.9	0.14–0.41	0.27	
Benzo[k]fluoranthene	ND–172		ND–5.0	4.53	0.012	ND–1.6	0.02–0.14	0.08–0.61	0.2–1.6	ND–0.14	0.09	

Table 10. (contd)

Compound	PAH concentration (µg/kg)										
	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[22]	[23]
Benzo[ghi]perylene	ND-153	0.1-0.3	ND-1.1	1.25	ND	ND	ND-0.01	0.01-0.06	ND-1.6	ND-2.14	0.15
Benzo[a]pyrene	ND-212	0.1-1.0	ND-2.5		0.015	ND-4.6	0.02-0.61	0.10-0.12	ND-3.2	0.10-0.44	0.10
Benzo[e]pyrene	ND-80.9	0.2-2.3		7.49						ND-1.89	
Chrysene ^c	0.3-140		ND-2.0	5.57	ND	ND- 8.5	ND-1.18	2.36-24.7	0.2-4.9	ND	0.84
Dibenz[a,h]anthracene ^d	ND-8.8	0.1-0.4	ND-2.0	4.75		ND			ND-3.3	ND-0.69	0.04
Fluoranthene	1.1-376	0.2-1.3	ND-4.6	1.19	0.099	ND-25.3	0.55-2.68	1.85-10.8	0.8-7.1	0.79-5.47	1.92
Fluorene						ND					0.67
Indeno[1,2,3-cd]pyrene	ND-171	0.1-0.4	ND-0.9	8.73		ND			ND-2.3		0.04
1-Methylphenanthrene	0.5-57.6										
Naphthalene						0.9-55.2					1.28
Perylene	ND-27.9										
Phenanthrene	3.5-618	0.5-1.9	0.4-6.1	18.5		ND-3.9			1.0-6.9		4.64
Pyrene	1.2-452	0.1-1.7	0.3-3.8	5.55	ND	ND-0.4	ND-5.38	1.27-5.29	1.3-17.9	ND	2.44

ND, not detected; Tr, traces

^a Reported including benzo[b]fluorene in some publications.^b Reported in some publications as sum of benzo[j+b+k]fluoranthenes.^c Reported including triphenylene in some publications.^d Reported including dibenz[a,c]anthracene in some publications.

Table 10. (contd)

- [1] Poultry and eggs, Netherlands (de Vos et al., 1990)
- [2] Meat and meat products, Netherlands (de Vos et al., 1990)
- [3] Smoked beef, Netherlands (de Vos et al., 1990)
- [4] Unsmoked beef, Netherlands (de Vos et al., 1990)
- [5] Bacon, United Kingdom (Crosby et al., 1981)
- [6] Smoked meat, United Kingdom (McGill et al., 1982)
- [7] Unsmoked meat, United Kingdom (McGill et al., 1982; Maga, 1986)
- [8] Smoked sausages, United Kingdom (McGill et al., 1982)
- [9] Unsmoked sausages, United Kingdom (McGill et al., 1982)
- [10] Meat, United Kingdom (Dennis et al., 1983)
- [11] Mesquite wood-cooked patties (70–90% lean), USA (Maga, 1986)
- [12] Hardwood charcoal-cooked patties (70–90% lean), USA (Maga, 1986)
- [13] Grilled sausages, Sweden (Larsson et al., 1983)
- [14] Smoked poultry, pork and beef products, sausages and frankfurters, USA (Joe et al., 1984)
- [15] Smoked poultry, beef and pork products, Spain (Gomaa et al., 1993)
- [16] Mean value of 190 samples of hen eggs, Kuwait (Husain et al., 1997)
- [17] Eggs, triplicate samples from different brands homogenized, Italy (Lodovici et al., 1995)
- [18] Pork (stewed, smoked), chicken (breast, wing, liver, heart — all stewed), grilled chicken and grilled duck, China (Province of Taiwan) (Chen et al., 1996)
- [19] Beef, pork, chicken, rabbit, cured meats, Italy, triplicate samples from different brands homogenized, values in selected meat products are shown as range (Lodovici et al., 1995)
- [20] Barbecued beef and pork, triplicate samples from different brands homogenized, values in selected meat products are shown as range, Italy (Lodovici et al., 1995)
- [21] Liquid smoke flavourings and seasonings, Spain (Gomaa et al., 1993)
- [22] Smoked meat products, three samples each of bacon, chicken, sausages, mortadella, range of mean values, Brazil (Camargo & Toledo, 2002b)
- [23] Meat and meat products, 30 samples, mean values, Spain (Falco et al., 2003)

fish) were found to be below 1.0 µg/kg in most samples (Garcia-Falcon et al., 1999). In another study, the mean benzo[a]pyrene concentration in 15 smoked sausage samples was found to be 0.022 µg/kg. Except in two samples (treated with wood smoke), the benzo[a]pyrene concentration in all samples was below the 0.03 µg/kg limit set in European Union legislation (Garcia-Falcon et al., 1999).

6.1.2 Fish and other marine foods

The levels of individual PAHs in fish and other marine foods are summarized in Table 11. The PAH levels in mussels and oysters collected from the coastal sites of France and the United States were compared under the monitoring programmes conducted by France and the United States, which showed striking similarities, with higher concentrations for high-molecular-mass PAHs (Beliaeff et al., 2002). A benzo[a]pyrene concentration ranging from not detected to 18 µg/kg in smoked fish was found. The differences were probably due to factors such as the type of smoke generator, the temperature of combustion and the degree of smoking (Draudt, 1963). The highest concentration of benzo[a]pyrene (130 µg/kg) in seafood was found in mussels from the Bay of Naples (Bourcart & Mallet, 1965), and a level of about 60 µg/kg was detected in smoked eel skin. Most of the fish analysed contained 0.1–1.5 µg/kg (Steinig, 1976). Benzo[a]pyrene was also detected at levels up to 3.3 µg/kg in 21 samples of smoked fish, oysters and mussels of various origins (Prinsen & Kennedy, 1977). A total concentration of three PAHs (benz[a]anthracene, chrysene and benzo[b]fluoranthene) ranged from not detected to 44.9 µg/kg in five species of fish from three sites in the Arabian Gulf (Iman & Inaam, 2002). In another study, high levels of PAHs were found in canned smoked mussels and oysters in oil; higher levels were found in the oil fraction (analysed separately) than in the meat (Lawrence & Weber, 1984a). High levels of PAHs were also found in canned oysters (Joe et al., 1984).

PAH concentrations in fish, bivalves (mussels, oysters, clams, scallops, limpets, etc.), crustaceans (lobsters) and other aquatic species in waters polluted by industrial emissions as well as background levels (collected from unpolluted waters) for these aquatic species are summarized in IPCS (1998). High levels of PAHs are reported in catfish (Vassilaros et al., 1982), English sole (Malins et al., 1985) and whole holothurians (Milano et al., 1986). Similarly high values were reported in mussels (Sirota & Uthe, 1981), snails (Rostad & Pereira, 1987) and lobsters (Sirota et al., 1983; Uthe & Musial, 1986). The background levels in fish (Black et al., 1981; Vassilaros et al., 1982; Milano et al., 1986; Rainio et al., 1986; DouAbdul et al., 1987), mussels (Mix & Schaffer, 1983; Hungspreugs et al., 1984; Takatsuki et al., 1985; Rainio et al., 1986; Boom, 1987; Compaan & Laane, 1992), clams (Mix & Schaffer, 1983; Smith et al., 1984; Takatsuki et al., 1985; Bender & Huggett, 1988), oysters (Kagi et al., 1985; Marcus & Stokes, 1985; McFall et al., 1985; Takatsuki et al., 1985; Bender & Huggett, 1988) and lobsters (Sirota & Uthe, 1981; Sirota et al., 1983; Uthe & Musial, 1986) were low.

In 1989 and 1990, the levels of 19 PAHs and alkylated derivatives of naphthalene were measured in salmon, herring, cod, rockfish and halibut in the area of the Gulf of Alaska where oil spilled from the tanker *Exxon Valdez*. As only the sums of

Table 11. PAH concentrations found in fish and marine foods, including grilled, barbecued and smoked products

Compound	PAH concentration (µg/kg)														
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]
Acenaphthene								<2– 5.13	2.22– 22.3						0.11
Acenaphthylene															0.22
Anthracene				0.9	1.3– 64.3	1.4– 49.6		<2– 78.4	ND– 5.88	ND– 0.6	ND– 1.9	<0.05	ND– 4.4		0.11
Benz[a]anthracene	1.3	ND– 11.2	ND– 6.3		ND– 86	Tr– 0.09	0.14	<2	0.14– 5.31	0.8– 3.0	0.8– 20.9		ND– 9.3	0.08– 0.14	0.38
Benzo[b]fluoranthene ^a	2.0	ND– 3.9	ND– 3.6	0.35			0.13		0.13– 5.77	3.0– 12.2	1.2– 24.3		ND– 1.8	0.03– 0.26	0.39
Benzo[k]fluoranthene	0.7	ND– 6.7	ND– 5.1	0.10			0.04					<0.002	ND– 5.0	0.01– 0.03	0.15
Benzo[ghi]perylene	0.9	ND– 2.8	ND– 1.8	4.3	ND– 25	Tr– 0.39	0.12		0.17– 30.9	0.4– 0.8	0.3– 5.7		0.1– 3.3	ND	0.26
Benzo[c]phenanthrene					ND– 15	0.01– 0.09									
Benzo[a]pyrene	1.4	ND– 5.5	ND– 5.4	0.10	ND– 18	Tr– 0.35	0.13	<2– 7.63	ND– 5.33	0.4– 1.0	0.2– 12.2	<0.004		0.01– 0.03	0.24
Benzo[e]pyrene		ND– 2.8	ND– 3.0				0.12		2.4– 6.3	0.7– 7.6			ND– 3.9		
Chrysene ^b	2.9	ND– 13.0	N–9.4				0.65	<2	ND– 15.9	3.2– 8.8	3.9– 30.8	<0.03	0.0– 5.8	ND– 0.91	0.67

Table 11. (contd)

Compound	PAH concentration (µg/kg)														
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]
Dibenz[a,h]-anthracene ^c							0.03		0.21–39.3	0.1–0.2	<0.1–0.5		0.1–1.9		0.08
Fluoranthene	1.8	1.4–79.9	1.7–48.4	2.4			0.1	<2–123.5	ND–32.7	5.1–17.5	4.5–18.7		1.2–15.8	0.02–0.43	0.97
Fluorene								<2–18.5	ND–65.7						0.22
Indeno[1,2,3-cd]pyrene	1.6	ND–7.1	ND–2.4	2.7	ND–37	ND–0.33			0.28–28.6	0.3–0.6	0.2–6.4		ND–1.2		0.03
Naphthalene								<2–67.4	2.06–156						0.93
Perylene		ND–1.2	ND–1.0							0.2–2.7	0.1–3.1	<0.05			
Phenanthrene	3.5	5–330	10.4–277					<2–101	5.84–87.2	2.1–4.2	1.9–19.6		2.1–23.3		2.33
Pyrene		1.3–67.8	2.1–38.4				0.79	<2–145	ND–68.0	3.1–12.4	2.6–11.2	<0.03	3.8–24.1	ND	0.82

ND, not detected; Tr, traces

^a Reported in some publications as sum of benzo[j+b+k]fluoranthenes.^b Reported including triphenylene in some publications.^c Reported including dibenz[a,c]anthracene in some publications.

Table 11. (contd)

- [1] Fish, Netherlands (de Vos et al., 1990)
- [2] Herring, whitefish, mackerel, eel, salmon, salmon trout, various fillets; all smoked; Sweden (Larsson, 1982)
- [3] Fish and fish products: sprats, herring, rainbow trout, caviar, herring paste, salmon paste; all smoked or canned; Sweden (Larsson, 1982)
- [4] Kippers, United Kingdom (Crosby et al., 1981)
- [5] Fish (smoked), United Kingdom (McGill et al., 1982)
- [6] Fish, unsmoked, United Kingdom (McGill et al., 1982)
- [7] Fish, United Kingdom (Dennis et al., 1983)
- [8] Fresh fish from the Arabian Gulf (andag, sheim, gato, sheiry, faskar, chaniedah) after an oil spill (Al-Yakoob et al., 1993)
- [9] Fresh fish and shrimps, Kuwait, after Gulf War (Saeed et al., 1995)
- [10] Fresh oysters, various origins (Speer et al., 1990)
- [11] Canned or smoked oysters and mussels, Germany (Speer et al., 1990)
- [12] Clam, Australia (Smith et al., 1987)
- [13] Smoked fish products — trout, shrimp, herring, salmon, oysters, whitefish and cisco chubs (Gomaa et al., 1993)
- [14] Trout, cod and dried cod, triplicate samples from different brands homogenized, values obtained for selected foods are shown as range, Italy (Lodovici et al., 1995)
- [15] Fish and shellfish, 16 samples, mean values, Spain (Falco et al., 2003)

the concentrations were considered, there was no apparent difference from concentrations in fish samples taken from unpolluted control sites in 1989. In 1990, slightly elevated PAH concentrations were found at the polluted sampling site. Nevertheless, the fish from the area were considered to be safe for human consumption by these investigators (Saxton et al., 1993).

In another special exposure situation, the average daily PAH intake of the inhabitants of Kuwait due to consumption of seafood after the war in the Persian Gulf was calculated to be 0.23 μg on the basis of the concentrations monitored in local fish and shrimps (Saeed et al., 1995).

6.1.3 Vegetables

The levels of PAHs found in vegetables in recent studies are listed in Table 12. In a study in Brazil, the levels of summed PAHs in vegetables (lettuce, tomato, cabbage, potato, carrot, bean and pea) were found to be in the range of 1.26–23.1 $\mu\text{g}/\text{kg}$. The highest levels of PAHs were found in lettuce, followed by tomato and cabbage. However, no PAHs were detected in bean samples (Camargo & Toledo, 2002b). Fluoranthene was the only PAH that was reported to have been found in vegetables (unspecified) in Canada at levels ranging from not detected to 1.8 $\mu\text{g}/\text{kg}$ (Environment Canada, 1994). Kale was found to contain high concentrations of fluoranthene (120 $\mu\text{g}/\text{kg}$), pyrene (70 $\mu\text{g}/\text{kg}$), chrysene (62 $\mu\text{g}/\text{kg}$) and benz[a]-anthracene (15 $\mu\text{g}/\text{kg}$), and PAH concentrations up to 7 $\mu\text{g}/\text{kg}$ were determined in other vegetables (Vaessen et al., 1984).

The differences in PAH content have been attributed to variations in the ratio of surface area:weight, in location (rural or industrialized) and in growing season. Washing (at 20 °C) vegetables contaminated by vehicle exhaust did not reduce the PAH contamination (Grimmer & Hildebrandt, 1965). In a comparative study on PAH levels in vegetables (lettuce, tomato and cabbage) grown near roadways and rural areas, total PAH levels in samples grown near roadways were found to be almost double those in rural areas (Camargo & Toledo, 2003). In another study, higher concentrations of PAHs were detected in vegetables (lettuce) grown close to a highway; the levels of individual PAHs decreased with distance from the road. Washing the vegetables reduced their content of high-molecular-mass PAHs, but not of phenanthrene (Larsson & Sahlberg, 1982). In another study, the profiles of PAHs in lettuce were similar to those in ambient air, indicating that deposition of airborne particles was the main source of contamination (Wickström et al., 1986).

PAH concentrations were determined in fenugreek, spinach, beet, amaranthus, cabbage, onion, lettuce, radish, tomato and wheat grown on soil that had been treated with sewage sludge. The levels of individual PAHs in lettuce leaves were 1–2 orders of magnitude lower than those in the sewage sludge and the soil on which the lettuce was grown (Lenin, 1994).

PAH levels in carrots and beans grown near a German coking plant were below 0.5 $\mu\text{g}/\text{kg}$ wet weight. The levels of fluoranthene were 1.6–1.7 $\mu\text{g}/\text{kg}$, and those of pyrene, 1.0–1.1 $\mu\text{g}/\text{kg}$. Vegetables with large, rough leaf surfaces, such

Table 12. PAH concentrations in vegetables

Compound	PAH concentration (µg/kg)													
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]
Acenaphthene													0.01	
Acenaphthylene													0.07	
Anthracene		0.09– 0.19		<0.1– 0.3									0.02	1.8– 17.1
Benz[a]anthracene	15			0.7– 4.6	0.05– 3.17	0.05– 3.2	0.4	0.3	0.006– 2.39	0.35– 0.68	0.25– 0.42	ND– 0.28	0.04	2.2– 9.34
Benzo[a]fluorene ^a		0.08– 2.6												
Benzo[b]fluorene		0.11– 2.8												
Benzo[b]fluoranthene ^b	4.2	0.05– 1.4	5.6	0.3– 6.2	ND– 1.42	0.05– 3.0		0.2	0.001– 0.45	0.08– 0.12	ND– <0.70			
Benzo[ghi]fluoranthene			6.1	0.5– 7.3		0.9– 3.2	0.2		0.008– 0.92	0.14– 0.47	0.07– 0.22	ND– 0.48	0.03	
Benzo[ghi]perylene		3.7				ND– 11	0.1		0.002– 0.14	0.21– 0.28	<0.07– 0.20	ND– 0.27	0.02	
Benzo[c]phenanthrene	7.7	0.13– 2.1	10	0.5– 10.8	ND– 1.39	3.7– 10	0.1		ND– 0.05	1.75– 3.45	0.45– 1.19	ND– 3.44	0.02	
Benzo[a]pyrene	9.2				0.05– 1.5									
Benzo[e]pyrene												ND– 0.39	0.01	0.98– 7.2

Table 12. (contd)

Compound	PAH concentration (µg/kg)													
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]
Phenanthrene		0.47– 12		1.8– 7.5									0.19	
Pyrene	70	0.9– 18		3.4– 10.4					ND– 0.69	2.53– 3.94	ND– 2.47	ND	0.14	

ND, not detected

^a Reported including benzo[b]fluorene in some publications.

^b Reported in some publications as sum of benzo[*j+b+k*]fluoranthenes.

^c Reported including triphenylene in some publications.

^d Reported including dibenz[*a,c*]anthracene in some publications.

[1] Kale, Netherlands (Vaessen et al., 1984)

[2] Lettuce, Finland (Wickström et al., 1986)

[3] Lettuce, Germany (Table 53 in IPCS, 1998)

[4] Lettuce, Sweden (Larsson & Sahlberg, 1982)

[5] Lettuce and cabbage, United Kingdom (McGill et al., 1982)

[6] Lettuce, India (Lenin, 1994)

[7] Potatoes, Netherlands (de Vos et al., 1990)

[8] Tomatoes, Netherlands (Vaessen et al., 1984)

[9] Cauliflower, lettuce, tomatoes, squash, beet greens and potatoes, triplicate samples are homogenized together, values obtained are shown as range, Italy (Lodovici et al., 1995)

[10] Lettuce, tomato, cabbage from roadway plantation area, six samples each, range of mean values, Brazil (Camargo & Toledo, 2003)

[11] Lettuce, tomato, cabbage from rural area, six samples each, range of mean values, Brazil (Camargo & Toledo, 2003)

[12] Beans, peas, peanut, potato and carrot, three samples each, range of mean values, Brazil (Camargo & Toledo, 2002b)

[13] Lettuce, tomato, potato, green beans and cauliflower, 16 samples, mean values, Spain (Falco et al., 2003)

[14] Potato, 26 samples; scallion, 26; Chinese cabbage, 24; cabbage, 23; cucumber, 23; tomato, 22; eggplant, 20; wax gourd, 19; and celery, 17, mean values obtained for the vegetables are shown as range, northern China (Zhong & Wang, 2002)

as spinach and lettuce, had PAH levels that were 10 times higher, perhaps due to deposition from ambient air (Crössmann & Wüstemann, 1992).

6.1.4 Fruits and confections

The levels of PAHs found in fruits and confections are summarized in Table 13. Higher concentrations of PAHs were found in fresh fruit than in canned fruit or juice, and especially high concentrations of phenanthrene (17 µg/kg) and chrysene (69 µg/kg) were found in nuts (de Vos et al., 1990). In 1982–1983 in the United Kingdom, high PAH levels were found in samples of puddings, biscuits and cakes, which were probably derived from vegetable oil. Similar concentrations of individual PAHs were detected in samples of British chocolate (Dennis et al., 1991). In a study conducted on the Italian diet, peeled apples contained considerably lower levels of PAHs than unpeeled apples (Lodovici et al., 1995).

6.1.5 Cereals and cereal products

The levels of PAHs found in cereals and cereal products such as flour, bran, bread, biscuits, breakfast cereals and other ready-to-eat foods (such as pizza) are summarized in Table 14. Wheat, corn, oats and barley grown in areas near industries contained higher levels of PAHs than crops from more remote areas. Drying with combustion gases increased the contamination by 3- to 10-fold; use of coke as fuel resulted in much less contamination than use of oil (Bolling, 1964). The highest concentrations of total PAHs, up to 160 µg/kg, were found in smoked cereals (Tuominen et al., 1988). Surveillance data in recent studies indicate lesser contamination by PAHs. The PAH concentrations in rye grown near a highway with high traffic density decreased slightly 7–25 m away from the road (Larsson, 1982).

6.1.6 Beverages

PAH levels in beverages are summarized in Table 15. In one study, 16 PAHs were measured in various types of tea, and total PAH concentrations ranged from 323 to 8800 µg/kg, with the highest concentration found in black tea. Three- to four-ring PAHs were dominant in all tea samples (Lin et al., 2005). Mean PAH levels in tea infusion (five black teas, one green tea and one decaffeinated tea) ranged from 28.7 to 112 ng/l, and higher levels of phenanthrene (119–658 ng/l) were found in all samples (Kayali-Sayadi et al., 1998). The mean total PAH (10 compounds) content was relatively lower in mate tea (tea infusion) samples (0.7 µg/kg) than in brewed coffee (10.12 µg/kg) in Brazil (Camargo & Toledo, 2002a).

Mean PAH levels in the brew of six different coffee samples ranged from 1.67 to 2.87 ng/l, and one brew (torrefied coffee, coffee roasted with sugar) was found to contain higher levels of PAHs (Kayali-Sayadi et al., 1999). The benzo[a]pyrene content of 55 commercial samples of ground roasted coffee was <0.1–0.5 µg/kg, with an average of 0.2 µg/kg (de Kruijf et al., 1987). Benzo[a]pyrene was found at 0.8 µg/kg in coffee powder, 0.01 µg/l in brewed coffee, 9.51 µg/kg in tea leaves and 0.02 µg/l in brewed tea (Lintas et al., 1979). In 40 samples of tea leaves from

Table 13. (contd)

Compound	PAH concentration (µg/kg)								
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]
Phenanthrene	7.8			3.2					0.38
Pyrene			0.83		0.59–2.37	ND–3.46	ND	ND	0.03

ND, not detected

^a Reported in some publications as sum of benzo[*j*+*b*+*k*]fluoranthenes.

^b Reported including triphenylene in some publications.

^c Reported including dibenz[*a,c*]anthracene in some publications.

[1] Fresh fruit, Netherlands (de Vos et al., 1990)

[2] Canned fruit and juices, Netherlands (de Vos et al., 1990)

[3] Fruit and sugar, United Kingdom (Dennis et al., 1983)

[4] Sugar and sweets, Netherlands (de Vos et al., 1990)

[5] Puddings, biscuits and cakes, United Kingdom (Dennis et al., 1991)

[6] Apples, peeled apples and citrus fruits, triplicate samples are homogenized together, values obtained for selected foods are shown as range, Italy (Lodovici et al., 1995)

[7] Apple, pear and grape, range, six samples each, Brazil (Camargo & Toledo, 2003)

[8] Cane sugar, range, three brands (three batches of each brand were pooled and analysed), Brazil (Camargo & Toledo, 2002b)

[9] Fruits (apples, oranges and pears), 12 samples, mean values, Spain (Falco et al., 2003)

Table 14. PAH concentrations in cereals and cereal products (flour, bread, biscuits, bran and breakfast cereals)

Compound	PAH concentration (µg/kg)														
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]
Acenaphthene		1.6					0.7						0.6/ 0.7		
Anthanthrene												0.05– 0.08			
Anthracene		9.4					1.3						0.5		
Benzo[a]anthracene	0.1– 4.2	1.1	0.69	0.11– 0.21	2.5/ 3.7	0.6/ 0.3	<0.1/ 0.2	0.03– 0.31	0.11	0.4	ND– 0.2	0.14– 0.25	<0.1/ <0.1	0.3– 0.8	0.06– 0.15
Benzo[b]fluoranthene ^a	0.1– 0.5		0.28	0.07– 0.09	0.9	0.2/ 0.1		0.03– 0.04	0.04					0.1/ 0.2	0.02– 0.05
Benzo[ghi]fluoranthene														0.1/ 0.2	0.02– 0.05
Benzo[k]fluoranthene			0.50	0.1– 0.14				0.02– 0.03	0.02						
Benzo[ghi]perylene			0.54	0.13– 120				ND– 0.01	0.06		ND– 0.2				0.02– 0.07
Benzo[c]phenanthrene												0.20– 0.35			0.06– 0.08
Benzo[a]pyrene	ND– 0.3	5.4	0.40	0.10– 0.12	0.5/ 0.8	0.2	0.3/ 0.4	0.02– 0.02	0.03						
Benzo[e]pyrene			0.42	0.06– 0.17			0.1/ 0.7				<0.1	0.17– 0.30	0.2/ 0.4	0.1	0.03– 0.05

Table 14. (contd)

Compound	PAH concentration (µg/kg)														
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]
Chrysene ^b								0.52– 1.88	3.01		ND– 0.1	0.16– 0.29	0.2/ 0.4		0.06– 0.16
Dibenz[<i>a,h</i>]anthracene ^c	ND– 1.2		0.06	0.01– 0.02	3.6							0.07– 0.13			
Fluoranthene	0.8– 26	130	0.71	0.58– 0.69	18/ 28	1.9/ 1.4	1.5/ 13	0.12– 3.95	0.18				1.8/ 3.0	1.5– 7.4	0.22– 0.60
Fluorene		5.9					2.3/ 2.7			2.9	0.9– 1.3	0.32– 0.57	1.3/ 1.7		
Indeno[1,2,3- <i>cd</i>]pyrene	ND– 0.4		1.08	0.24– 0.33	1.4	0.2								3.2	0.08– 0.15
5-Methylchrysene											0.16– 0.29				
1-Methylphenanthrene										0.3					
Perylene	0.1– 0.4	0.7				94				2.6			<0.1/ 0.1	0.1– 0.3	
Phenanthrene	1.1– 48	47	0.10	0.38– 0.62	20/ 21	2.2/ 3.4	1.6/ 5.4	ND	0.58	0.1			9.9/ 10		
Pyrene											1.3– 1.5		1.6/ 5.5	2.6– 8.5	0.26– 1.18

Table 14. PAH concentrations in cereals and cereal products (flour, bread, biscuits, bran and breakfast cereals) (contd)

Compound	PAH concentration (µg/kg)														
	[16]	[17]	[18]	[19]	[20]	[21]	[22]	[23]	[24]	[25]	[26]	[27]	[28]	[29]	[30]
Acenaphthene		0.6													0.16
Acenaphthylene															0.89
Anthracene															0.14
Benz[a]anthracene	0.33–1.26	0.1	0.04–0.19	0.64	0.8	0.10–0.14	0.5	0.1	0.4		0.2	0.12–0.41	0.37–0.86	0.10–0.13	0.72
Benzo[b]fluoranthene ^a	0.1–0.27		0.02–0.06	0.25	1.2	0.04–0.06	0.5	0.6	1.0	0.05	0.4	ND–0.21	0.28–0.67	0.17–0.21	0.41
Benzo[ghi]fluoranthene	0.1–0.27														
Benzo[k]fluoranthene			0.03–0.08	0.35	0.6	0.04–0.1	0.1	0.3	0.5	0.08	0.1	0.08–0.11	0.10–0.18	0.12–0.12	0.23
Benzo[ghi]perylene	0.15–0.31		0.06–0.19	0.39	0.5	0.04–0.21	0.5	0.9	0.6		1.1	ND	ND–0.36	1.06–1.16	0.65
Benzo[c]phenanthrene	0.15–0.28								0.7						
Benzo[a]pyrene			0.02–0.09	0.43	0.8	0.05–0.15	0.2	0.3	0.8		0.3	0.07–0.21	0.23–0.36	0.19–0.27	0.26
Benzo[e]pyrene	0.15–0.34		0.10–0.23	0.35		0.06–0.12						ND	1.25–1.75	ND	
Chrysene ^b	0.28–0.81				1.0		2.0		1.3	0.4	0.5	ND	ND	ND	1.11

Table 14. (contd)

Compound	PAH concentration (µg/kg)														
	[16]	[17]	[18]	[19]	[20]	[21]	[22]	[23]	[24]	[25]	[26]	[27]	[28]	[29]	[30]
Dibenz[a,h]-anthracene ^c			<0.01–0.11	0.05		<0.01–0.01									
Fluoranthene	0.82–6.17	3.8	0.07–0.40	0.66	2.8	0.23–2.03	3.7	0.6	2.5		1.9	ND–2.17	1.96–2.92	1.68–2.73	1.43
Fluorene		2.0													0.26
Indeno[1,2,3-cd]pyrene	0.30–0.65		0.06–0.24	0.84	0.6	0.11–0.25	0.3	0.6	0.5		0.4				0.09
Naphthalene															1.56
Phenanthrene		14			3		4.2	3.0	2.1		2.9				3.74
Pyrene	1.41–10.9	2.6	0.04–0.88	0.67		0.23–0.87						ND	ND	ND	2.71

ND, not detected

^a Reported in some publications as sum of benzo[*j+b+k*]fluoranthenes.^b Reported including triphenylene in some publications.^c Reported including dibenz[*a,c*]anthracene in some publications.

[1] Barley malt, three samples, Canada (Lawrence & Weber, 1984b)

[2] Bran, Finland (Tuominen et al., 1988)

[3] Bran, United Kingdom (Dennis et al., 1991)

[4] High bran and granary bread, United Kingdom (Dennis et al., 1991)

[5] Bran, two samples, Canada (Lawrence & Weber, 1984b)

[6] Corn bran/flaked milled corn, one sample each, Canada (Lawrence & Weber, 1984b)

[7] Oats, Finland: rolled oats, two samples; and milled oats, three samples (Tuominen et al., 1988)

Table 14. (contd)

- [8] Bread, pasta, rice and corn, triplicate samples are homogenized together, values obtained in selected foods are shown as range, Italy (Lodovici et al., 1995)
- [9] Pizza, triplicate samples are homogenized together, Italy (Lodovici et al., 1995)
- [10] Whole-grain oats, one sample, Canada (Lawrence & Weber, 1984b)
- [11] Whole-grain rye, Sweden (Larsson, 1982)
- [12] Wheat grain, United Kingdom (Jones et al., 1989)
- [13] Wheat, Finland (Tuominen et al., 1988)
- [14] Wheat, Canada (Lawrence & Weber, 1984b)
- [15] Breakfast cereal, United Kingdom (Dennis et al., 1991)
- [16] Bran-enriched cereals, United Kingdom (Dennis et al., 1991)
- [17] Bolted rye flour, Finland (Tuominen et al., 1988)
- [18] White flour, United Kingdom (Dennis et al., 1991)
- [19] Granary flour, United Kingdom (Dennis et al., 1991)
- [20] Bread, Netherlands (de Vos et al., 1990)
- [21] White bread, United Kingdom (Dennis et al., 1991)
- [22] Noodles, pizza, Netherlands (de Vos et al., 1990)
- [23] Potato products, Netherlands (de Vos et al., 1990)
- [24] Rice, macaroni, Netherlands (de Vos et al., 1990)
- [25] Soups, Netherlands (de Vos et al., 1990)
- [26] Biscuits, Netherlands (de Vos et al., 1990)
- [27] Cereals (rice, pasta, flour, ground maize), range, three brands of each food (three batches of each brand were pooled and analysed), Brazil (Camargo & Toledo, 2002b, 2004)
- [28] Bread and biscuits, range, three brands of each food (three batches of each brand were pooled and analysed), Brazil (Camargo & Toledo, 2002b, 2004)
- [29] Pizza, six samples, Brazil (Camargo & Toledo, 2002b, 2004)
- [30] Cereals, eight samples, mean values, Spain (Falco et al., 2003)

Table 15. (contd)

Compound	PAH concentration (µg/kg, except where otherwise noted)									
	[1]	[2] ^d	[3] ^d	[4]	[5]	[6]	[7]	[8]	[9]	[10]
Fluoranthene	1.18	4.2–30.1	0.74– 2.27	25.4– 1480	5.86	8.364	1.21–5.53	0.30– 0.35	ND	ND
Fluorene				13.7–491						
Indeno[1,2,3- <i>cd</i>]pyrene				ND–148						
Naphthalene				0.59–429						
Phenanthrene		119– 658		64.0– 3460						ND
Pyrene	ND	18.4– 168	6.80–10.9	24.9– 1190	11.03	ND	ND–9.33	ND	ND	

ND, not detected

^a Reported in some publications as sum of benzo[*j+b+k*]fluoranthenes.^b Reported including triphenylene in some publications.^c Reported including dibenz[*a,c*]anthracene in some publications.^d Results reported in ng/l.

[1] Coffee beverage, triplicate samples are homogenized together, Italy (Lodovici et al., 1995)

[2] Black, green and decaffeinated tea infusion, seven samples (35 g tea dust making 500 ml of infusion), Spain (Kayali-Sayadi et al., 1998)

[3] Brewed coffee, six samples (50 g coffee powder making 300 ml of brew), Spain (Kayali-Sayadi et al., 1999)

[4] Various types of tea, 14 samples, China (Lin et al., 2005)

[5] Ground coffee, 18 samples, mean values, Brazil (Camargo & Toledo, 2002a)

[6] Chocolate, Italy (Lodovici et al., 1995)

[7] Brewed coffee (250 ml of water added to 25 g of coffee), 18 samples, Brazil (Camargo & Toledo, 2002b, 2004)

[8] Tea, 18 samples (500 ml of water added to 25 g of tea), Brazil (Camargo & Toledo, 2002b, 2004)

[9] Cola soft drink, range, three samples (three batches of each brand were pooled and analysed), Brazil (Camargo & Toledo, 2004)

[10] Drinks, number of samples not known, first figure is median value and the second is maximum, Netherlands (de Vos et al., 1990)

India, China and Morocco, the concentration of benzo[a]pyrene was generally 2.2–60 µg/kg, although concentrations up to 110 µg/kg were found in smoked teas (Prinsen & Kennedy, 1978).

In samples of whiskey and beer, the concentrations of 6 of 11 PAHs studied were below or slightly above 0.01 µg/kg. Pyrene was found at the highest level (0.24 µg/kg) (Dennis et al., 1991).

6.1.7 Oils, fats and related products

The levels of PAHs in various vegetable oils, vegetable cream, margarine and mayonnaise are listed in Table 16. The occurrence of PAHs in edible fats and oils was reviewed by Moret & Conte (2000). Vegetable oils are reported to be naturally free of PAHs, and contamination is due to technological processes such as smoke drying of oil seeds or environmental sources such as exhaust gases from traffic. Most work on the oils and fats was concentrated on the levels of benzo[a]pyrene. The PAH content of native olive oils was particularly high (Speer et al., 1990). The PAH content of coconut, soya bean, maize and rapeseed oil was radically reduced during refining, particularly by treatment with activated charcoal (Larsson et al., 1987). This method is now widely used (Dennis et al., 1991).

Benzo[a]pyrene was detected in 30 vegetable oils from Italy and France in 1994, including 17 grapeseed oils and one pumpkinseed oil. The average concentration was 59 µg/kg, and the maximum value was 140 µg/kg. Benzo[b]fluoranthene, benzo[k]fluoranthene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene were also found in measurable amounts. The source of these high levels was the smoke in drying ovens (State Chemical Analysis Institute, 1995). High numbers of PAHs in high concentrations have been reported in olive pomace oil (Guillen et al., 2004b). High concentrations of PAHs were found in olive, olive residue oils and seed oils; alkylated compounds accounted for more than half in some samples, and olive residue oils were found to contain a high proportion of heavy PAHs (Guillen et al., 2004b). In a study in Spain, PAHs in 47 samples of crude as well as refined vegetable oils were analysed. PAH levels ranged from 0.3 to 1145 µg/kg; phenanthrene was found in one crude coconut oil at a very high level of 1145 µg/kg (Barranco et al., 2003). All samples of Brazilian corn oil analysed showed contamination by benzo[a]pyrene, and the levels varied widely among brands and within different batches of the same brand, in the range of 0.85–25.2 µg/kg (Toledo & Camargo, 1998). In another study consisting of three samples each of 17 brands of olive oil in Brazil, mean benzo[a]pyrene levels ranged from not detected to 107, with one brand having the highest level of 164 µg/kg (Pupin & Toledo, 1996a). In a study involving the benzo[a]pyrene content in vegetable oils collected from the Brazilian market, the ranges of mean benzo[a]pyrene concentrations in 5 brands (five samples each) of corn oil, 16 brands (five samples each) of soya bean oil, 4 brands (four samples each) of sunflower oil, 2 brands (four samples each) of rice oil and 2 brands (four samples each) of palm oil were found to be 3.6–24.7 µg/kg, ND–2.1 µg/kg, ND–1.6 µg/kg, 1.6–2.1 µg/kg and ND–6.2 µg/kg, respectively (Pupin & Toledo, 1996b). In another study, benzo[a]pyrene was found in the range of ND–1.16 µg/kg in six Spanish virgin olive oils and in the

Table 16. PAH concentrations in oils, fats and related products

Compound	PAH concentration (µg/kg)											
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]
Acenaphthene		<0.02– 45					0.29		0.59– 2.49	<1.5– 45		
Acenaphthylene									0.86– 4.36			
Anthanthrene	Tr–0.1						0.03– 0.53					
Anthracene		<0.02– 460	<0.1– 0.1	ND–4.8	ND– 8		0.04– 0.92		Tr– 2.10	0.4 – 230		
Benz[a]anthracene			0.7–6.1	ND–6.1	ND	0.30– 7.46		0.03	0.86– 78.5	<0.1– 97	0.22– 3.98	0.28– 0.96
Benzo[b]fluorene		<0.02– 45			ND		0.03– 2.1					
Benzo[b]fluoranthene ^a	Tr–0.1	<0.02– 91		ND–8.9	ND	0.20– 2.39		0.26	0.56– 85.3	<0.2– 67	0.16–3.0	0.09– 0.37
Benzo[ghi]fluoranthene		<0.02– 1.3			ND		0.14– 4.9					
Benzo[k]fluoranthene					ND	0.24– 3.17		0.06	0.53– 98.8	<0.3– 16	0.20– 3.40	0.16– 0.55
Benzo[ghi]perylene		<0.02– 10	0.5–1.7	ND–4.2		0.06– 5.23	0.02– 1.4	ND	0.49– 65.8	0.40– 30	0.38– 5.21	0.17– 1.16
Benzo[a]pyrene	Tr–0.3	<0.02– 24	0.5–2.3	ND–4.1	ND	0.29– 4.92	0.05– 2.2	0.10	0.60– 106	<0.3– 67	0.19–6.0	0.17– 0.83

Table 16. (contd)

Compound	PAH concentration (µg/kg)											
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]
Benzo[e]pyrene		<0.02– 23	0.7–2.4	ND–3.8	ND	0.26– 6.06	0.09– 2.1		1.89– 87.6		0.42– 6.11	0.36– 0.87
Chrysene ^b				0.1–8.6	ND	0.39– 10.3		0.38	0.49– 63.3	<0.2– 217	0.26– 7.36	0.31– 0.97
Coronene		<0.02							Tr– 7.43			
Cyclopenta[cd]pyrene		<0.02– 1.4			ND		0.10– 1.1					
Dibenz[a,h]anthracene ^c	0.7–1.1	<0.02– 1.1		ND–0.2		<0.01– 0.82			0.24– 12.9	<0.3– 3.6	0.05– 1.02	0.04– 0.11
Fluoranthene	0.2–7.5	<0.02– 460	1.2–4.8	0.2– 18.2	3–15	0.21– 12.4	0.52– 9.0	0.17	1.98– 19.9	1.1– 464	0.09– 4.50	0.44– 1.56
Fluorene		<0.02– 200			ND– 7		0.08– 1.6		0.43– 2.10	<2.5– 264		
Indeno[1,2,3-cd]pyrene	Tr–0.5	<0.02– 0.8	5 0.3– 1.7	ND–4.3		0.59– 6.78	0.03– 1.1		0.27– 80.6	<0.3– 18	0.49– 9.14	0.43– 1.17
1-Methylphenanthrene		<0.02– 190					0.08– 1.8					
Perylene	Tr–0.2	<0.02– 5.9	0.1–0.4	ND–0.9			0.02– 0.57		0.46– 36.2			
Phenanthrene		0.09– 1400	0.9–1.6	ND– 69.4	4–38		0.29– 6.0		1.10– 6.19	<0.4– 1145		

Table 16. (contd)

Compound	PAH concentration (µg/kg)											
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]
Pyrene	0.2–1.4	<0.02– 330	1.1–4.2	0.1– 13.6	2–14	0.58– 17.2	0.59– 15	ND	0.57– 8.47	<0.3– 452	0.29– 6.03	0.44– 1.88

Table 16. PAH concentrations in oils, fats and related products (contd)

Compound	PAH concentration (µg/kg)										
	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[22]	[23]
Acenaphthene	<0.1–11										0.12
Acenaphthylene											1.17
Anthanthrene	<0.1–2.7			<0.10							
Anthracene	<0.2–5.6			0.60– 7.50							0.19 0.80–4.80
Benz[a]anthracene	<0.1–5.2	2.10– 15.2	1.02– 2.22	0.20– 3.00	0.22– 1.36	0.45–3.6	0.14– 2.14	0.24– 0.40	0.32– 4.83	0.57	1.0–1.90
Benzo[b]fluoranthene ^a	<0.2–9.2	2.09– 22.7	1.25– 1.37	<0.1	0.18– 1.03	0.29– 1.15	ND– 0.68	0.09– 0.26	0.14– 2.43	0.18	1.0–2.80
Benzo[k]fluoranthene	<0.1– 11.4	0.50– 9.18	0.27– 0.27	0.10– 0.30	0.07– 0.34	0.11– 0.84	0.06– 0.20	0.08– 0.12	0.08– 1.07	0.16	
Benzo[ghi]perylene	<0.2– 10.6	ND	0.22– 0.57	0.10– 0.70						0.22	ND–0.80

Table 16. (contd)

Compound	PAH concentration (µg/kg)										
	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[22]	[23]
Benzo[a]pyrene	<0.2–5.2	1.85–25.2	0.81–0.87	0.10–0.40	0.11–1.23	0.32–1.39	0.14–0.63	0.01–0.32	0.12–6.99	0.27	0.20–1.20
Benzo[e]pyrene		4.56–37.2	4.06–6.23	<0.10–0.60							0.20–1.10
Chrysene ^b	<0.2–7.5	ND–15.7	ND	0.70–6.00	ND–0.62	ND–1.61	ND–0.94	ND	ND–1.37	0.92	3.7–6.9
Coronene				0.10–0.40							
Dibenz[a,h]anthracene ^c	<0.1–9.2	2.39–42.9		<0.10–0.20	ND	ND–4.37	ND	ND	ND–6.76	0.12	ND–0.10
Fluoranthene	<0.1–1.6	12.2–25.0	2.66–5.85	2.90–22.5	ND–1.95	ND–3.19	0.07–0.78	0.03–0.07	0.11–2.76	0.87	8.30–16.1
Fluorene	<0.2–2.1									0.16	
Indeno[1,2,3- <i>cd</i>]pyrene	<0.2–9.7			0.10–0.30						0.10	ND–0.90
Naphthalene	<0.2–52									0.51	
Perylene				0.10–0.20							<0.10–0.30
Phenanthrene	<0.2–4.6			15.0–81.0						1.47	31.3–62.6
Pyrene	<0.1–1.7	ND	ND	3.20–21.0	ND–0.84	ND–2.36	ND–0.40	ND	ND–7.03	1.68	5.20–13.6

Table 16. (contd)

ND, not detected; Tr, traces

^a Reported in some publications as sum of benzo[*j*+*b*+*k*]fluoranthenes.

^b Reported including triphenylene in some publications.

^c Reported including dibenz[*a,c*]anthracene in some publications.

- [1] Corn oil, canola, soya bean oil, Canada (Lawrence & Weber, 1984b)
- [2] Corn oil, coconut oil (crude and deodorized), olive oil, soya bean oil, sunflower oil, sesame oil, flax oil, wheat seed oil (Hopia et al., 1986)
- [3] Coconut oil (pure) (Sagredos et al., 1988)
- [4] Various olive oils, safflower oils, sunflower oils, maize germ oils, sesame oil, linseed oil, wheat germ oil, Germany (Speer et al., 1990)
- [5] Olive oils and virgin olive oils, 13 samples, Italy (Menichini et al., 1991)
- [6] Various unspecified oils, United Kingdom (Dennis et al., 1991)
- [7] Four cooking margarines, seven table margarines (Hopia et al., 1986)
- [8] Olive oil, triplicate samples are homogenized together, Italy (Lodovici et al., 1995)
- [9] Grapeseed oil, rapeseed oil, sunflower oil, cocoa butter, soya bean oil, peanut oils, seven samples, Switzerland (Kolarovic & Traitter, 1982)
- [10] Corn oil, coconut oil (crude and refined), crude sunflower oil, palm oil, palm stearine, palm kernel oil, olive oil, two olive residue oils, 47 samples, Spain (Barranco et al., 2003)
- [11] Margarine, United Kingdom (Dennis et al., 1991)
- [12] Low-fat spread, United Kingdom (Dennis et al., 1991)
- [13] Margarine, New Zealand (Thomson et al., 1996)
- [14] Soya bean oil, nine samples (three batches of the three most consumed brands), Brazil (Camargo & Toledo, 2004)
- [15] Margarine, nine samples (three batches of the three most consumed brands), Brazil (Camargo & Toledo, 2004)
- [16] Vegetable oils, 10 samples, analysed by donor-acceptor complex chromatography cleanup method, Netherlands (van Stijn et al., 1996)
- [17] Margarine, 18 samples, Brazil (Camargo & Toledo, 2000)
- [18] Vegetable cream, 15 samples, Brazil (Camargo & Toledo, 2000)
- [19] Spread, six samples, Brazil (Camargo & Toledo, 2000)
- [20] Shortening, three samples, Brazil (Camargo & Toledo, 2000)
- [21] Mayonnaise, 21 samples, Brazil (Camargo & Toledo, 2000)
- [22] Oils and fats, six samples, Spain (Falco et al., 2003)
- [23] Olive oils, seven samples, Germany (Speer et al., 1990)

range of ND–1.99 µg/kg in 12 vegetable oils associated with fish (tuna, sardines, mackerel) (Troche et al., 2000).

Lard and drippings were found to contain levels of individual PAHs ranging from <0.01 µg/kg for dibenz[*a,h*]anthracene to 6.9 µg/kg for fluoranthene (Dennis et al., 1991). High PAH levels were found in margarine samples in studies in Finland (Hopia et al., 1986), the Netherlands (Vaessen et al., 1988), New Zealand (Thomson et al., 1996) and the United Kingdom (Dennis et al., 1991).

6.1.8 Dairy products

PAHs in dairy products are summarized in Table 17. Milk and infant formula samples from Japan contained total PAHs (12 PAHs) in the range of 0.23–2.01 µg/kg and 1.68–2.28 µg/kg, respectively (Kishikawa et al., 2003). In a study in France, milk samples collected from the tanks at farms located near potential emission sources from industries indicated that eight PAHs were detected in similar profiles at low concentrations, except fluorene and naphthalene, for which source molecule interaction was pointed out (Nathalie et al., 2002). Concentrations of 12 selected PAHs were estimated in milk, milk powder and other dairy products in Canada (Lawrence & Weber, 1984b), the Netherlands (de Vos et al., 1990) and the United Kingdom (Dennis et al., 1983, 1991). The concentrations ranged from <0.01 µg/kg for benzo[*k*]fluoranthene and dibenz[*a,h*]anthracene to 2.7 µg/kg for pyrene. Canadian infant formula was found to contain fluoranthene at 8.0 µg/kg, pyrene at 4.8 µg/kg, benz[*a*]anthracene at 1.7 µg/kg, benzo[*b*]fluoranthene at 0.7 µg/kg, benzo[*a*]pyrene at 1.2 µg/kg, perylene at 0.6 µg/kg, anthanthrene at 0.3 µg/kg and indeno[1,2,3-*cd*]pyrene at 1.2 µg/kg (Lawrence & Weber, 1984b). Slightly lower levels were detected in British infant formula samples in 1982–1983 (Dennis et al., 1991). High concentrations of PAHs were found in the milk of reared cows and sheep in Kuwait; mean concentrations of phenanthrene, fluoranthene, pyrene and chrysene were significantly higher in sheep milk samples than in cow milk samples, whereas levels of other PAHs were comparable or not detected (Husain et al., 1997).

PAHs were detected in considerable amounts in smoked cheese (Prinsen & Kennedy, 1977; Lintas et al., 1979; McGill et al., 1982; Osborne & Crosby, 1987). Numerous PAHs have been identified in the exterior zone of smoked cheese, and concentrations of low-molecular-mass PAHs were higher than those of high-molecular-mass PAHs (Guillen et al., 2004a). The benzo[*a*]pyrene content of a smoked Italian Provolone cheese was 1.3 µg/kg (Lintas et al., 1979). Concentrations of fluoranthene, benz[*a*]anthracene, benzo[*c*]phenanthrene, benzo[*a*]pyrene, benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene of 0.01–5.6 µg/kg fresh weight and 0.01–0.06 µg/kg fresh weight were found in a smoked cheese sample and in unsmoked cheese, respectively, from the United Kingdom (McGill et al., 1982). In other unsmoked cheese samples from the United Kingdom, the individual PAH levels ranged between <0.01 µg/kg for dibenz[*a,h*]anthracene and 1.5 µg/kg for pyrene. Similar concentrations of PAHs were found in British butter and cream samples (Dennis et al., 1991). In Finnish butter samples, most of the measured PAHs (phenanthrene, 1-methylphenanthrene, fluoranthene, pyrene, benzo[*a*]fluorene, benzo[*ghi*]fluoranthene, cyclopenta[*cd*]pyrene, perylene, anthanthrene,

Table 17. (contd)

Compound	PAH concentration (µg/kg)													
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]
Dibenz[a,h]anthracene ^c	ND	ND	ND	0.01	0.04	ND– 0.04	0.02– 0.10	ND– 0.06	ND, 1.5	ND, 1.6	3.0	ND	ND	
Dibenz[a,e]pyrene									ND	ND				
Fluoranthene	2.34– 6.12	2.60– 2.90	3.00– 3.49	0.11	0.87	ND– 0.25	0.12– 0.25	ND– 0.13	ND	ND	8.0	14.2	3.39	0.15
Fluorene				0.02	0.07									
Indeno[1,2,3-cd]pyrene				0.01	0.04	ND– 0.03	0.04– 0.20	ND– 0.03	ND	ND	1.2	ND	ND	
Naphthalene				0.27	0.88									
Perylene									ND	ND	0.6			
Phenanthrene				0.40	1.56	0.09– 0.48	0.30– 0.49	0.04– 0.99				10.0	3.08	
Pyrene	ND	ND	ND	0.24	1.48	ND– 0.27	0.08– 0.24	ND– 0.29			4.8	139	35.5	1.77

ND, not detected; Tr, traces

^a Reported in some publications as sum of benzo[*j*+*b*+*k*]fluoranthenes.^b Reported including triphenylene in some publications.^c Reported including dibenz[*a,c*]anthracene in some publications.

[1] Milk, nine samples (three batches of the most consumed three brands), range of mean values, Brazil (Camargo & Toledo, 2004)

[2] Cheese, nine samples (three batches of the three most consumed brands), range of mean values, Brazil (Camargo & Toledo, 2004)

[3] Yoghurt, nine samples (three batches of the three most consumed brands), range of mean values, Brazil (Camargo & Toledo, 2004)

[4] Milk, four samples, mean values, Spain (Falco et al., 2003)

[5] Yoghurt and cheese, four samples, mean values, Spain (Falco et al., 2003)

Table 17. (contd)

- [6] Milk (whole, skimmed, condensed and fortified), 14 samples, Japan (Kishikawa et al., 2003)
- [7] Infant formula, three samples, Japan (Kishikawa et al., 2003)
- [8] Human milk, 51 samples, Japan (Kishikawa et al., 2003)
- [9] Milk, Netherlands, number of samples not known, first figure is median value and the second is maximum value (de Vos et al., 1990)
- [10] Dairy products, Netherlands, number of samples not known, first figure is median value and the second is maximum value (de Vos et al., 1990)
- [11] Infant formula, Canada (Lawrence & Weber, 1984b)
- [12] Sheep milk, Kuwait, 30 samples, mean values (Husain et al., 1997)
- [13] Cow milk, Kuwait, 47 samples, mean values (Husain et al., 1997)
- [14] Butter, triplicate samples are homogenized together, Italy (Lodovici et al., 1995)

benzo[ghi]pyrene and indeno[1,2,3-cd]pyrene) occurred at levels ≤ 0.1 $\mu\text{g/kg}$. The maximum level was for fluoranthene, at 1.4 $\mu\text{g/kg}$ (Hopia et al., 1986). In butter samples from Italy, levels of chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene and benzo[ghi]perylene were <0.05 $\mu\text{g/kg}$, while concentrations reported for pyrene, benz[a]anthracene and fluoranthene were 1.769, 0.666 and 0.146 $\mu\text{g/kg}$, respectively (Lodovici et al., 1995).

(a) Human milk

A study in Japan involving 51 samples of human milk reported total PAH levels in the range of ND–2.15 $\mu\text{g/kg}$ (Kishikawa et al., 2003). In an Austrian study (Lechner et al., 1991), benzo[a]pyrene was not detected in any of 41 samples of human milk from the region. Human milk samples were reported to contain PAHs at levels of 0.003–0.03 $\mu\text{g/kg}$ (Heeschen, 1985). In a study conducted in Germany, human milk samples were found to contain a number of PAHs at levels ranging from 5 to 15 ng/kg, with benzo[a]pyrene being detected at a concentration of 6.5 ng/kg (IPCS, 1998).

6.2 Profiles of PAHs in food

There were large differences in the choice of PAHs determined in various food groups, making comparisons between results almost impossible. There have been wide variations in the concentration data for a given PAH in a food item. However, from the tabulated occurrence data, it is evident that meat and fish products, particularly grilled and barbecued products, oils and fats, cereals and cereal-based foods are the major foods contaminated. Data were not available to study geographical as well as seasonal variations. It is also observed that some determinations were carried out following episodes of contamination for a given food or incidence of environmental pollution. Concentration data for four dibenzopyrenes as well as 5-methylchrysene, among those PAHs considered to be genotoxic and carcinogenic (see section 10.2), were either limited or not available.

The Scientific Committee on Foods has made an attempt to draw the profiles of PAHs in foods (EC, 2002). As different sets of PAHs were analysed under different studies, to determine whether it is possible to distinguish the PAH profiles on the basis of different sources, the Scientific Committee on Foods (EC, 2002) selected two subsets of foods and compared foods contaminated only by atmospheric contamination with foods in which PAHs are formed during processing. Since benzo[a]pyrene was regarded as a marker for contamination of food by PAHs, total PAHs:benzo[a]pyrene concentration ratios were calculated for different foods. The following conclusions were drawn:

- The lower-molecular-mass, i.e. three- and four-ring, PAHs (fluorene, anthracene, phenanthrene, fluoranthene and pyrene) show markedly higher variability than higher-molecular-mass PAHs.
- The variability for almost all the higher-molecular-mass PAHs (from benzo-fluorenes upwards) was within a factor of 10 when the raw data from all the selected studies were used. However, a significant part of the variability is

likely due to the differences in the analytical procedures used in different investigations and to any poor accuracy possibly present in some investigations and/or for some PAHs.

- The variability markedly decreased after excluding outlying data (10% of all data), being within 1 order of magnitude for all these higher-molecular-mass PAHs. This holds especially for the carcinogenic PAHs measured (benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene), whose profiles in different foods were constantly within a factor of 5.
- When the comparison of profiles in different foods was based on analyses performed in the same laboratory with the same procedure, as was the case with the investigation of Dennis et al. (1991), the variability for the higher-molecular-mass PAHs (from benz[a]anthracene upwards) was at most within a factor of 5.
- The median profile of measured carcinogenic PAHs (benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene) in foods was very similar to those in coke oven fumes and in urban air, within an approximate factor of 2.
- The PAH median profile was also similar to that found in the coal tars used in a recent carcinogenicity study, well within 1 order of magnitude. In particular, for the measured carcinogenic PAHs, the PAH:benzo[a]pyrene concentration ratios in coal tar and in foods (median values) were in the range 0.5–1.1.
- When processed foods, foods contaminated by deposition only and all foods were compared, similar profiles were observed, except for anthracene and phenanthrene being more abundant in processed foods. As for the measured carcinogenic PAHs, the profiles appeared to be similar, any difference being within a factor of approximately 2.
- It thus appears that benzo[a]pyrene can be used as an indicator of occurrence and concentration in food of the higher-molecular-mass PAHs (from benzofluorenes upwards), at least in terms of an order of magnitude. Benzo[a]pyrene cannot be used as an indicator of lower-molecular-mass PAHs.

The Scientific Committee on Foods (EC, 2002) noted that the above conclusions are in agreement with the results of a validation study performed by Kazerouni et al. (2001). To verify that benzo[a]pyrene is a good marker for other PAHs, they analysed samples from each major food group for 15 PAHs (2–5 rings), including seven PAHs considered to be carcinogenic (4 and 5 rings). The Pearson correlation coefficient between concentrations of benzo[a]pyrene and the sum of the carcinogenic PAHs was 0.98 ($P = 0.0001$), while it decreased to 0.87 ($P = 0.0001$) in the comparison of benzo[a]pyrene and the total of 15 PAHs.

6.3 Conclusion and recommendations on analysis of PAHs in foods

- Solid-phase extraction cleanup followed by HPLC with fluorescence and photo-diode array detection is adequate for the routine analysis of PAHs. GC-MS methods serve as confirmatory/alternative methods.
- Method validation studies carried out so far include selected matrices and PAHs only. It is necessary that validation studies be extended to a wide range of matrices covering all the PAHs. Interlaboratory collaborative studies should be extended to cover all PAHs and high-risk foods.
- CRMs should be developed covering a wide range of PAHs in various matrices.
- Methods should be assessed and adopted as official methods for monitoring as well as for use in regulatory purposes.
- Monitoring and surveillance studies should continue to collect data on the whole PAH profile to enable evaluation of contamination and any further changes in the PAH profiles.

7. INTAKE ESTIMATES

There are various routes of exposure to PAHs for humans, including air, water, food, cigarette smoking, dermal contact and occupational exposure. Humans are exposed to varying mixtures of PAHs from these sources. PAHs in food and water come from environmental contamination and the uptake of PAHs from soil and water and from some food preparation and cooking processes (e.g. smoking, drying, barbecuing, grilling).

The assessment provided below for the present meeting of the Committee is focused primarily on the exposure to PAHs for humans from the consumption of food and water. Exposure from other sources, such as air and smoking, are more difficult to estimate. Some information on these sources has been provided. Intakes from a range of PAHs have been considered.

7.1 Dietary intake estimates

7.1.1 Introduction and background to intake estimates

PAHs as a group have not been assessed by the Committee in recent times. At the fifth meeting of the Committee (Annex 1, reference 5), a small assessment was conducted on "Substances that may be contaminated with carcinogenic aromatic compounds." This assessment focused on smoking of foods and the need to control smoking processes to control contamination. No intake data were included in the assessment.

Benzo[a]pyrene was previously assessed by the Committee at its thirty-seventh meeting in 1990 (Annex 1, reference 94). This assessment included

estimates of intake of benzo[a]pyrene. The Committee concluded the following from the meeting:

- Dietary intakes varied considerably.
- The estimated daily intakes were about 4 orders of magnitude lower than the level reported to be without effect on the incidence of tumours in rats.
- Effects on human health are likely to be small.
- There was a need to minimize the exposure to benzo[a]pyrene as far as possible.

The evaluation by the Committee at its thirty-seventh meeting reported that intakes of benzo[a]pyrene varied considerably, with some consumers being exposed to high levels. Reported benzo[a]pyrene intakes from food ranged between 0.16 and 3.3 µg/day. The major contributors to dietary intake were cereals and vegetable oils. No tolerable level was established for benzo[a]pyrene. The Committee recognized that benzo[a]pyrene was only one of a group of PAHs, of which others should be evaluated.

Food can become contaminated by PAHs in the soil in which food grows or deposition of particles from the air, from water used in growing or cooking or via certain processing and cooking methods, such as smoking, drying, grilling or barbecuing. Concentrations in some animal foods (e.g. meat, eggs, milk and poultry) are low due to the metabolism of PAHs in these organisms. Some marine animals, such as lobsters, oysters and mussels, can absorb and accumulate PAHs from contaminated water (EC, 2002). PAHs are lipophilic and therefore tend to accumulate in lipid tissue of plants and animals (IPCS, 1998; EC, 2002).

Levels of PAHs in soils tend to be higher in metropolitan areas and areas near industrialization and transportation (WHO Regional Office for Europe, 2003).

There is some variation in exposures to PAHs with area of residence (higher in industrial areas), level of soil contamination, types of foods consumed, food preparation practices and the water source used for drinking and preparing foods.

The varying toxicities of the different PAHs are rarely considered in the intake estimates presented in the literature, and toxic equivalency factors (TEFs) are usually not included in the assessments.

There have been three recent, comprehensive assessments conducted on PAHs, all of which included some data on PAH intakes. These have been conducted by the IPCS (1998), the Scientific Committee on Food (EC, 2002) and the European Commission SCOOP task force 3.2.12 on the collection of occurrence data on PAHs in food (SCOOP, 2004). Intake assessments from these reviews form the basis of this assessment, together with estimates of intake from the literature not covered by these assessments and intake estimates submitted for review by the Committee for Australia, Brazil, New Zealand and the United Kingdom.

The Committee usually calculates international estimates of intake of contaminants using the GEMS/Food regional diets (WHO, 2003) and extrapolates data on concentrations of the contaminant from regions in which data are collected to

regions for which no data exist. In the present case, no data on concentrations of PAHs for individual samples were submitted to the Committee or available in the literature. Therefore, no distributions of concentrations of PAHs or mean concentrations of PAHs in foods were available in the required format to be used in calculating intakes of PAHs at the regional level. Should PAHs be reassessed by the Committee in the future, the Committee recommends that raw data from individual samples be submitted to allow estimates of the regional intakes to be made.

The WHO web site "SIGHT" (Summary Information on Global Health Trends) was also searched for information on PAH intakes. This web site contains summary information from studies conducted around the world on substances in food and intakes for those countries who have submitted relevant data to WHO. There was no information on PAH intakes from this source.

There are no international limits for PAHs in food set by the Codex Alimentarius Commission. There are limits set for smoked foods in the European Union.

Ideally, all sources of PAHs should be considered in order to determine total estimated exposures to PAHs and the risk to human health. Primarily the intakes of PAHs from food and water are considered below. Some estimates of intake from other sources, such as inhalation, have been provided. In order to obtain a good estimate of intake, a wide range of foods should be included in the intake assessment, because it has been demonstrated by numerous sources that PAHs occur in a very broad range of foods and beverages.

Food is a significant source of PAHs for humans, of which the source is mainly environmental pollution, food packaging and processing (Husain et al., 1997). Human exposure to PAHs is almost all (99%) from food (Garcia-Falcon et al., 1996). Cigarette smoking also contributes to PAH exposure, although it has been reported that food consumption may be a greater contributor than cigarette smoking (Lawrence & Weber, 1984a).

Benzo[a]pyrene has often been regarded as an indicator of PAHs in food. The review by the Scientific Committee on Food (EC, 2002) stated that benzo[a]pyrene is a good indicator of only the higher-molecular-mass PAHs, from the benzofluor- enes upwards.

There are certain PAHs that researchers tend to classify as being "carcinogenic" or of more concern toxicologically, including benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene, dibenz[*a,h*]anthracene and indeno[1,2,3-*cd*]pyrene (Gomaa et al., 1993; Chen et al., 1996; Järvenpää et al., 1996; EC, 2002; UKFSA, 2002), which may differ from the classification of carcinogenic made by the Committee. These are usually the PAHs with five or six fused rings. These PAHs are also stated as United States Environmental Protection Agency priority pollutants and as probable human carcinogens by IARC. Intake assessments of individual PAHs also tend to focus more on these PAHs than on others. The most abundant PAHs in food tend to be smaller compounds that are less harmful (Järvenpää et al., 1996; Phillips, 1999).

7.1.2 Methods

Estimates of intake for PAHs have been determined in a variety of ways. This may include intakes for individual PAHs, intakes of "summed" PAHs or intakes of "carcinogenic" PAHs. Summed PAHs vary in the PAHs covered, and "carcinogenic" PAHs depend on the authors' classification of carcinogenic. Some studies cover a broader range of PAHs than others, although there do tend to be certain PAHs that are included in most assessments, such as benzo[a]pyrene. These are the PAHs perceived to be of more concern toxicologically than others. Many of the intake estimates are for benzo[a]pyrene only, reflecting the fact that many reports in the literature state that benzo[a]pyrene is an indicator of all PAHs. Phillips (1999) stated that it is often preferable to determine a number of PAHs, because benzo[a]pyrene is only one of a number of potentially carcinogenic PAHs.

For this review of PAHs by the Committee, dietary intake estimates were reviewed only for the 13 PAHs that were determined by the Committee to be clearly carcinogenic and genotoxic (see section 10.2). There were many intake estimates that covered a broader range of PAHs. These have not been reviewed here, nor have the intakes estimated for "summed" PAHs or groups of "carcinogenic" PAHs.

Estimates of dietary intake of PAHs have been calculated on a chronic basis. The acute oral toxicity for PAH is moderate to low (IPCS, 1998; EC, 2002). There are many estimates of chronic intake reported in the literature. No estimates of acute intake (from one meal or one day) were found in the literature.

Most intake estimates have included foods with PAH concentrations resulting from background contamination of the environment during growth of the food and also as a result of food processing, preparation and cooking. No separate estimates of intakes from food from the different sources have been made.

The literature has given no indication of population subgroups that are particularly susceptible to exposure to PAHs. Generally, PAH intakes have been estimated for adults or "whole" populations covering a broad range of ages. Intakes of PAHs by children were assessed separately in a small number of studies.

Most of the reported studies in the literature provide point estimates of PAH intakes. Some used individual dietary records, producing a distribution of estimated intakes from which population summary statistics (e.g. mean intakes) were derived.

(a) Limitations of comparing intake estimates

It is difficult to compare intake estimates made for each PAH because of the different ways in which each assessment has been undertaken.

Intakes of PAHs from the diet have been considered in detail. These assessments may or may not have included drinking-water. Some estimates of PAH intake from drinking-water alone have also been included. Intakes of PAHs from air and exposure from other sources have been included on a smaller scale. A

comparison of intakes between sources has been included, with an estimate of which source is the major contributor to PAH intake.

Some estimated intakes are presented as "total population" (e.g. per capita or all survey respondents) and others as "consumers only." The results can be different between the groups if the number of consumers is less than the total number of respondents in a survey. In studies in which the food groups included are commonly consumed by the majority of the population, there will be little difference, if any, between the number of respondents and the number of consumers, and the resulting estimated intakes will be virtually identical. For intake estimates where the number of consumers is lower than the number of respondents, the results for the respondents will be lower than those for the consumers, as they are averaged, including "zero" intakes from non-consumers.

Intake estimates calculated using "food availability" data tend to be an overestimate, as they do not take into consideration losses of food due to waste, actual consumption amounts or variation of consumption within the population. Duplicate-diet studies, 7-day records or individual dietary records tend to produce better estimates of intake. Intakes from household budget surveys are based on food purchases and do not consider wastage, and they may not consider distribution and actual consumption by individual household members. They may assume equal distribution among household members. They usually do not include foods purchased and consumed away from home.

Some PAHs, when analysed, have concentrations too small to be detected by the analytical method being used. Therefore, these "not detected" (ND) results need to be assigned a numerical concentration in order to enable an intake from the food in question to be calculated. The intake assessments reviewed have used various approaches in dealing with ND results. These range from assigning a zero concentration to the result to assigning a concentration equal to the limit of reporting (LOR). Assigning a zero concentration may result in an underestimate of estimated intake, as there may be PAHs occurring in foods from background contamination. Some assessments assign a concentration equal to the limit of detection (LOD), limit of quantification (LOQ) or the LOR. This is the upper end of the range of possible concentrations that could be in the food and may lead to an overestimate of estimated intake. Some studies have used a concentration equal to half the LOD or LOQ, based on the assumption that the concentration will be somewhere between zero and the LOD or LOQ.

It was not possible to estimate intakes of PAHs from food additive uses (smoke flavouring) alone using maximum permitted levels, as no data were available on consumption amounts of smoke flavourings. Residues from food additive uses may have been picked up via analysis of smoked foods; however, intakes from smoke flavouring were not specifically highlighted in the assessments reviewed.

(b) *Toxic equivalency factors (TEFs)*

It is recognized that different PAHs have different potencies and toxicological effects. There are a small number of intake estimates in the literature that have used TEFs. However, this methodology is not commonly used for PAHs. There are no internationally recognized TEFs for PAHs, and there is no international consensus on the use of this methodology to incorporate TEFs into intake assessments for PAHs. Additionally, no TEFs were developed by the Committee. Intake estimates using TEFs have not been included in this assessment.

(c) *Reference health standards*

Tolerable daily intakes were not set by the Committee for PAHs; therefore, estimates of intake have been expressed only in units per day or units per kilogram of body weight.

7.1.3 *International estimates of intake*

Many estimates of PAH intake have been made internationally. Estimated intakes of individual PAHs from food were between 0.1 and 10 µg/day (IPCS, 1998). Intake of PAHs over a lifetime of 70 years was reported to be equivalent to approximately 300 mg of benzo[a]pyrene (Lutz & Schlatter, 1992).

The intake assessments reviewed and presented below include food and drinking-water; however, not all reported intakes include drinking-water. The majority of the estimates also included processed and cooked foods, which can have higher concentrations of PAHs.

The Committee reviewed estimates of intake for a range of PAHs from 18 countries, including data submitted for Australia, Brazil, New Zealand and the United Kingdom. A summary of the data submitted on PAH intakes is shown in Table 18. Australia submitted estimated dietary intakes for summed PAHs and benzo[a]pyrene based on individual dietary records, as did New Zealand. Brazil submitted a number of intake estimates based on consumption figures from household surveys or per capita estimates of intake. The United Kingdom submitted estimated intakes of PAHs from a 2000 total diet study (TDS).

Table 18. Summary of submissions on PAH intakes

Country	FBS/HES/sales data	Model diets	Individual dietary records
Australia			✓
Brazil	✓		
New Zealand			✓
United Kingdom		✓	

FBS, Food balance sheet; HES, Household economic survey

The Scientific Committee on Food opinion on PAHs (EC, 2002) and the European Union SCOOP report (SCOOP, 2004) were also submitted for review by the Committee. These reviews contained a range of estimates of intake, which have been assessed and summarized below where they have included the PAHs assessed by the Committee.

Certain reviews have focused on particular PAHs primarily due to the level of toxicological concern. Most of the intake assessments in the literature tend to include those that were included in the reviews by IPCS and the Scientific Committee on Food, with some additions.

(a) *National estimates of PAH intake submitted for consideration by the Committee*

(i) *Australia*

Estimated intakes of PAHs for Australia were based on the concentrations of PAHs in the foods assessed in the 2000 United Kingdom TDS. An estimate of intake for the sum of the PAHs assessed as well as an estimate for benzo[a]pyrene were conducted. Only the benzo[a]pyrene intakes are presented here. The estimates were calculated using concentrations of PAHs in foods from the United Kingdom TDS and food consumption data from a 1995 Australian National Nutrition Survey ($n = 13\,858$). The survey respondents were aged 2 years and above. The survey used a 24-h recall methodology. Individual dietary records and body weights from individuals were used for the intake calculations, resulting in a distribution of intakes, from which population summary statistics were derived. The mean body weight for all respondents was 67 kg. A wide range of foods covering the main foods consumed in the Australian diet was included in the assessment. Lower- and upper-bound estimates of intake were derived for the Australian population aged 2 years and above: the lower bound where ND results were assigned a concentration of zero, and the upper bound where ND results were assigned a concentration equal to the LOR. Intake estimates for Australia are shown in Table 19. Only a small number of survey respondents were not consumers of benzo[a]pyrene for the lower-bound estimates, where consumers of foods with a zero concentration were not counted as consumers of PAHs. This means that there is a small difference in the intakes between respondents and consumers only.

Table 19. Estimated intakes of benzo[a]pyrene for Australia for consumers^a of PAHs

PAH	Mean intake		95th-percentile intake	
	Lower bound, µg/day (µg/kg bw per day)	Upper bound, µg/day (µg/kg bw per day)	Lower bound, µg/day (µg/kg bw per day)	Upper bound, µg/day (µg/kg bw/day)
Benzo[a]- pyrene	0.03 (0.005)	0.15 (0.0025)	0.07 (0.0013)	0.27 (0.0049)

^a Number of consumers: lower bound = 13 727; upper bound = 13 858.

(ii) Brazil

Information submitted to the Committee for review from Brazil contained some intake data. Pupin & Toledo (1996a) estimated intakes of benzo[a]pyrene from vegetable oils in Brazil. This was calculated using oil consumption figures based on total estimated oil consumption for the country, divided by the number of people in the population. Estimated intake of benzo[a]pyrene from soya bean oil was 0.09 µg/day and from corn oil was 0.4 µg/day, assuming the same level of consumption.

Intakes of benzo[a]pyrene from olive oils were estimated by Pupin & Toledo (1996b). Based on per capita consumption of olive oil, daily intake of benzo[a]pyrene was 0.0006 µg/day. However, based on per capita consumption for only regular consumers of olive oil in Brazil, the estimated intake of benzo[a]pyrene was 0.01 µg/day.

Intakes of a number of PAHs from a wide range of foods in Brazil were estimated by Camargo & Toledo (no date). Food consumption data were obtained from a national household economic survey of about 16 000 households surveyed over 12 months. An average diet was constructed, and the mean consumption data were used to estimate PAH intakes with analytical concentrations determined in a range of foods. Estimated intakes are shown in Table 20 for the PAHs of interest. Estimates were derived for a lower-bound mean (ND = 0), an upper-bound mean (ND = LOD) and a maximum intake (where the highest concentration from the group of foods was used, and ND results were assigned the LOD).

Table 20. Estimated intakes of PAHs in Brazil from a range of foods

PAH	Intake (µg/person per day)		
	Lower-bound mean intake	Upper-bound mean intake	Maximum intake
Benz[a]anthracene	0.42	0.47	0.65
Benzo[b]fluoranthene	0.40	0.46	0.78
Benzo[k]fluoranthene	0.24	0.26	0.40
Benzo[a]pyrene	0.40	0.42	0.77
Chrysene	0.08	0.52	0.71
Dibenz[a,h]anthracene	0.61	0.76	1.47

(iii) New Zealand

Estimated intakes of PAHs in New Zealand were also based on the concentrations of PAHs in the foods assessed in the 2000 United Kingdom TDS. An estimate of intake for the sum of the PAHs assessed as well as an estimate for benzo[a]pyrene were conducted. Only the intakes for benzo[a]pyrene are presented here. The estimates were calculated based on the concentrations of PAHs

in foods in the United Kingdom TDS and food consumption data from a New Zealand 1997 National Nutrition Survey ($n = 4636$ respondents). The survey respondents were 15 years of age and above. The survey used a 24-h recall methodology. Individual dietary records and body weights from individuals were used for the intake calculations, resulting in a distribution of intakes, from which population summary statistics were derived. The mean body weight for all respondents was 71 kg. A wide range of foods covering the main foods consumed in the New Zealand diet were included in the assessment. Lower- and upper-bound estimates of intake were derived for the New Zealand population aged 15 years and above: the lower bound where ND results were assigned a concentration of zero, and the upper bound where ND results were assigned a concentration equal to the LOR. Results are shown in Table 21. Not all survey respondents were consumers of benzo[a]pyrene at the lower bound, where consumers of foods with a zero concentration were not counted as consumers of PAHs. Children were not included in the assessment.

Table 21. Estimated intakes of PAH and benzo[a]pyrene for New Zealand for consumers^a of PAHs

PAH	Mean intake		95th-percentile intake	
	Lower bound, $\mu\text{g/day}$ ($\mu\text{g/kg}$ bw per day)	Upper bound, $\mu\text{g/day}$ ($\mu\text{g/kg}$ bw per day)	Lower bound, $\mu\text{g/day}$ ($\mu\text{g/kg}$ bw per day)	Upper bound, $\mu\text{g/day}$ ($\mu\text{g/kg}$ bw per day)
Benzo[a]- pyrene	0.04 (0.0005)	0.16 (0.0022)	0.10 (0.0012)	0.27 (0.0037)

^a Number of consumers: lower bound = 4594; upper bound = 4636.

A study in New Zealand on the PAH intake from margarines was also located in the literature (Thomson et al., 1996). For this study, 16 margarines were analysed for 16 PAHs. Average consumption of margarines for people aged 15 years and over was used to calculate the estimated intakes of PAHs from margarines. The mean consumption for margarines in New Zealand was reported as 8.3 g/day. Mean concentrations of each PAH were used for the intake calculation. The estimated intakes of seven PAHs from margarines are shown in Table 22. Estimated intakes in $\mu\text{g/kg}$ bw per day were calculated using the mean body weight for New Zealanders aged 15 years and over, as stated in the other New Zealand study outlined above. Intakes of the PAHs assessed from a broad range of foods will obviously be higher than just the intakes from margarines, as shown in Table 20.

(iv) United Kingdom

The United Kingdom estimated intakes of PAHs using a TDS approach (UKFSA, 2002). Two TDSs have included PAHs in the past — one in 1979 by the Ministry of Agriculture, Fisheries and Food and the other in 2000 by the United Kingdom Food Standards Agency. These surveys have been referred to

elsewhere in the literature. The 1979 survey results were presented by Dennis et al. (1983) (also cited in IPCS, 1998 and EC, 2002), and the Committee on Toxicology of Chemicals in Food, Consumer Products and the Environment (COT, 2002) has reviewed the results of the 2000 study and made a comparison with the 1979 study after recalculating the estimates for 1979 based on recent food consumption data and the 1979 analytical concentrations. Major differences between the two surveys are shown in Table 23. The 2000 study has also been cited in the SCOOP (2004) report.

Table 22. Estimated intakes of New Zealand adults for seven PAHs from margarines only

PAH	Estimated intake, µg/day (µg/kg bw per day)
Benz[a]anthracene	0.005 (0.00007)
Benzo[b]fluoranthene	0.009 (0.0001)
Benzo[k]fluoranthene	0.012 (0.0002)
Benzo[a]pyrene	0.005 (0.0001)
Chrysene	0.008 (0.0001)
Dibenz[a,h]anthracene	0.007 (0.0001)
Indeno[1,2,3-cd]pyrene	0.009 (0.0001)

Table 23. Differences in the 1979 and 2000 United Kingdom TDS for PAHs

Parameter	1979 TDS (Dennis et al., 1983)	2000 TDS (UKFSA, 2002)
Number of PAHs assessed	11	19
Determination of PAH concentration	Determined by analysis	Determined by analysis
Number of food groups analysed	9	20
Type of sample analysed	Composite samples for groups of foods	Composite samples for groups of foods
Food consumption data set used	Average total daily consumption of food and beverages of 1.46 kg (Buss & Lindsay, 1978)	Dietary and Nutritional Survey of British Adults, National Diet and Nutrition Survey of young people aged 4–18 years, National Diet and Nutrition Survey of children aged 1.5–4.5 years
Treatment of ND results	ND = 0 i.e. lower bound	ND = LOR i.e. upper bound

Estimates of intake were calculated for 19 individual PAHs in the 2000 study, as well as an estimate for the sum of the 19 PAHs. Eleven PAHs were considered for the original 1979 study. Only intakes of those PAHs assessed by the Committee are presented below.

The estimated intakes of PAHs from the 1979 study (Dennis et al., 1983) are shown in Table 24 as lower-bound estimates, and Table 25 shows the upper-bound estimates for the 2000 study (ND = LOR) (UKFSA, 2002) and, for comparison, the 1979 lower-bound results recalculated using more up-to-date consumption data. Results for the 2000 TDS are presented for the various population groups assessed (adults and children 15–18 years, 11–14 years, 7–10 years, 4–6 years, 3.5–4.5 years, 2.5–3.5 years and 1.5–2.5 years).

Estimates of PAH intakes in children and toddlers were also estimated in the 2000 United Kingdom TDS. Children had higher intakes per kilogram of body weight than adults for all PAHs assessed, between 2- and 2.5-fold higher. Estimated intakes per kilogram of body weight decreased with increasing age. For adults, an average body weight of 60 kg was used. For school children and toddlers, the body weight of each individual consumer was used. Estimated intakes of PAHs in units per day were not calculated.

Table 24. Estimated lower-bound^a intakes of PAHs from the 1979 United Kingdom TDS

PAH	Intake (µg/day)
Benz[a]anthracene	0.22
Benzo[b]fluoranthene	0.18
Benzo[k]fluoranthene	0.06
Benzo[a]pyrene	0.25
Chrysene	0.50
Dibenz[a,h]anthracene	0.03
Indeno[1,2,3-cd]pyrene	0

From Dennis et al. (1983)

^a Lower bound = ND results assigned a concentration of zero.

Three PAHs from the 1979 study had their estimated intakes recalculated using the diets used for the 2000 survey, in order to estimate a trend over time for PAH intakes using the different concentration data from the two surveys. Results for two of the three PAHs could be compared over time. The trends over time from 1979 and 2000 are not entirely accurate due to the use of the same consumption data set and different food groupings; however, they provide a guide as to the trend in PAH intakes. COT (2002) did the comparison and found that lower-bound intakes of benzo[a]pyrene and benz[a]anthracene are 4–5 times lower in 2000 than in 1979, both at the mean and 97.5th-percentile intakes. The upper-bound intakes for 2000 were lower than the lower-bound intakes for 1979, about 1.5-fold for benzo[a]pyrene and 3-fold for benz[a]anthracene. Phillips (1999) attributes

decreases in PAH concentrations in foods to a reduction in coal burning in the United Kingdom since introduction of the Clean Air Act of 1956 and a conversion to cleaner fuels for power generation and domestic heating.

Table 25. Estimated intakes of PAHs from United Kingdom TDSs

PAH	TDS	Population group	Mean intake, upper bound (ng/kg bw per day)	97.5th percentile intake, upper bound (ng/kg bw per day)
Benz[a]anthracene	2000	Adults	0.8	1.4
		15–18 years	0.8	1.4
		11–14 years	1.0	1.6
		7–10 years	1.4	2.2
		4–6 years	1.7	2.6
		3.5–4.5 years	1.7	2.5
		2.5–3.5 years	1.8	2.8
		1.5–2.5 years	1.8	3.1
Benz[a]anthracene	1979 ^a	Adults	2.6 ^b	4.8 ^b
		15–18 years	2.5	4.7
		11–14 years	3.1	5.3
		7–10 years	4.5	7.0
		4–6 years	5.5	8.6
		3.5–4.5 years	5.4	8.8
		2.5–3.5 years	5.7	9.8
		1.5–2.5 years	5.9	11.0
Benzo[b]fluoranthene	2000	Adults	1.5	2.6
		15–18 years	1.4	2.4
		11–14 years	1.7	2.9
		7–10 years	2.5	3.8
		4–6 years	3.2	4.8
		3.5–4.5 years	3.0	4.6
		2.5–3.5 years	3.3	5.2
		1.5–2.5 years	3.6	6.0

Table 25. (contd)

PAH	TDS	Population group	Mean intake, upper bound (ng/kg bw per day)	97.5th percentile intake, upper bound (ng/kg bw per day)
Benzo[k]fluoranthene	2000	Adults	1.3	2.2
		15–18 years	1.2	2.0
		11–14 years	1.4	2.5
		7–10 years	2.1	3.3
		4–6 years	2.7	4.3
		3.5–4.5 years	2.6	3.9
		2.5–3.5 years	2.8	4.7
		1.5–2.5 years	3.2	5.4
Benzo[a]pyrene	2000	Adults	1.6	2.7
		15–18 years	1.4	2.5
		11–14 years	1.8	3.0
		7–10 years	2.6	4.0
		4–6 years	3.3	5.0
		3.5–4.5 years	3.1	4.8
		2.5–3.5 years	3.4	5.4
		1.5–2.5 years	3.8	6.2
Benzo[a]pyrene	1979 ^a	Adults	2.4 ^b	4.4 ^b
		15–18 years	2.3	4.2
		11–14 years	2.8	4.7
		7–10 years	4.1	6.8
		4–6 years	5.0	7.8
		3.5–4.5 years	4.9	7.8
		2.5–3.5 years	5.1	8.8
		1.5–2.5 years	5.3	9.6

Table 25. (contd)

PAH	TDS	Population group	Mean intake, upper bound (ng/kg bw per day)	97.5th percentile intake, upper bound (ng/kg bw per day)
Chrysene	2000	Adults	1.6	2.7
		15–18 years	1.4	2.6
		11–14 years	1.8	3.0
		7–10 years	2.6	3.9
		4–6 years	3.3	4.8
		3.5–4.5 years	3.2	4.8
		2.5–3.5 years	3.3	5.2
		1.5–2.5 years	3.5	5.9
Dibenz[a,h]anthracene	2000	Adults	0.5	0.9
		15–18 years	0.5	0.9
		11–14 years	0.6	1.0
		7–10 years	0.9	1.4
		4–6 years	1.1	1.8
		3.5–4.5 years	1.0	1.6
		2.5–3.5 years	1.1	1.8
		1.5–2.5 years	1.2	2.1
Dibenz[a,h]anthracene	1979 ^a	Adults	0.3 ^b	0.6 ^b
		15–18 years	0.3	0.6
		11–14 years	0.4	0.7
		7–10 years	0.6	0.9
		4–6 years	0.7	1.1
		3.5–4.5 years	0.7	1.2
		2.5–3.5 years	0.7	1.3
		1.5–2.5 years	0.7	1.4

Table 25. (contd)

PAH	TDS	Population group	Mean intake, upper bound (ng/kg bw per day)	97.5th percentile intake, upper bound (ng/kg bw per day)
Indeno[1,2,3-cd]pyrene	2000	Adults	1.4	2.4
		15–18 years	1.3	2.2
		11–14 years	1.6	2.6
		7–10 years	2.2	3.6
		4–6 years	2.9	4.5
		3.5–4.5 years	2.8	4.3
		2.5–3.5 years	3.0	4.9
		1.5–2.5 years	3.4	5.8

From UKFSA (2002)

^a Recalculated using updated food consumption data used for the 2000 TDS.

^b All results for 1979 are lower bound (ND = 0).

(b) Estimated intakes of PAHs reported in the literature

There have been many assessments of PAH intakes for groups of PAHs or individual PAHs in the literature, from a range of different countries. These estimates are summarized below for the PAHs of relevance to this assessment. Many of these estimates have been included in the detailed assessments conducted by ICPS (1998), the Scientific Committee on Food (EC, 2002) or SCOOP (2004).

Benzo[a]pyrene intakes have been estimated from a number of sources, in isolation of any other PAHs. Benzo[a]pyrene is the most common PAH for which intakes have been estimated.

(i) Austria

In Austria, intakes of a range of PAHs were estimated by using a duplicate-diet study of 10 persons for a week (Pfannhauser, 1991). Results are shown in Table 26. No more details on how these estimates were calculated were provided.

(ii) Belgium

Estimated mean intakes of benzo[a]pyrene for Belgium were 0.014 µg/day (0.00023 µg/kg bw per day) for all respondents (SCOOP, 2004). This was based on a 7-day food record completed by 341 teenagers aged 14–18 years. A range of foods where there was at least one detected concentration (fats and oils, sauces, nuts, canned fish, mussels, leafy vegetables, chocolate and chocolate spread)

were included in the intake estimate; however, there were only a very limited number of samples analysed for the foods included. ND results were assigned a concentration of half of the LOQ. Therefore, this estimate is neither very robust nor representative of the whole population.

Table 26. Estimates of PAH intakes in Austria

PAH	Mean intake (µg/day per person)	Range of intakes (µg/day per person)
Benz[a]anthracene	<0.02	<0.02–0.14
Benzo[a]pyrene	0.05	<0.01–0.36
Benzo[b]fluoranthene	0.005	<0.05–1.02
Benzo[j]fluoranthene	<0.03	<0.03–0.90
Benzo[k]fluoranthene	0.04	<0.02–0.30
Chrysene	0.20	<0.03–0.90
Dibenz[a,h]anthracene	<0.02	<0.01–0.10
Indeno[1,2,3-cd]pyrene	<0.02	<0.02–0.31

(iii) *China*

Four PAHs (anthracene, flupranthene [*sic*, assumed to be fluoranthene], benz[a]anthracene and benzo[a]pyrene) were analysed in a range of core vegetables from northern China, and estimated intakes were calculated (Zhong & Wang, 2002). Coal is a major fuel for cooking, heating, industry and greenhouse warming in China and could therefore influence the PAH concentrations in foods and resulting intakes. Nine vegetables were chosen (potato, cabbage, Chinese cabbage, scallion, celery, cucumber, tomato, eggplant, wax gourd), which represent 80% of the vegetables in the northern Chinese diet. Two hundred samples were taken over a year. The number of samples of each vegetable was weighted based on the proportion of the diet it made up. Samples were analysed raw. To estimate the intakes, the mean concentration of the PAH was multiplied by the mean daily consumption of the vegetables. No information on how the consumption figures were derived was provided. Estimated intakes of relevant PAHs are shown in Table 27.

Table 27. Estimated mean intakes of PAH from vegetables in China

PAH	Intake (µg/person per day)
Benz[a]anthracene	1.52
Benzo[a]pyrene	2.04

(iv) Denmark

Intake of benzo[a]pyrene in Denmark has been estimated to be 0.016 µg/day (0.0002 µg/kg bw per day) (SCOOP, 2004). This estimate is based on a national food consumption survey in 1995, which used a 7-day weighed food record. There were 1837 respondents aged 15–80 years. Consumption of 205 food items across a broad range of major food groups was used, representing 30% of the total diet. Concentration data were from a small number of foods from Denmark, supplemented with some concentration data from Norway. This is a moderately robust estimate; however, it does not include children and is limited by the small number of samples.

(v) Finland

Intakes for a range of individual PAHs and the sum of the PAHs included in the study were calculated for Finland. The estimates of relevant PAHs are shown in Table 28 (SCOOP, 2004). These estimates are based on mean food consumption data from the 2002 Dietary Survey of Finnish Adults aged 24–64 years. The survey used a 24-h recall. Mean concentrations of the PAHs were used for the intake calculations, where ND analytical results were assigned half the LOQ. About half of the meats in the intake estimate were smoked, for which there were a large number of targeted samples from producers who had problems with high PAH concentrations in their products. Therefore, this may be an overestimate of mean intakes. However, Finland may have higher intakes than other countries in general, as they have a higher consumption of smoked foods as a part of their regular diet (SCOOP, 2004).

Table 28. Estimated mean intakes of PAHs in Finland

PAH	Intake (µg/person per day)
Benz[a]anthracene	0.76
Benzo[a]pyrene	0.27
Chrysene + triphenylene	1.11
Dibenz[a,h]anthracene	0.05
Indeno[1,2,3-cd]pyrene	0.15

(vi) France

Estimated intakes of PAHs for the French population have been calculated for benzo[a]pyrene as well as a sum of the six PAHs studied (SCOOP, 2004). Adults (1474 respondents aged 15–65 years) and children (1018 respondents aged 3–14 years) were assessed separately. Estimated intakes for benzo[a]pyrene are shown in Table 29. Mean and high-percentile intakes were estimated. The intakes were based on food consumption data from the 1999 National Individual Food Consumption survey, which used a 7-day diary record. Food samples were

grouped into 14 categories. Concentration data were for 978 samples collected between 1999 and 2002. ND results were assigned half the LOQ, which accounted for approximately 50% of samples. Intake estimates were calculated for each respondent in the survey, from which population group means and percentiles were derived.

Table 29. Estimated intakes of benzo[a]pyrene in France

PAH	Population group	Mean, µg/day (µg/kg bw per day) ^a	95th percentile, µg/day	97.5th percentile, µg/day
Benzo[a]pyrene	Adults	0.089 (0.001)	0.150	0.168
	Children	0.069 (0.002)	0.121	0.143

^a Mean body weight = 60 kg adults; 31 kg children.

Adults had higher intakes than children on a total daily basis; however, when intakes were expressed per kilogram of body weight, children had higher intakes than adults.

(vii) Germany

One estimate of benzo[a]pyrene intake for Germany was 0.02–0.14 µg/day (State Committee for Air Pollution Control, 1992).

Another estimate conducted for the former German Democratic Republic reported that the average citizen ingests up to 85 mg of benzo[a]pyrene during a lifetime (Kolarovic & Traitter, 1982). For a 60-year-old person at 60 kg bw, this would equate to around 3.88 µg/day or 0.06 µg/kg bw per day.

Mean intakes of benzo[a]pyrene ranged between 0.5 and 0.6 µg/day for German subjects in a field study conducted by Scherer et al. (2000). The methodology for estimating this intake was not reported in detail; however, it seems to have been derived based on six 7-day food records of 69 subjects (not specified if weighed or estimated food amounts) and a single concentration value for six main food groups combined. The authors noted that estimated intakes of benzo[a]pyrene from food did not correlate to the benzo[a]pyrene biomarkers assessed. This was determined to be a function of the limitations associated with the estimate for dietary PAH intake. The authors compared their estimates of intake from the diet with another conducted by the duplicate-diet method, for which estimated intakes of benzo[a]pyrene were between 0.12 and 0.18 µg/day, which was 2–3 times lower.

Other intakes for a range of PAHs have been estimated for Germany (SCOOP, 2004). These are shown in Table 30 for the relevant PAHs. The consumption data were from the national consumption study from 1985 to 1988, with 24 632 respondents aged 4 years and above. A 7-day dietary record was used for the survey. Foods included in the estimated intakes depended on which PAH was being assessed. The foods included were bacon, ham, frankfurters, smoked meat,

Table 30. Estimated intakes for a range of PAHs for all respondents for a range of population groups in Germany

PAH	Intakes, ng/day (ng/kg bw per day) ^a							
	4–6 years (girls)		10–12 years		15–17 years		18 years and above	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95
Benz[a]anthracene	6.59 (0.32)	13.30 (0.64)	15.36 (0.38)	17.09 (0.42)	13.99 (0.23)	19.02 (0.31)	28.30 (0.40)	93.11 (1.32)
Benzo[b]fluoranthene	21.89 (1.06)	75.79 (3.67)	29.31 (0.72)	103.05 (2.52)	30.83 (0.50)	123.84 (2.00)	31.52 (0.45)	127.43 (1.81)
Benzo[k]fluoranthene	7.26 (0.35)	23.75 (1.15)	9.98 (0.24)	31.89 (0.78)	10.44 (0.17)	37.53 (0.61)	11.12 (0.16)	43.02 (0.61)
Benzo[a]pyrene	23.61 (1.14)	96.38 (4.66)	52.01 (1.27)	178.73 (4.36)	53.39 (0.86)	226.99 (3.66)	66.64 (0.95)	258.39 (3.67)
Chrysene	16.32 (0.79)	44.55 (2.15)	24.88 (0.61)	57.72 (1.41)	25.81 (0.42)	62.96 (1.02)	32.67 (0.46)	113.26 (1.61)
Dibenz[a,h]anthracene	4.64 (0.22)	16.33 (0.79)	6.08 (0.15)	22.67 (0.55)	6.67 (0.11)	27.06 (0.44)	6.64 (0.09)	28.20 (0.40)
Indeno[1,2,3-cd]pyrene	11.62 (0.56)	34.75 (1.68)	15.92 (0.39)	45.21 (1.10)	15.91 (0.26)	49.10 (0.79)	17.66 (0.25)	64.92 (0.92)

P95, 95th percentile

^a When ND = ½ LOD or LOQ.

smoked fish, canned mussels, vegetable oils, margarine, coconuts, corn, some fruits, dried plums and water. Benzo[a]pyrene was the only PAH for which all these foods were included. For most of the other PAHs assessed, bacon, ham, frankfurters, smoked meat or coconuts were not included in the intake estimates.

Little information was provided on how the estimated intakes were calculated. A wide range of results were presented, including for all population, consumers only, four age groups (4–6 years [girls], 10–12 years, 15–17 years and 18 years and above), and mean and 95th percentiles for three mean concentrations based on different treatment of ND results (ND = zero; ND = $\frac{1}{2}$ LOD or LOQ; mean of positive samples only). Only the results for the means derived when ND = $\frac{1}{2}$ LOD or LOQ are presented in Table 30. The full results can be obtained from the SCOOP (2004) report. The results for consumers only have not been reported here, as the methodology for calculating these (which appears to be the summation of deterministically calculated intakes from each food group with different numbers of consumers for each group) is not statistically sound.

(viii) Greece

Voutsas & Samara (1998) reported intakes of PAHs from vegetables in Greece. PAH levels were determined in five different vegetables (cabbage, carrot, lettuce, leek and endive) by analysis. Sampling was undertaken in four sites (in an area where industry and agriculture are both prominent), twice a year for seasonal vegetables. Vegetables were prepared to a ready-to-consume state before analysis. Sixteen PAHs were analysed. Intakes were assessed for benzo[a]pyrene alone, carcinogenic PAHs and the sum of the 16 PAHs assessed. The food consumption data used for the intake calculations were the mean daily availability per capita in Greece from two household budget surveys conducted during 1981–1982 and 1987–1988, which were representative of Greek households. The authors have estimated intakes from just the five vegetables assessed (representing 3.9% of the total diet), but also from all vegetables (representing 15.3% of the total diet), assuming all vegetables have the same PAH concentrations as the five assessed. The estimated intakes for benzo[a]pyrene are shown in Table 31. Of the five vegetables studied, endive followed by lettuce were the highest contributors to PAH intake.

Table 31. Estimated intakes of benzo[a]pyrene from fresh vegetables in Greece

Vegetable	Intake ($\mu\text{g/day}$)	
	Mean ^a	Range
Five vegetables assessed	0.0012	0.0006–0.0039
All vegetables	0.0048	0.0022–0.0152

^a Based on mean food consumption and a median concentration of benzo[a]pyrene.

Other estimates of a range of PAH intakes have been estimated for Greece from olive oils and vegetable oils only (SCOOP, 2004). The relevant intakes are shown in Table 32. Mean and high-level intakes were calculated for both the total population and consumers only. The "high"-level intake was not defined as to what percentile it represents. The consumption data were average availability of foods in the Greek population collected from a household budget survey from 1998 to 1999 for 6258 households.

Table 32. Estimated intakes of PAH from olive oils and vegetable oils in Greece

PAH	Intake (µg/day)			
	Total population		Consumers only	
	Mean	High level ^a	Mean	High level ^a
Benz[a]anthracene	0.16	0.81	0.61	1.69
Benzo[b]fluoranthene	0.08	0.38	0.28	0.75
Benzo[k]fluoranthene	0.04	0.17	0.13	0.34
Benzo[a]pyrene	0.10	0.46	0.33	0.88
Chrysene	1.16	5.67	4.24	11.57
Dibenz[a,h]anthracene	0.03	0.14	0.10	0.28
Indeno[1,2,3-cd]pyrene	0.04	0.15	0.10	0.27

^a High level not defined.

Due to the limited number of foods considered in each of the Greek studies, neither of them is representative of PAH intakes from the total diet.

(ix) Italy

Four studies were located in the literature that have estimated PAH intakes in Italy (Menichini et al., 1991; Menichini, 1992; Lodovici et al., 1995; Turrio-Baldassarri et al., 1996). Only two contained any of the 13 PAHs considered. Estimated intakes of PAHs for Italy are shown in Table 33.

One estimate by Turrio-Baldassarri et al. (1996) assessed intakes of PAHs based on a 5-day model diet that was derived from data collected from a survey of 10 000 households. A broad range of foods and beverages covering all the major foods in the diet were included in the intake assessment.

Intakes of benzo[a]pyrene in Italy have also been estimated by another study (Menichini, 1992). No details of how this study was conducted were provided.

(x) Netherlands

Three estimates of PAH intake from the literature have been reviewed for the Netherlands. A Netherlands TDS (de Vos et al., 1990) was based on mean food

consumption data derived from a 14-day food consumption survey of 18-year-old men. Concentrations of 17 PAHs were analysed for 221 foods/23 food groups covering the major foods consumed making up 98% of the diet of 18-year-old males. Other estimates of intakes from the Netherlands are also reported (Vaessen et al., 1984). No details of this study were provided. Estimated intakes for relevant PAHs for the Netherlands are shown in Table 34.

Table 33. Estimates of PAH intakes in Italy

PAH	Intake (µg/day per person)				
	[1]		[2]		
			National	North-western	North-eastern
Benz[a]anthracene			0.41	0.34	0.23
Benzo[a]pyrene	0.1–0.3 ^a 0.2 ^b		0.17	0.16	0.19
Benzo[b+j+k]fluoranthene			1.10	0.42	0.50
Chrysene			1.46	1.70	1.36
Dibenz[a,h]anthracene			0.08	0.17	<0.05
Indeno[1,2,3-cd]pyrene			0.16	0.14	0.13

[1] Menichini, 1992; [2] Turrio-Baldassarri et al., 1996.

^a Adult smoker.

^b Mean.

Table 34. Estimates of PAH intake in the Netherlands

PAH	Intake (µg/day per person)				
	[1]			[2]	
	ND = 0 LB mean	ND = LOD UB mean	ND = LOD Maximum	Market basket	Duplicate diet
Benz[a]anthracene	0.20	0.36	0.65		
Benzo[a]pyrene	0.12	0.29	0.42	0.5	0.5
Benzo[b]fluoranthene	0.31	0.36	0.59		
Benzo[k]fluoranthene	0.10	0.14	0.24		
Benzo[b+j+k]fluoranthene	>0.41	>0.50	>0.83		
Chrysene					5.0
5-Methylchrysene	0.58	0.73	2.58		
Dibenzo[a,e]pyrene	0.01	0.63	0.64		
Indeno[1,2,3-cd]pyrene	0.08	0.46	0.55	0.4	<0.3

LB, lower bound; LOD, limit of detection; ND, not detected; UB, upper bound

[1] de Vos et al., 1990; [2] Vaessen et al., 1984.

Estimated intakes for a range of PAHs for the Netherlands have also been reported in the SCOOP (2004) report. Intakes for the PAHs being assessed by the Committee are shown in Table 35. These estimates were based on a 2-day dietary survey, with intakes estimated for the whole population ($n = 6250$) and children aged 1–6 years ($n = 530$). Concentration data used were those from the 2000 United Kingdom TDS (UKFSA, 2002).

Table 35. Estimated intakes for all respondents for a range of PAHs for the whole Netherlands population and children aged 1–6 years based on United Kingdom TDS data

PAH	Intake ($\mu\text{g/day}$)	
	Whole population	Children 1–6 years
Benz[a]anthracene	0.060	0.035
Benzo[b]fluoranthene	0.085	0.049
Benzo[k]fluoranthene	0.065	0.038
Benzo[a]pyrene	0.088	0.051
Chrysene	0.117	0.072
Dibenz[a,h]anthracene	0.025	0.014
Indeno[1,2,3-cd]pyrene	0.077	0.045

(xi) Norway

Intakes of PAHs from a range of foods have been estimated in Norway (SCOOP, 2004). A summary of the estimates of benzo[a]pyrene intake is shown in Table 36. Estimates for adults were based on a 1997 national dietary survey using a quantitative food frequency questionnaire. The dietary survey for children was conducted in 2000, using 4-day diary records. A small range of foods and beverages were included in the estimate, including meats, fish, bread, cereals, coffee and tea. Many of the meats and fish included were smoked.

Table 36. Mean intake of benzo[a]pyrene in Norway

Population group	Mean intake ($\mu\text{g/day}$)
Men, 16–79 years ($n = 1291$)	0.026
Women, 16–79 years ($n = 1381$)	0.017
Children, 13 years ($n = 1009$)	0.023
Children, 9 years ($n = 815$)	0.020

(xii) Spain

Falco et al. (2003) estimated intakes of PAHs in Spain. Sixteen PAHs were analysed in a broad range of foods covering the major food groups in the Spanish diet. The only PAH presented individually was benzo[a]pyrene. Estimated mean intakes for benzo[a]pyrene are shown in Table 37. Foods with concentrations less than the LOD were assigned half the LOD for calculation purposes. Food consumption information was derived from various sources. Daily intakes of the PAH were calculated by multiplying the concentration in each food by the average consumption amount for an individual. The intakes from all foods were then summed to obtain an intake for the PAH assessed. Five subpopulation groups were assessed: children, adolescents, male adults, female adults and seniors.

Table 37. Estimated mean intakes of benzo[a]pyrene for various population groups in Spain

Intake units	Children	Adolescents	Adult males	Adult females	Seniors
µg/day	0.110	0.123	0.128	0.097	0.100
µg/kg bw per day	0.005	0.002	0.002	0.002	0.002

Estimated intakes for Spain were also reported in the SCOOP (2004) report. These are reported in Table 38. These estimated intakes were based on purchase records from 6000 households. The estimates were based on only a few foods, which are indicated in Table 38. Concentrations less than the LOD or LOQ were assigned half the LOD or half the LOQ, respectively.

Table 38. Estimated mean intakes of a range of PAHs for Spain

PAH	Intake for all population (µg/day)	Foods assessed
Benz[a]anthracene	0.0454	Olive oil, mussels and tea
Benzo[b]fluoranthene	0.0798	Olive oil and mussels
Benzo[k]fluoranthene	0.0286	Olive oil and mussels
Benzo[a]pyrene	0.0333	Range of foods
Dibenz[a,h]anthracene	0.0132	Olive oil and mussels
Indeno[1,2,3-cd]pyrene	0.0138	Olive oil and mussels

(xiii) Sweden

The only estimate of intake of PAHs for Sweden was for benzo[a]pyrene. This was an estimated intake of 0.08 µg/day (Larsson, 1986). No further information

about the study or the methodology of how this figure was calculated was provided.

(xiv) United States of America

Three estimates of benzo[a]pyrene intake have been made for the USA: a mean of 0.14 µg/day and maximum of 1.15 µg/day (Butler et al., 1993); a median of 0.05 µg/day and maximum of 0.15 µg/day (Kazerouni et al., 2001); and a range of 0.16–1.6 µg/person per day (Santodonato et al., 1980). The study conducted by Butler et al. (1993) included 9–10 homes where portions of each meal consumed by one member of a household were collected for three lots of 2 weeks and analysed. The Kazerouni et al. (2001) study was based on consumption data from a food frequency questionnaire from 228 respondents as part of the National Health and Nutrition Examination Survey and analytical concentrations in foods. No details of how the Santodonato et al. (1980) study was conducted were provided.

(c) Foods contributing to estimated intakes of PAHs

Some of the studies assessed by the Committee of foods contributing to the intake of PAHs are from assessments based on “summed” PAHs and also in some cases for benzo[a]pyrene. There are fewer reports of contributing foods for the other relevant individual PAHs assessed by the Committee. Therefore, any information on major foods contributing to PAH intake has been included below.

The major foods contributing to intakes of PAHs reported by the Scientific Committee on Food (EC, 2002) were cereals and fats and oils. The main contributor determined in the IPCS (1998) review was reported to be cereals, due to its significant contribution to the diet. For the studies summarized above, it also appears to be the trend that cereals and cereal products and fats and oils, particularly vegetable oils, are major contributors to the intake of PAHs.

Major contributors to estimated intakes for Australia for summed PAHs (based on lower-bound concentrations determined in United Kingdom TDS foods) were bread and related products (18%), cereals and cereal products (16%), fruits and vegetables (15%), non-alcoholic beverages (10%), meat and poultry (5%) and liquid milk (5%). Major contributors to estimated intakes for Australia for benzo[a]pyrene were bread and bread products (40%), cereals and cereal products (30%) and fats and oils (28%).

In Brazil, one study (Camargo & Toledo, 2001) showed oil and fat products as the main contributor to PAH intakes for 10 of the 11 PAHs studied. For the other PAH, the meat group was the major contributor. Camargo & Toledo (no date) confirmed this in another study, showing 22% of the intake coming from vegetable fats and oils (soya bean oil and margarine), sugar (15%) and smoked meats (15%). The contribution from sugar is thought to arise from PAHs developing during the burning of the sugar cane before harvest.

Major contributors to estimated intakes for New Zealand for summed PAHs (based on lower-bound concentrations determined in United Kingdom TDS foods) were bread and related products (18%), fruits and vegetables (16%), cereals and cereal products (13%), non-alcoholic beverages (9%) and fats and oils (9%). Major contributors to estimated intakes for New Zealand for benzo[a]pyrene were fats and oils (40%), bread and bread products (36%) and cereals and cereal products (21%).

From the United Kingdom TDS in 1979 (Dennis et al., 1983), fats and oils contributed 50% to benzo[a]pyrene and 34% to summed PAH intakes. Cereals contributed 30% to benzo[a]pyrene and 31% to summed PAH intakes. Fats and oils contributed due to their high concentrations of PAHs and cereals due to the consumption amount in the diet. Vegetables contributed 8% to benzo[a]pyrene intakes and 12% to summed PAH intakes. In the most recent 2000 United Kingdom TDS (COT, 2002), bread and cereals were again major contributors, at 24% for benzo[a]pyrene and 35% for summed PAH intakes; all vegetables contributed 12% to benzo[a]pyrene intakes and 13% to summed PAH intakes. The contribution from fats and oils was lower than previously estimated, at 6% for benzo[a]pyrene intakes and 3% for summed PAH intakes. This is attributed to the possibility that the number of ND results has influenced the total intakes and therefore estimated contributions. Beverages contributed 28% to benzo[a]pyrene intakes and 8% to summed PAH intakes, and milk and dairy 12% to benzo[a]pyrene intakes and 9% to summed PAH intakes.

In Belgium, the main contributor to estimated intake of benzo[a]pyrene was vegetable oils and products (44%) (SCOOP, 2004).

The intake assessment for China (Zhong & Wang, 2002) was based only on consumption of vegetables. However, within the vegetables studied, scallion (24%) was the most important source of summed PAH intakes, followed by potato (13%) and cabbage (13%).

In Denmark, the major contributors to benzo[a]pyrene intakes were bread (47%), potatoes (14%) and vegetable oil (10%) (SCOOP, 2004).

In Finland, the major contributors to the individual PAH intakes were smoked meat products, bread and cereals. This pattern is seen for 15 of the 16 PAHs studied, including benzo[a]pyrene. For dibenz[a,h]anthracene, the major contributors were tea (12%) and coffee (12%), followed by beer (10%) and smoked turkey (10%) (SCOOP, 2004). For summed PAHs, smoked meat products contributed 71% of the intake, and bread and cereals, 10%.

The main foods contributing to intakes of PAHs for France were cereal products (38% for adults and 43% for children) followed by meat products (27% for adults and 25% for children) (SCOOP, 2004).

For Germany, across the PAHs studied (SCOOP, 2004), a general scan of the presented data indicated that the major contributors were fish and vegetable oils. However, many of these estimates did not include meat and meat products or cereals, found to be high contributors in many other studies. Where meats, such as frankfurter-type sausages, were included in the intake estimates, these were

among the high contributors. Depending on the PAH, fruits often came up as a contributor as well. For benzo[a]pyrene, the main contributors were meat and meat products, followed by vegetable oils. No cereals were included in the intake estimate. Owing to the nature of the data presented, where estimates from food groups did not sum to the total intake presented for each PAH, actual percent contributions could not be calculated. These contributors were based on the results where ND results were assigned zero, therefore highlighting more effectively what foods actually contributed to PAH intakes.

The studies in Greece were targeted to include either vegetables or vegetable oils; therefore, contributors from the whole diet cannot be determined. Where vegetable oils and meats were assessed for benzo[a]pyrene intakes, vegetable oils contributed 90% of the intake (SCOOP, 2004).

In Italy (Lodovici et al., 1995), cereal products (30%), meat/eggs (15%), milk products (11%), vegetables including potatoes (11%), barbecued meat (6%) and fruit (14%) were found to be highest contributors to PAH intake, because these were the most commonly consumed foods. Exact numbers were not presented in the report; the numbers provided above are approximate based on the contribution graph in the article. Barbecued foods contributed little to the estimated intakes, because the urbanized Italian population does not commonly consume these foods.

The main contributors to benzo[a]pyrene intakes for one Netherlands study (de Vos et al., 1990) were fats and oils (47%) and cereals (36%), followed by sugars and sweets (14%). For the sum of the 17 PAHs assessed, cereals contributed 27%, sugars and sweets 18% and fats and oils 14%. Another study for the Netherlands (SCOOP, 2004) found the main contributors to summed PAH intakes for the whole population to be bread (16%), vegetables (12%), meat and meat products (10%), fats and oils (10%), cereals (9%) and sugars and preserves (9%). The contribution of the fats and oils group was attributed to the elevated PAH concentrations in vegetable oils. For 1- to 6-year-olds, the major contributors were dairy products (16%), bread (15%) and sugars and preserves (9%).

For intakes of 16 PAHs in Norway, the major contributors for men 16–79 years were bread (39%), meat (24%), fish (16%) and smoked meat (12%) (SCOOP, 2004). For women 16–79 years, the main contributors were bread (39%), meat (22%), fish (18%) and smoked meat (12%). For 13-year-old children, the major contributors were meat (37%), bread (27%) and smoked meat (21%). For 9-year-old children, the major contributors were meat (36%), bread (29%) and smoked meat (23%).

Only a very small number of food groups were assessed in the Spanish study (SCOOP, 2004) — mainly olive oil and mussels. For benzo[a]pyrene, there were five food groups. Of these, the main contributors to intake were mussels (40%), olive oil (33%) and meat products (23%).

For the Swedish study (Larsson, 1986), cereals contributed 34% to the summed PAH intakes, vegetables contributed 18% and fats and oils 16%. Significant intakes were also found for smoked meat products.

In the USA diet, grilled/barbecued meats were a high contributor, at 21% of the intake of benzo[a]pyrene, after the bread and cereals group, at 29% (Kazerouni et al., 2001). Grilled/barbecued meats are a commonly consumed food in the USA.

As noted in the IPCS (1998) PAH assessment, the main contributors to PAH intakes were cereals (Dennis et al., 1983; Larsson, 1986) and fats and oils. Cereals have a low concentration of PAHs; however, their consumption is high. Smoked meats and fish, despite having higher concentrations of PAHs, were low contributors due to their low consumption in the diet (Larsson, 1986).

For the majority of studies, smoked fish and meats and barbecued foods contributed little to PAH intakes, as they were minor parts of the diets of many countries. However, they can be major contributors where these foods are a regular part of the diet.

Vegetable oils contribute due to their higher concentrations of PAH, which are generally formed during the production of the oils from drying of the seeds.

(d) Intake of PAHs from water

Intake of PAHs can occur via consumption of drinking-water. Many estimates of intake for PAHs from water relate to "summed" PAHs and also, in many cases, to benzo[a]pyrene. There are fewer reports of intake from water for the other relevant individual PAHs being assessed by the Committee.

The Committee on Pyrene and Selected Analogues (1983) stated that the intake of PAHs by humans from drinking-water is insignificant (at only 0.1% of intake) in comparison with intake from food. Surface water and groundwater may have higher concentrations of PAHs, particularly in contaminated sites; however, treatment to obtain drinking-water removes 95% of PAHs.

Drinking-water samples have been demonstrated to contain benzo[a]pyrene at up to 2.0 ng/l (IPCS, 1998). Some individual PAHs range in concentration up to several hundred micrograms per litre.

Intake of PAHs from drinking-water has been estimated at 0.006 µg/day, with a range of 0.0002–0.12 µg/day (based on 2 litres of water per day) (WHO Regional Office for Europe, 2003), and at 0.027 µg/day for total (summed) PAHs (Santodonato et al., 1981). Intake of benzo[a]pyrene from drinking-water has been estimated to be 0.0002 µg/day (IPCS, 1998) and 0.0001–0.001 µg/day (Santodonato et al., 1981; Lioy et al., 1988) .

It is specified in the WHO *Guidelines for Drinking-water Quality* (WHO, 2004) that the level of benzo[a]pyrene in drinking-water corresponding to an upper-bound excess lifetime risk of cancer of 10^{-5} is 0.7 µg/l. Guidelines for other PAHs have not been set due to insufficient data.

Estimates of PAH intakes from drinking-water alone tend to be smaller than estimated intakes from food.

7.2 *Estimates of non-dietary exposure*

Exposure to PAHs can occur via inhalation (including from environmental contamination or cigarette smoking) and dermal contact. Many estimates of non-dietary exposure relate to "summed" PAHs and also, in many cases, to benzo[a]pyrene. There are fewer reports of non-dietary exposure for the other relevant PAHs being assessed by the Committee.

PAHs occur in the environment as a result of both natural and human-made sources, including volcanoes, forest fires, residential fires, motor cars and industry (WHO Regional Office for Europe, 2003). Concentrations in the air vary with the season (Pastorelli et al., 1999), being higher in winter than in summer due to residential wood fires, and vary with the area, such that concentrations are higher in urban or industrialized areas in comparison with rural areas (IPCS, 1998).

PAH concentrations in the air range between 1 and 30 ng/m³ in urban areas and are up to 200 ng/m³ in cities with a lot of traffic. Indoor air usually has concentrations of between 1 and 50 ng/m³ (IPCS, 1998). Concentrations of benzo[a]pyrene indoors are 1.0 ng/m³ in homes with cigarette smokers and 0.4 ng/m³ in homes of non-smokers. Benzo[a]pyrene concentrations are 1.07 ng/m³ in commercial buildings in a smoking environment and 0.39 ng/m³ in non-smoking buildings (Scherer et al., 2000). In countries where there is a move away from wood fires to gas or oil heating, concentrations of PAHs in the air are decreasing, mainly due to changes in heating systems and kinds of fuel used (WHO Regional Office for Europe, 2003).

PAH intake from air varies depending on the area in which humans live, in relation to traffic, pollution and industry, as well as human activities, such as smoking and type of heating used. Intakes of PAHs from ambient air are estimated to be 0.16 µg/day (median), with a range of 0.02–3 µg/day (WHO Regional Office for Europe, 2003). Another estimate of intake of PAHs via inhalation is between 10 and 50 ng/day, after assessments over 14 days (IPCS, 1998). Benzo[a]pyrene intakes from air have been reported to range between 0.0005 and 1 µg/day, depending on the environmental conditions (IPCS, 1998), and between 0.002 and 0.06 µg/day (EC, 2002). Intakes of other PAHs from air are reported to range between 0.0005 and 0.2 µg/day (IPCS, 1998). Passive smokers breathing indoor air for 20 h/day, with a cigarette smoking room next door, based on human intake of 18 m³ of air per day, had intakes of 6.4 µg/day for 14 PAHs and 0.33 µg/day for benzo[a]pyrene (German Ministry of Environment, 1979). Scherer et al. (1990) also estimated an intake of 11 ng of benzo[a]pyrene per day from air.

In developing countries, the release of PAHs during residential heating and cooking is an important cause of contamination when biomass is burnt in relatively simple stoves (IPCS, 1998).

Occupationally exposed persons have higher exposures to PAHs than the general population. Occupational exposure occurs through inhalation and dermal absorption. Concentrations of PAHs in the air are up to 470 µg/m³ in petroleum refineries and up to 430 µg/m³ in copper mines. Whereas concentrations in the air

at an aluminium smelter were up to $9.6 \mu\text{g}/\text{m}^3$, concentrations in the workers' urine remained low (IPCS, 1998).

Cigarette smokers appear to have higher exposures to benzo[a]pyrene (based on biomarkers) than non-smokers (Scherer et al., 2000). Smoking a packet of cigarettes per day increases exposure by 2–5 $\mu\text{g}/\text{day}$ (WHO Regional Office for Europe, 2003). Benzo[a]pyrene intake from 20 cigarettes per day has been reported as being between 0.15 and 0.75 $\mu\text{g}/\text{day}$ (Scherer et al., 1990) and as 0.21 $\mu\text{g}/\text{day}$ (EC, 2002). Passive smoking for 3 h can contribute to an additional exposure to benzo[a]pyrene of 0.0018 μg (Scherer et al., 2000) and for 5 h/day, an additional 0.04 $\mu\text{g}/\text{day}$ (EC, 2002).

There are limited reported estimates of dermal exposure to PAHs. There are reports of between 20% and 75% of pyrene exposure being attributed to the dermal route in occupationally exposed people (WHO Regional Office for Europe, 2003). Use of coal tar-containing shampoos can result in an exposure to benzo[a]pyrene at 0.45 $\mu\text{g}/\text{kg}$ bw via absorption, assuming a concentration of benzo[a]pyrene at 56 mg/kg, 70 kg bw and 3% dermal absorption (van Schooten et al., 1994).

7.3 Comparative estimates of intake from different sources

It is difficult to estimate the relative proportions of PAH intake contributed by different sources. This is due to the range of methodologies used to estimate each source and the different population groups used to estimate intakes from each source, which means that intakes from different sources cannot simply be added together and contributions calculated. Comparative estimates have generally been expressed on a total/summed PAH basis.

For non-occupationally exposed people, the main sources of PAH exposure are diet, air, cigarette smoking and coal tar-containing medications, with diet estimated to contribute 120–2800 ng/day, cigarette smoking about 200 ng/day (from one packet), air about 2% of total exposure, water 1% of exposure and passive smoking <1% of exposure (Scherer et al., 2000). Phillips (1999) reported that for non-smokers, more than 70% of exposure comes from the diet.

The Scientific Committee on Food review (EC, 2002) presented a comparison of PAH intakes from different sources (food, water and air). The findings are shown in Table 39. The data show that food is the major source of intake for a range of PAHs for non-smoking humans. For cigarette smokers, the proportion of smoking and food to PAH intake may be of a similar size (EC, 2002).

In an Italian study by Lodovici et al. (1995), the authors concluded that the daily intake of PAHs from food (at approximately 3 $\mu\text{g}/\text{day}$ for summed PAHs and 1.4 $\mu\text{g}/\text{day}$ for "carcinogenic" PAHs) was about 10 times more than the daily intake from air (0.37 $\mu\text{g}/\text{day}$ for summed PAHs and 0.13 $\mu\text{g}/\text{day}$ for "carcinogenic" PAHs). Based on the limited data with which to make these calculations, the authors tentatively concluded that food is the major source of PAH exposure for humans.

Table 39. Estimate of mean daily intake by different routes for an adult non-smoker

PAH	Mean intake (ng/person per day)		
	Food	Drinking-water	Air
Anthracene	<30–640		20
Benz[a]anthracene ^a	<20–410	0.2–10	20
Benzo[a]pyrene ^a	50–290	0.2–2	20
Benzo[e]pyrene	200		20
Benzo[b]fluoranthene ^a	5–360	0.1–2	20
Benzo[j]fluoranthene ^a	<30	0.02–0.2	
Benzo[k]fluoranthene ^a	40–140	0.02–2	20
Benzo[b+j+k]fluoranthene	<70–1100		60
Benzo[ghi]perylene	120–360	0.2–2	20
Chrysene ^a	200–1530	200	20
Dibenz[a,h]anthracene ^a	<10–80		2
Fluoranthene	600–1660	2–200	100
Indeno[1,2,3-cd]pyrene ^a	<20–460	0.2–2	20
Phenanthrene	<330–4510		400
Pyrene	600–1090	0.2–200	100

From EC (2002)

^a PAHs determined by the Committee to be genotoxic and carcinogenic (see section 10.2).

For benzo[a]pyrene, with a total mean estimated intake of 0.2 µg/person, 90% of the intake was estimated to come from food, less than 1% from drinking-water and the rest from air (EC, 2002).

The Committee on Pyrene and Selected Analogues (1983) reported intakes of total (summed) PAHs to be 0.207 µg/day from air, 0.027 µg/day from water and 1.6–16 µg/day from food. For benzo[a]pyrene, reported intakes were 0.0095–0.0435 µg/day from air, 0.0011 µg/day from water and 0.16–1.6 µg/day from food. These figures concur with other reports that food is the major source of PAH intakes.

Reference to a study in North America by the WHO Regional Office for Europe (2000) had similar findings, with mean total intake of PAHs of 3.12 mg/day, of which food contributed about 96%, air 2%, water 0.2% and soil 0.4%.

7.4 Summary of intake estimates

There is a large amount of variation in the way in which the estimates of PAH intakes have been calculated. This includes differences in the food consumption

data, the analytical methodology, food groupings, the range of foods included, sample collection, the treatment of ND results, the range of PAHs considered and the methodology used to calculate the estimated intake. Therefore, direct comparisons cannot be made between PAH intakes of different countries, or even within countries that have conducted more than one intake study.

However, the range of estimated intakes can give an indication of the possible range of PAH intakes that are likely for the PAHs assessed. For 3 of the 13 PAHs assessed, there were no intake estimates determined on a national level. These were dibenzo[a,h]pyrene, dibenzo[a,i]pyrene and dibenzo[a,j]pyrene. Table 40 provides a summary of the range of intake estimates from food (and water, where included in the study) for the other 10 PAHs assessed from all of the studies reviewed. The table includes summaries from intake assessments that incorporated data on specific foods as well as the total diet. The minimum and maximum intakes from any study for each PAH are shown. Some of the studies reviewed reported intakes in units of $\mu\text{g}/\text{person per day}$, and others in units of $\mu\text{g}/\text{kg bw per day}$. Therefore, the range of intakes presented in $\mu\text{g}/\text{person per day}$ may not be from the same study as those expressed in $\mu\text{g}/\text{kg bw per day}$ and therefore are not directly divisible by an average body weight.

Estimated intakes of benzo[a]pyrene ranged from <1 to $2.0 \mu\text{g}/\text{day}$ and from 0.0001 to $0.006 \mu\text{g}/\text{kg bw per day}$. For the other nine PAHs, intakes ranged from <1 to about $12 \mu\text{g}/\text{day}$ and from 0.0001 to $0.015 \mu\text{g}/\text{kg bw per day}$.

The main conclusions made by the Scientific Committee on Food (EC, 2002) were that intakes of PAHs spanned 3 orders of magnitude, up to some micrograms per day, with the highest estimated intakes being from the lower-molecular-mass PAHs (three and four rings). Comparison of intake estimates reported in this review by the Committee also demonstrated that estimated intakes varied greatly. Of the 10 PAHs with intake data, the PAHs with the largest estimated intakes ($>2 \mu\text{g}/\text{day}$: 5-methylchrysene and chrysene) had molecular masses lower than that of benzo[a]pyrene.

The Committee decided to apply a surrogate approach to the evaluation, in which benzo[a]pyrene was used as a marker of exposure to the 13 PAHs being assessed. Therefore, intakes of benzo[a]pyrene were assessed in isolation of the other PAHs. It was decided that intakes from studies that included a broad range of food groups covering the whole diet would provide better estimates of benzo[a]pyrene intake. Therefore, a separate determination of the range of intakes was conducted using only those studies (Table 41). These studies included foods that were "ready to eat" (e.g. cooked meat) and therefore included the likely concentrations of PAHs that arise due to cooking of food. From this analysis, mean intakes of benzo[a]pyrene ranged from 0.0014 to $0.42 \mu\text{g}/\text{day}$ and from 0.0002 to $0.005 \mu\text{g}/\text{kg bw per day}$.

Intakes of benzo[a]pyrene were similar to those estimated in the previous review by the Committee, with the upper end of the range being slightly higher.

Table 40. Summary of estimated intakes of 10 of the 13 PAHs considered by the Committee to be carcinogenic and genotoxic^a

PAH	No. of estimates of intake (No. of countries)	Lowest reported intake, µg/day (µg/kg bw per day)	Range of reported means, µg/day (µg/kg bw per day)	Range of reported 95th percentiles, µg/day (µg/kg bw per day)	Highest reported intake, µg/day (µg/kg bw per day)
Benz[a]anthracene	12 (9)	0.005 (0.00007)	0.0006–0.47 (0.00007–0.0018)	0.013–0.093 (0.00031–0.00132)	1.7 (0.003)
Benzo[b]fluoranthene	11 (9)	0.009 (0.0001)	0.005–0.46 (0.00045–0.0036)	0.075–0.127 (0.0081–0.0036)	1.0 (0.004)
Benzo[j]fluoranthene	1 ^b (1)	<0.030 (<0.0005) ^c	<0.030 (<0.0005) ^c	–	0.9 (0.015) ^c
Benzo[k]fluoranthene	11 (9)	0.007 (0.0002)	0.007–0.26 (0.00016–0.0032)	0.023–0.043 (0.00061–0.0015)	0.4 (0.005)
Benzo[a]pyrene	32 (18)	0.0006 (0.0001)	0.0006–2.04 (0.0001–0.005)	0.096–0.27 (0.0012–0.0047)	2.04 (0.006)
Chrysene	8 (8)	0.008 (0.0001)	0.008–5.0 (0.0001–0.0035)	0.045–0.113 (0.001–0.0021)	11.6 (0.006)
Dibenz[a,h]anthracene	10 (9)	0.005 (0.0001)	0.0046–0.76 (0.00009–0.0012)	0.016–0.028 (0.0004–0.00079)	1.5 (0.002)
Dibenzo[a,e]pyrene	1 (1)	0.010 (0.00017) ^c	0.01–0.63 (0.00017–0.011) ^c	–	0.64 (0.011) ^c
Indeno[1,2,3-cd]pyrene	9 (8)	0.009 (0.0001)	0.009–0.46 (0.0001–0.0034)	0.034–0.064 (0.00079–0.0017)	0.55 (0.006)
5-Methylchrysene	1 (1)	0.580 (0.0097) ^c	0.58–0.73 (0.0097–0.012) ^c	–	2.6 (0.040) ^c

^a The range of intakes presented in µg/day may not be from the same study as those expressed in µg/kg bw per day and therefore are not directly divisible by an average body weight.

^b Two additional estimates of intake were presented as benzo[b+j+k]fluoranthene; therefore, they have not been included in this summary.

^c No estimates of intake reviewed were expressed in µg/kg bw per day for this PAH. Therefore, intakes in µg/day were divided by 60 kg to determine a likely intake in µg/kg bw per day.

Table 41. Summary of estimated intakes of benzo[a]pyrene from studies covering the range of major food groups in the diet

PAH	No. of estimates of intake (No. of countries)	Range ^a of reported means, µg/day (µg/kg bw per day)	Range ^a of reported 95th percentiles, µg/day (µg/kg bw per day)	Highest reported intake, µg/day (µg/kg bw per day)
Benzo[a]pyrene	16 (13) ^b	0.0014–0.42 (0.0002–0.005)	0.07–0.27 (0.0012–0.0049)	0.77 ^c (0.0062)

^a The range of intakes presented in µg/day may not be from the same study as those expressed in µg/kg bw per day and therefore are not directly divisible by an average body weight.

^b Australia, Austria, Belgium, Brazil, Denmark, Finland, France, Italy, Netherlands (*n* = 4), New Zealand, Norway, Spain, United Kingdom.

^c Equates to an intake of 0.013 µg/kg bw per day for a 60-kg person.

7.5 Conclusions on dietary intake

It is difficult to compare intakes of PAHs from different studies for a number of reasons. Overall, the Committee concluded that there was considerable variation in the intake assessments; however, from the range of estimated intakes of benzo[a]pyrene, the Committee selected the value of 0.004 µg benzo[a]pyrene/kg bw per day as being representative of a mean intake for use in the risk characterization for the present evaluation.

The highest reported intake of benzo[a]pyrene from any study in µg/day was 0.77 and in µg/kg bw per day was 0.0062 (from different studies) (Table 41). If the intake of 0.77 µg/day were divided by a body weight of 60 kg, this would result in a higher estimate on a body weight basis of 0.013 µg/kg bw per day. On the basis of these data, the Committee identified a high-level intake of 0.010 µg benzo[a]pyrene/kg bw per day for use in the risk characterization for the present evaluation.

Major contributing foods for the majority of intake assessments tend to be cereals and vegetable fats and oils, followed by vegetables. Cereals are a contributor because they make up a major part of the diet, and vegetable fats and oils are major contributors due to their relatively higher concentration of PAHs. Generally, despite their usually higher concentrations of PAHs, smoked fish and meats and barbecued foods do not contribute significantly, particularly where they are a small component of the diet. They do, however, make larger contributions leading to higher PAH intakes where these foods make up a larger part of the diet.

It was also noted by the Committee that there may be some regions of the world that have higher intakes than the "whole of diet" estimates presented in Table 41. One study reviewed from northern China calculated intakes of PAHs from vegetables only. This area of China uses a lot of coal fuel for cooking, heating and greenhouse warming. The intake of benzo[a]pyrene in this study was estimated at about 2 µg/day. It was noted that a consumption of vegetables of about 440 g/day was used to calculate the estimated intake, although data submitted to the Committee from the Chinese National Nutrition and Health Survey

(2005) indicated that actual consumption of vegetables is about 270 g/day. Therefore, actual intake of benzo[a]pyrene from vegetables alone for this part of China is likely to be about 1 µg/day. This is in the same order of magnitude as the intake of 0.77 µg/day reported as being the highest intake from the major foods in the diet.

A small number of studies included intake estimates for children. From the intake estimates reviewed, the results showed that children have higher intakes of PAHs per kilogram of body weight compared with adults (UKFSA, 2002; Falco et al., 2003; EC, 2004), approximately 2–2.5 times higher. Children usually have higher intakes per kilogram of body weight based on their lower body weight and higher food consumption per kilogram of body weight compared with adults.

Trends in intakes of PAHs over time can be determined only for the United Kingdom (from TDSs conducted in 1979 and 2000). While not directly comparable due to the different methodologies used to estimate the intakes, generally it could be concluded, for the three PAHs compared, that intakes have decreased between 1979 and 2000 (COT, 2002).

8. PREVENTION AND CONTROL

The amount of PAHs formed during cooking or processing of food depends markedly on the conditions used. Direct contact of oil seeds or cereals with combustion products during drying processes has been found to result in the formation of PAHs and should therefore be avoided. Charcoal grilling and smoking are the two major processing methods that can result in the formation of high amounts of total carcinogenic PAHs, while no carcinogenic PAHs are detected in steaming and liquid smoke flavouring (Chen, 1997). Simple practices such as selecting preferentially lean meat and fish and avoiding contact of foods with flames for barbecuing, using less fat for grilling and cooking at lower temperature for a longer time result in a significantly reduced contamination of foods by PAHs (Lijinsky & Ross, 1967; Knize et al., 1999). Broiling (heat source above) can significantly reduce PAH levels. Fat should not drip down onto an open flame, sending up a column of smoke that coats the food with PAHs. The use of medium to low heat and placement of the meat farther from the heat source can greatly reduce contamination with PAHs. The intensity of flavour is not necessarily associated with the depth of the brown colour of grilled foods. It is therefore not necessary to overcook the food to get the flavour. However, cooking must always remain effective as regards inactivation of any possible contaminating bacteria or endogenous toxins. If proper precautions against fat pyrolysis are taken and out-of-the-ordinary fuels such as crumpled paper are not used, PAH contamination is substantially reduced (Fretheim, 1983). It is suggested that PAHs in roasted meat products may be removed by being in contact with the packaging film and then eliminated by UV radiation (Chen & Chen, 2005).

The PAH contamination of smoked foods can be significantly reduced by replacing direct smoking (with smoke developed in the smoking chamber, traditionally in smokehouses) with indirect smoking. The latter is obtained by an

external smoke generator that, in modern industrialized kilns, is operated automatically under properly controlled conditions (Karl & Leinemann, 1996).

JECFA has specified a maximum limit of 2 mg/kg for benzo[a]pyrene in smoke flavourings (FAO, 2001), while the European Economic Community has set a maximum benzo[a]pyrene limit of 0.03 mg/kg of foodstuff or beverages as consumed, as a result of the use of smoke flavourings (EEC, 1988; CEC, 2002). The levels of PAHs in crude edible oils vary widely, and refining (based on the deodorization step) reduces the concentration of a number of the lower-molecular-mass compounds such as fluoranthene, while no corresponding effect is observed for the higher-molecular-mass PAHs. The level of the latter may be reduced by treatment with activated charcoal (Larsson et al., 1987), and this refining method has been reported to be widely used (Dennis et al., 1991). A study on the effect of processing on the reduction of benzo[a]pyrene levels in corn oil showed that the grinding process of the corn grains may also influence the level of PAHs in the refined oils: lower levels of benzo[a]pyrene were found in crude oils obtained from wet grinding grains as compared with those from dry grinding (Camargo & Toledo, 1999).

The waxy surface of vegetables and fruits can concentrate low-molecular-mass PAHs mainly through surface adsorption. The concentrations of PAHs are generally greater on plant surface (peel, outer leaves) than on internal tissue. Consequently, washing or peeling may remove a significant proportion of the total PAHs. Particle-bound high-molecular-mass PAHs that remain on the surface are easily washed off, whereas low-molecular-mass compounds that are in the vapour phase can penetrate the waxy layer of fruits and vegetables and are less efficiently removed by washing.

In summary, effective prevention and control measures include the following:

- Avoid dripping fat directly onto the heat source.
- Maintain a certain distance between the food and the heat source.
- Minimize contact of foods with combustion gases.
- Replace direct smoking with indirect smoking.
- Avoid fire drying of seeds, and seek alternative drying techniques.
- Wash or peel vegetables and fruits prior to consumption.

9. DOSE-RESPONSE ANALYSIS AND ESTIMATION OF CARCINOGENIC RISK

9.1 Contribution of above data to assessment of risk

9.1.1 Pivotal data from biochemical and toxicological studies

Because several PAHs are genotoxic and carcinogenic, a threshold approach to the evaluation is not appropriate. At its sixty-fourth meeting, the Committee

agreed on general considerations for formulation of advice on compounds that are both genotoxic and carcinogenic (see Annex 4).

9.1.2 Pivotal data from human clinical/epidemiological studies

An association between PAH exposure and cancer risk has been mainly considered in the occupational setting, as an environmental pollutant and as a component of tobacco smoke. The most consistent evidence concerns the association between PAH exposure from several occupations and risk of lung cancer, ascertained in several cohort studies, where PAH level was estimated by means of measurements of time-weighted air concentrations at the workplace. However, this does not contribute to dietary risk assessment of PAHs, other than qualitatively. Regarding the association between oral exposure to PAHs and cancer risk, a small study related a high consumption of wine in tar-impregnated bottles with an increased risk of gastric cancer. Consumption of well done cooked meat has been associated with increased risk of colorectal cancer and lung cancer. Although PAHs may occur in cooked meat, there are other potential carcinogens, such as nitrosamines and heterocyclic amines, that could explain these results. The evidence concerning oral exposure to PAHs is indirect, is unconvincing and does not include quantitative exposure data. Thus, it is not suitable for use in the risk assessment of PAHs.

9.1.3 Biomarker studies

Urinary excretion of 1-hydroxypyrene and levels of PAH adducts to DNA and proteins measured in blood have mainly been used to biomonitor occupational exposure. Levels of both 1-hydroxypyrene in urine and adducts in blood were consistently higher among exposed workers and, in most cases, were positively correlated with air concentrations of PAHs at the workplace. DNA adducts and albumin adducts were also correlated with measured airborne benzo[a]pyrene levels from ambient air pollution in non-occupationally exposed populations. However, these results are not useful to establish any relationship between the level of biomarkers and oral exposure to dietary PAH intake. Two small studies did not find a relationship between estimated dietary intake and any PAH biomarker. It must be noted that, so far, there is no study specifically designed to assess the relationship between dietary exposure to PAHs and the level of PAH biomarkers.

9.2 General modelling considerations

In line with the Committee's general considerations for formulation of advice on compounds that are both genotoxic and carcinogenic (see Annex 4), dose-response modelling of toxicological data was used to determine a basis for hazard characterization. The dose-response data from carcinogenicity studies conducted in rodents were used to derive a lower confidence limit (BMDL) of the benchmark dose (BMD) for a 10% incidence of tumours, as described in Annex 3 to the report of the 64th meeting (Annex 1, reference 174), reproduced here as Annex 5.

9.2.1 Selection of data

For the risk assessment of PAHs, dose–response modelling was applied to tumour incidence data from two oral studies: the mouse study of Culp et al. (1998) and the rat study of Kroese et al. (2001). In both studies, the animals were administered purified benzo[a]pyrene, while Culp et al. (1998) also applied two coal tar mixtures. The BMD and BMDL resulting from various models are reported in this monograph.

(a) Mouse study (Culp et al., 1998)

Dose–responses have been modelled for forestomach and lung tumours and for total tumour-bearing animals in the study of Culp et al. (1998), in which mice were fed diets containing benzo[a]pyrene and two coal tar mixtures. In the dose–response analysis of this study as presented below, the two highest dose levels of coal tar mixture 1 were omitted, due to the fact that all animals in these dose groups died before the end of the study.

Figure 2 shows the incidence of forestomach tumours as a function of the benzo[a]pyrene dose for mixture 1, mixture 2 and pure benzo[a]pyrene, fitted by the Proast M4 model, assuming the “slope” of the dose–response to be dependent on the mixture. Table 42 summarizes the results for other models fitted to the same data.

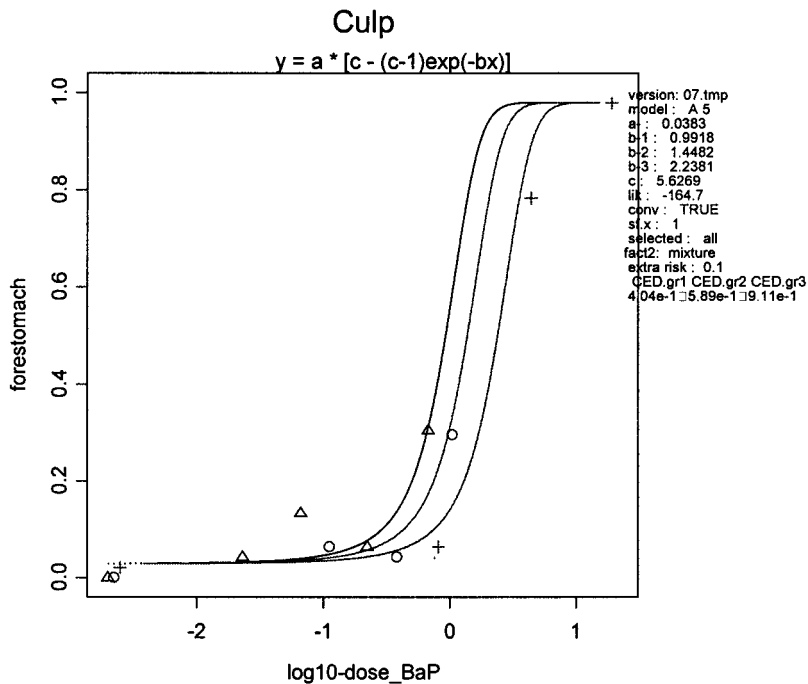
Figure 3 shows the incidence of forestomach tumours as a function of the benzo[a]pyrene dose for mixture 1 and mixture 2, applying the Proast M4 model to fit the data and assuming the “slope” of the dose–response to be dependent on the mixture. Pure benzo[a]pyrene is not included in this analysis, as it did not induce lung tumours. The fits of the other models are summarized in Table 43.

Figure 4 shows the incidence of tumour-bearing animals for mixture 1 and mixture 2, with the log-logistic model fitted to the data. No significant differences were found between the two mixtures regarding the dose–response; therefore, the data of the two mixtures were taken together. Pure benzo[a]pyrene is not included in this analysis, as no data for tumour-bearing animals were available. The fits of the other models are presented in Table 44.

(b) Rat study (Kroese et al., 2001)

The dose–responses for the liver tumours and for tumour-bearing animals observed in the oral (gavage) study in rats (Kroese et al., 2001) have been modelled. Males and females were both included in this analysis. As no significant difference between males and females was found, the dose–response modelling was based on the combined data of both sexes. Figure 5 shows the observed incidence of liver tumours with the Proast M4 model fitted to them. Table 45 summarizes the results for the other models.

Figure 2. Mouse forestomach tumours induced by benzo[a]pyrene and two coal tar mixtures, plotted against log-dose with Proast M4 fitted to the data, assuming different slopes for mixture 1, mixture 2 or benzo[a]pyrene



Right curve, plusses: pure benzo[a]pyrene; middle curve, circles: mixture 2; left curve, triangles: mixture 1. CED = BMD₁₀. gr1, r2, gr3 = mixture 1, mixture 2, pure benzo[a]pyrene , respectively.

Table 42. Summary of dose–response modelling results for mouse forestomach tumours when simultaneously fitting the same model to the data for two mixtures and pure benzo[a]pyrene

Model	log-lik	Number of parameters	Dose (mg/kg bw per day)		
			BMD (BMDL)	BMD (BMDL)	BMD (BMDL)
			Mixture 1	Mixture 2	Benzo[a]pyrene
One-stage	-168.40	4			
Two-stage	-168.28	5			
Log-logistic	-164.70	5	0.40 (0.31)	0.59 (0.45)	0.91 (0.62)
Log-probit	-168.19	5			

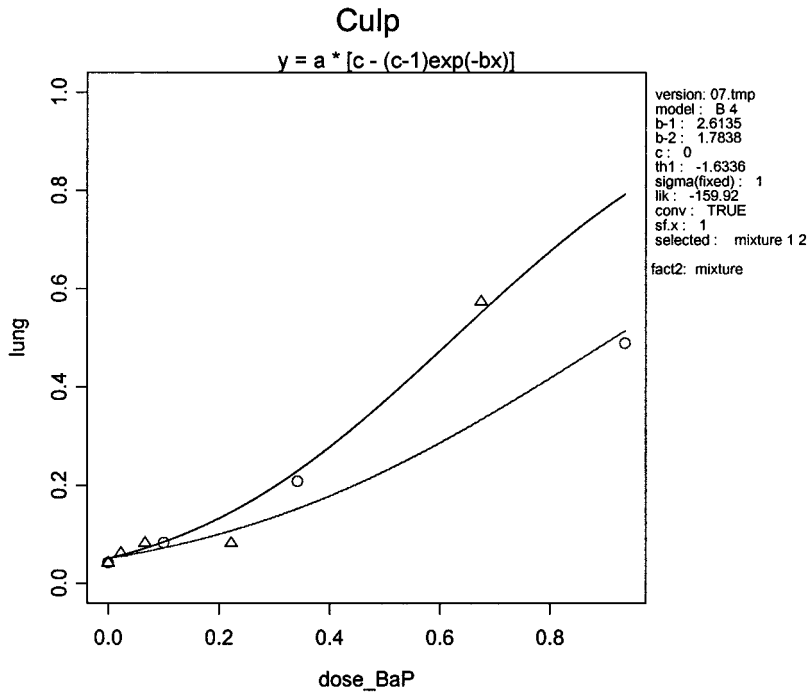
Table 42. (contd)

Model	log-lik	Number of parameters	Dose (mg/kg bw per day)		
			BMD	BMD	BMD
			(BMDL)	(BMDL)	(BMDL)
			Mixture 1	Mixture 2	Benzo[a]pyrene
Weibull	-165.41	5	0.21 (0.16)	0.37 (0.27)	0.40 (0.31)
Proast M2	-185.95	4			
Proast M4	-163.71	5	0.36 (0.29)	0.53 (0.43)	0.92 (0.74)
Saturated model ^a	-156.44	13			

log-lik, log-likelihood

^a The result of the saturated model is used to select the best-fitting other models (see Annex 5).

Figure 3. Observed incidences of lung tumours in mice fitted with the Proast M4 model, assuming different slopes between the two mixtures



Triangles: mixture 1, circles: mixture 2.

Table 43. Summary of dose-response modelling for mouse lung tumours resulting from administration of coal tar mixtures

Model	log-lik	Mixture-dependent parameter	Number of parameters	Dose (mg/kg bw per day)
				BMD (BMDL), combined data for mixtures 1 and 2
One-stage	-163.11	None	2	0.14 (0.11)
One-stage	-162.26	Slope	3	
Two-stage	-162.16	None	3	
Log-logistic	-161.63	None	3	0.24 (0.15)
Log-probit	-161.60	None	3	0.26 (0.20)
Log-probit		Slope	4	
Weibull	-162.01	None	3	0.22 (0.15)
Proast M2	-163.16	None	2	
Proast M4	-161.14	None	3	0.21 (0.16)
Proast M4	-159.92	Slope	4	

log-lik, log-likelihood

Figure 4. Incidence of tumour-bearing animals for mixture 1 and mixture 2, with the log-logistic model fitted to the data

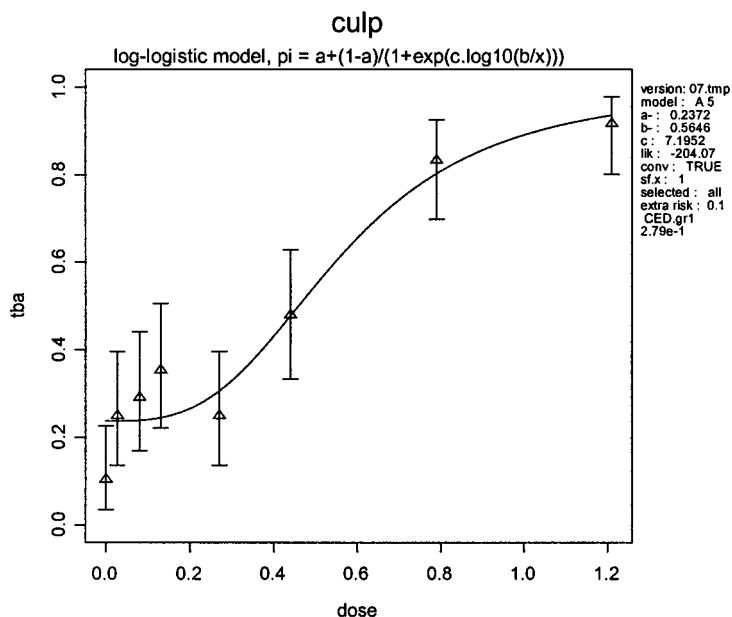


Table 44. Summary of dose-response modelling results for tumour-bearing animals, mixture 1 and mixture 2

Model	Mixture-dependent parameter	log-lik	Number of parameters	Dose (mg/kg bw per day)
				BMD (BMDL), combined data for mixture 1 and mixture 2
One-stage	None	-206.41	2	
Two-stage	Slope	-204.21	3	0.14 (0.12)
Log-logistic	None	-204.07	3	0.28 (0.20)
Log-logistic	Background	-202.64	4	
Log-logistic	Slope	-204.03	4	
Log-probit	Slope	-204.32	4	0.29 (0.23)
Weibull	Slope	-204.53	4	0.18 (0.11)
Proast M2	Slope			
Proast M4	None	-204.09		0.13 (0.10)
Saturated model ^a		-198.82	8	

log-lik, log-likelihood

^a The result of the saturated model is used to select the best-fitting other models (see Annex 5).

For the number of tumour-bearing rats, no statistically significant differences were found between males and females, and the models were fitted to the combined data of both sexes. Figure 6 shows the observed incidences with the log-logistic model fitted to them. Table 46 summarizes the results for the other models.

9.3 Potency estimates

Because a variety of PAHs are found together and because, at least to some extent, different PAHs act by the same mechanism, it is necessary to evaluate the toxicity of mixtures together. There are two general approaches to this problem. The surrogate approach uses a single component as the measure of concentration in relation to the response of the whole mixture. The second technique evaluates the mixture as the sum of its parts by scaling the dose of each component by its potency relative to that of a standard compound.

9.3.1 The surrogate approach

This method involves using a single compound to characterize the toxicity of the mixture and a single factor to account for the greater toxicity of the mixture. This approach is relatively simple to employ, since it does not require toxicological

Figure 5. Observed incidence of liver tumours in rats (both sexes) fitted with the Proast M4 model

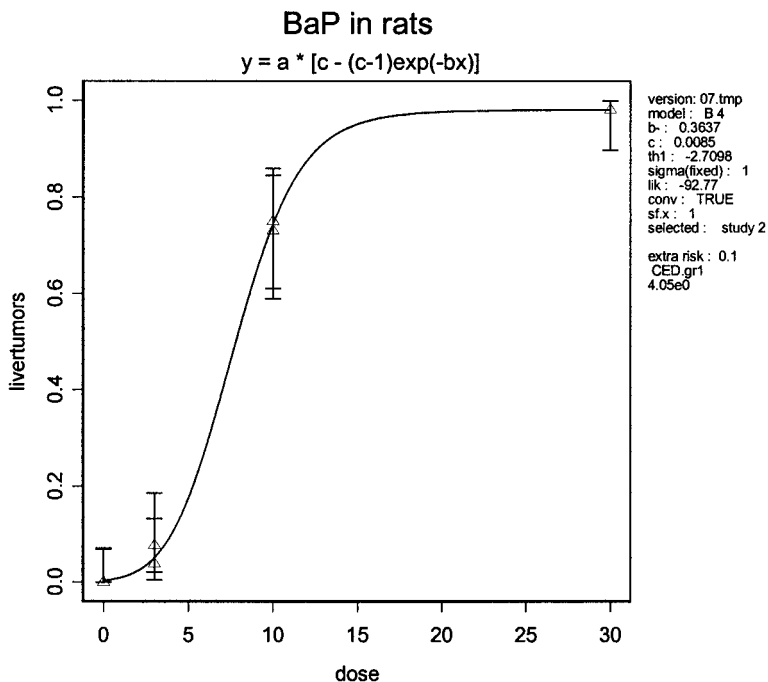


Table 45. Summary of dose-response modelling results for liver tumours in rats

Model	log-lik	Number of parameters	Dose (mg/kg bw per day)
			BMD (BMDL)
One-stage	-110.98	2	
Two-stage	-103.29	3	
Log-logistic	-92.59	3	3.5 (3.1)
Log-probit	-93.32	3	3.4 (2.9)
Weibull	-100.49	3	
Proast M2	-116.48	2	
Proast M4	-92.77	3	4.0 (3.4)
Saturated model ^a	-91.99	8	

log-lik, log-likelihood

^a The result of the saturated model is used to select the best-fitting other models (see Annex 5).

Figure 6. Observed fraction of tumour-bearing rats fitted with the log-logistic model

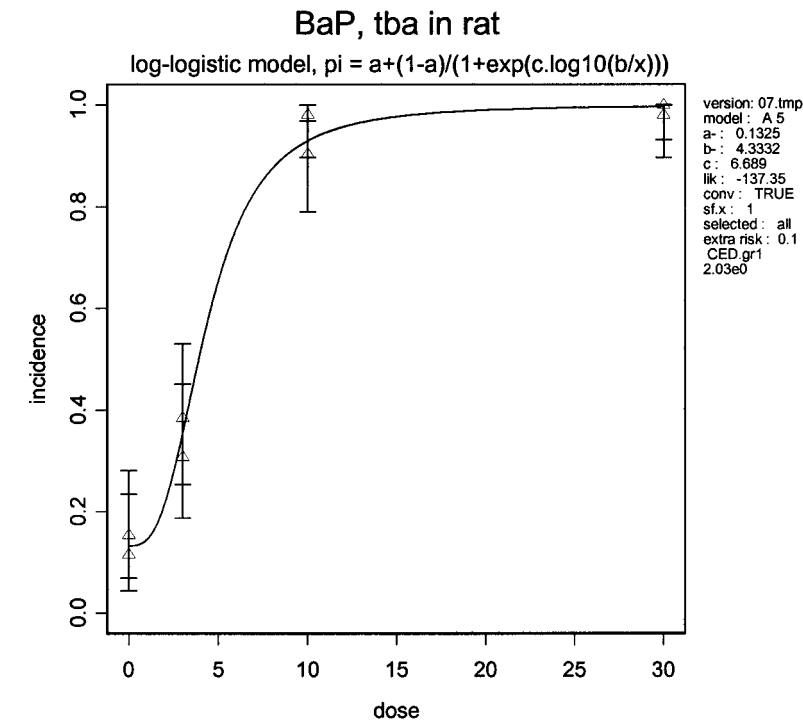


Table 46. Summary of dose–response modelling results for tumour-bearing rats (no significant difference between sexes)

Model	log-lik	Number of parameters	Dose (mg/kg bw per day)
			BMD (BMDL)
One-stage	-145.37	2	
Two-stage	-145.10	3	
Log-logistic	-137.35	3	2.03 (1.67)
Log-probit	-138.73	3	1.95 (1.56)
Weibull	-143.16	3	
Proast M2	-151.14	2	
Proast M4	-137.37	3	1.23 (1.04)
Saturated model ^a	-134.01	8	

log-lik, log-likelihood

^a The result of the saturated model is used to select the best-fitting other models (see Annex 5).

characterization of each component, nor does it require chemical characterization of all compounds in the potential source of exposure for which risk is being evaluated. Furthermore, given the uncertainty associated with toxicological evaluations, it may be more accurate, provided that the mixtures have a relatively constant composition. However, if the mixtures vary substantially in composition, then this approach may not work well. In particular, the surrogate technique cannot be used if the surrogate compound is not among the compounds in the mixture.

For application of the surrogate approach to PAH mixtures, the compound typically used is benzo[a]pyrene (see Table 47). The potencies of "typical" PAH mixtures have been found to be 16 times that of benzo[a]pyrene alone (Muller et al., 1996). However, this ratio is primarily based on environmental exposure to sources other than food. In at least some cases, the PAH mixture encountered in foods may be quite different. A factor of 10 has also been recommended to estimate mixture potency based on benzo[a]pyrene concentration (EC, 2002).

Table 48 summarizes the profiles of PAHs in food relative to benzo[a]pyrene. The maximum/minimum values are indicators of the correspondence of benzo[a]pyrene concentrations: the closer the ratio is to 1, the better is the correlation. Benzo[a]pyrene is a good marker for other genotoxic PAHs, but it does not correspond well with some of the lower-molecular-mass, non-genotoxic compounds.

Table 49 compares the PAH profiles relative to benzo[a]pyrene for the coal tar mixtures used in the mouse carcinogenicity study of Culp et al. (1998) with the mean and median concentrations of PAHs in food. While the concentrations of the mutagenic compounds are generally within a factor of 2, some of the lower-molecular-mass PAHs are present in much higher concentrations relative to benzo[a]pyrene in food than in the test mixtures used in the Culp et al. (1998) study.

9.3.2 The equivalency factor approach

The second analytical technique used to evaluate mixtures is based on the assumption of dose additivity, where the effective dose of the mixture is equal to the sum of the effective doses of each individual compound. Because different compounds differ in their ability to produce a toxic effect (Table 50), TEFs are used to scale each compound relative to some standard compound, which is typically chosen because it has a high relative potency and/or it has the best characterized dose-response relationship.

TEFs for PAH mixtures have been under development for many years, and most of the relevant data have already been reviewed (ATSDR, 1995; Muller et al., 1996; IPCS, 1998).

Although the equivalency factor methodology provides a practical approach to the problem of mixture evaluation, it has long been recognized that many uncertainties are associated with the technique. For compounds whose potencies are based on animal data (i.e. benzo[a]pyrene and a few of the others), there are the

Table 47. Average concentrations for PAH for a "mixture of standard composition" (relative to benzo[a]pyrene)

PAH	Average concentration ratio
Acenaphthene	0.71
Acenaphthylene	0.36
Anthanthrene	0.31
Anthracene	1.6
Benz[a]anthracene	1.2
Benzo[fluoro]anthracene	2.5
Benzo[a]pyrene	1.0
Benzo[e]pyrene	1.1
Benzo[ghi]perylene	1.0
Chrysene and triphenylene	2.0
Coronene	0.34
Dibenz[a,h]anthracene	0.28
Fluoranthene	3.8
Fluorene	1.6
Indeno[1,2,3-cd]pyrene	0.86
Phenanthrene	4.3
Pyrene	4.5

Data from Muller et al. (1996)

Table 48. PAH profiles in food relative to benzo[a]pyrene

Compound	Mean	Median	Min	Max	N	Max/min
Acenaphthene	1.9	1.9	0.3	3.5	2	12
Anthracene	5.8	1.8	<0.1	63	23	>788
Anthanthrene	<0.1	<0.1	0.08	0.2	4	2.6
Benz[a]anthracene	1.9	1.6	<0.5	6.0	39	>12
Benzo[a]fluorene	1.9	1.5	<1.0	3.7	5	>3.7
Benzo[b]fluoranthene	1.1	1.0	0.5	3.0	29	6.1
Benzo[b]fluorene	1.5	1.4	<0.8	2.7	5	>3.4
Benzo[c]phenanthrene	<2.3	<2.4	0.3	<4.0	6	<13
Benzo[e]pyrene	1.5	1.3	0.4	4.5	31	11
Benzo[ghi]perylene	1.3	1.1	<0.2	3.8	41	>19

Table 48. (contd)

Compound	Mean	Median	Min	Max	N	Max/min
Benzo[k]fluoranthene	0.8	0.7	0.2	1.6	29	8.0
Chrysene	2.8	2.3	1.2	6.0	21	5.2
Dibenz[a,h]anthracene	0.2	0.2	0.04	0.4	24	10
Fluoranthene	12.3	8.8	1.1	91	42	83
Fluorene	2.6	0.8	0.2	8.5	4	43
Indeno[1,2,3-cd]pyrene	1.2	0.9	<0.3	2.8	35	>9.2
Perylene	0.4	0.3	0.1	1.2	16	9.3
Phenanthrene	48.9	20.0	0.6	320	19	533
Pyrene	10.5	8.4	0.8	53	38	69

Data from EC (2002)

Max, maximum; min, minimum

Table 49. Comparison of PAH profiles in the coal tar mixtures with values in food

Compound	Coal tar mixture 1	Coal tar mixture 2	Mean	Median
Acenaphthene	1.1	0.5	1.9	1.9
Anthracene	1.4	1.1	5.8	1.8
Benz[a]anthracene	1.3	1.2	1.9	1.6
Benzo[b]fluoranthene	1.1	1.0	1.1	1
Benzo[k]fluoranthene	0.4	0.4	0.8	0.7
Chrysene	1.3	1.1	2.8	2.3
Dibenz[a,h]anthracene	0.1	0.1	0.2	0.2
Fluoranthene	2.7	2.3	12.3	8.8
Fluorene	2.0	1.7	2.6	0.8
Indeno[1,2,3-cd]pyrene	0.7	0.7	1.2	0.9
Phenanthrene	4.2	3.7	48.9	20
Pyrene	2.8	2.6	10.5	8.4

Note: The coal tar values are data from Culp et al. (1998) that were normalized relative to benzo[a]pyrene. The mean and median values are from EC (2002).

usual uncertainties associated with cancer risk assessment, such as low-dose extrapolation and species-to-species extrapolation. Many of the other compounds have been evaluated with in vitro assays that do not adequately reflect differences in metabolic activation and detoxification. No studies are available to demonstrate the relevance of the proposed TEFs to oral exposure.

Table 50. Relative potencies of PAHs

Compound	[1]	[2]	[3]	[4]
Acenaphthene		0.001	0.001	
Acenaphthylene		0.001	0.001	
Anthanthrene		0.320		0.28
Anthracene			0.01	
Benz[a]anthracene	0.145	0.1	0.1	0.014
Benzo[b]fluoranthene	0.141	0.1	0.1	0.11
Benzo[j]fluoranthene				0.045
Benzo[k]fluoranthene	0.061	0.1	0.1	0.037
Benzo[ghi]perylene	0.022	0.01	0.01	0.012
Benzo[a]pyrene	1.0	1.0	1.0	1.0
Benzo[e]pyrene	0.004		0.01	0
Chrysene	0.0044	0.01	0.01	0.026
Coronene				
Cyclopenta[cd]pyrene	0.023		0.1	0.012
Dibenz[a,c]anthracene			0.1	
Dibenz[a,h]anthracene	1.11	5	1.0	0.89
Dibenzo[a,e]fluoranthene				1.0
Dibenzo[a,e]pyrene				1.0
Dibenzo[a,h]pyrene				1.2
Dibenzo[a,i]pyrene				
Dibenzo[a,l]pyrene				100
Fluoranthene		0.001	0.001	
Fluorene		0.001	0.001	
Indeno[1,2,3-cd]pyrene	0.232	0.1	0.1	0.067
1-Methylphenanthrene			0.001	
Naphthalene		0.001	0.001	
Perylene			0.001	
Phenanthrene		0.001	0.001	0.00064
Pyrene	0.81	0.001	0.001	0

Adapted from IPCS (1998)

[1] Krewski et al. (1989)

[2] Nisbet & LaGoy (1992)

[3] Malcolm & Dobson (1994)

[4] Muller et al. (1995a, 1995b, 1996)

At least theoretically, interactions among PAHs involving tumour promotion could also be modelled using an equivalency factor approach. Table 51 lists TEFs for tumour promotion for several PAHs relative to fluoranthene, which is the most potent. However, since most tumour promotion assays involve dermal application, it is not possible to use these values to directly modify the potency of a mixture when the exposure is oral. However, these values could still be used to modify the potency relative to some standard (e.g. Muller et al., 1996) mixture where the relative increase in potency is known.

Table 51. TEFs for tumour promotion (gap junction inhibition)

PAH	TEF
Benzo[c]phenanthrene	0.31
Benzo[a]pyrene	0.09
Cyclopenta[cd]pyrene	0.32
Dibenz[a,c]anthracene	0.21
7,12-Dimethylbenz[a]anthracene	0.21
Fluoranthene	1
Fluorene	0.24
5-Methylchrysene	0.75
1-Methylpyrene	0.375
Phenanthrene	0.32
Picene	0.69
Pyrene	0.27

Data based on in vitro studies of Bláha et al. (2002)

10. COMMENTS

10.1 Absorption, distribution, metabolism and excretion

Absorption of PAHs from the diet is determined by the size and lipophilicity of the molecule and the lipid content of the food. PAHs are metabolized by oxidation of the aromatic rings, primarily by enzymes of the CYP1, CYP2 and CYP3 families, followed by formation of glutathione, glucuronide and sulfate conjugates. Oxidation can generate electrophilic metabolites that bind covalently to nucleic acids and proteins. Some PAHs and some metabolites of PAHs also bind to the AhR, resulting in up-regulation of several of the enzymes involved in PAH metabolism. This may lead to complex and potentially non-linear dose-response relationships for mixtures of PAHs.

10.2 Toxicological data

The relatively few studies of acute toxicity that were available indicated that PAHs have moderate to low acute toxicity. A limited number of short-term studies of toxicity with individual PAHs (acenaphthene, fluoranthene, fluorene, naphthalene, pyrene) administered orally to rats and mice were available. These studies predominantly showed toxicity in the liver and kidney, with no-observed-effect levels (NOELs) ranging from 53 to 175 mg/kg bw per day. The NOEL for benzo[a]pyrene was 3 mg/kg bw per day on the basis of toxicity in the liver in rats. The Committee considered that these studies were not pivotal for the present risk assessment.

The carcinogenicity of PAHs administered by dermal, subcutaneous, inhalation or oral routes has been assessed in a large number of studies. In most studies, the site of tumour development was related to the route of administration, e.g. gastric tumours after oral administration, skin tumours after dermal application. However, tumours at sites other than the site of application were also observed (e.g. liver tumours after oral exposure to benzo[a]pyrene, or lung tumours after oral exposure to coal tar mixtures containing PAHs). On the basis of all the available information, the Committee concluded that 13 PAHs (benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,l]pyrene, dibenzo[a,i]pyrene, indeno[1,2,3-cd]pyrene, 5-methylchrysene) are clearly carcinogenic in experimental animals. On the basis of observations in bioassays in animals treated parenterally, dibenz[a,h]anthracene, dibenzo[a,h]pyrene, dibenzo[a,l]pyrene, benzo[a]pyrene and benzo[b]fluoranthene seem to be the most potent carcinogens.

For the present evaluation, studies of carcinogenicity after oral administration are most relevant. Benzo[a]pyrene, when administered by the oral route, has been reported to produce tumours of the gastrointestinal tract, liver, lungs and mammary glands of mice and rats. Of the few other PAHs tested for carcinogenicity by the oral route, dibenz[a,h]anthracene and benz[a]anthracene have been reported to produce tumours of the gastrointestinal tract, lungs and liver in mice. No increases in incidences of tumours were seen in rats after oral administration of benz[a]anthracene, phenanthrene, fluorene or naphthalene. No other PAHs have been tested for carcinogenicity after oral administration. All these studies, however, had limitations as to their design and scope. The Committee paid particular attention to two new studies using oral administration: a study in mice in which the tumorigenic response to benzo[a]pyrene was compared with that to two mixtures of coal tar, and a study in rats given benzo[a]pyrene.

In the first of these studies, groups of 48 female B6C3F1 mice were fed diets containing benzo[a]pyrene at a concentration of 0, 5, 25 or 100 mg/kg of diet (equivalent to doses of 0, 0.7, 3.6 or 14 mg/kg bw per day) for 2 years. Papillomas and squamous cell carcinomas were observed in the forestomach, with a combined incidence of 1/48, 4/47, 36/47 and 46/47 in each group. The increased incidences at 25 and 100 mg/kg of diet were significant and dose-related. The combined incidences of papillomas and carcinomas were: oesophagus, 0/48,

0/48, 2/45, 27/46; and tongue, 0/48, 0/48, 2/46, 23/48. In the two latter tissues, only animals receiving the highest dose differed significantly from those receiving the solvent only. Groups of 48 female B6C3F1 mice were also fed diets containing 0, 0.01, 0.03, 0.1, 0.3, 0.6 or 1.0% coal tar mixture 1, which contained benzo[a]pyrene at a concentration of 2240 mg/kg (equivalent to doses of 0, 0.03, 0.09, 0.32, 0.96, 1.92 or 3.2 mg/kg bw per day), or 0, 0.03, 0.1 or 0.3% of coal tar mixture 2, which contained benzo[a]pyrene at a concentration of 3669 mg/kg (equivalent to doses of 0, 0.16, 0.52 or 1.1 mg/kg bw per day). A significantly increased incidence of alveolar and bronchiolar adenomas and carcinomas was found at 0.3, 0.6 and 1.0% of mixture 1 (27/47, 25/47 and 21/45 vs 2/47 in the control group) and at 0.1 and 0.3% of mixture 2 (10/48 and 23/47 vs 2/47 in the control group). For tumours of the forestomach, a significant increase was observed at 0.3, 0.6 and 1.0% of mixture 1 (14/46, 15/45 and 6/41 vs 0/47 in the control) and at 0.3% of mixture 2 (13/44). The total numbers of tumour-bearing animals were 5/48, 12/48, 14/48, 12/48, 40/48, 42/48 and 43/48 at 0, 0.01, 0.03, 0.1, 0.3, 0.6 and 1.0% of mixture 1, and 5/48, 17/48, 23/48 and 44/48 at 0, 0.03, 0.1 and 0.3% of mixture 2. This study indicated that benzo[a]pyrene alone induced only tumours of the alimentary tract, whereas the coal tar mixtures also induced liver and lung tumours.

Administration of oral doses of benzo[a]pyrene at 0, 3, 10 or 30 mg/kg bw per day by gavage to groups of 104 male and female Wistar rats on 5 days per week for 2 years resulted in a large variety of tumours, most prominent being those in the liver and forestomach. In the forestomach, the combined incidence of papilloma and carcinoma was, respectively, 1/52, 6/51, 30/51 and 50/52 for females and 0/52, 8/52, 43/52 and 52/52 for males. The incidences of combined adenoma and carcinoma in the liver were, respectively, 0/52, 2/52, 39/52 and 51/52 for females and 0/52, 4/52, 38/52 and 49/52 for males. In addition to these tumours, treatment with benzo[a]pyrene also induced soft tissue sarcomas (skin, mammary), as well as tumours of the auditory canal, oral cavity, small intestine and kidney. The total numbers of tumour-bearing animals were 8/52, 20/52, 47/52 and 51/52 for females and 6/52, 16/52, 51/52 and 52/52 for males.

On the basis of the available information, the Committee concluded that 15 individual PAHs are clearly genotoxic *in vitro* and *in vivo*. These genotoxic PAHs are benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[ghi]perylene, benzo[j]fluoranthene, benzo[k]fluoranthene, chrysene, cyclopenta[cd]pyrene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene. The Committee considered that four individual PAHs (anthracene, benzo[a]fluorene, naphthalene, pyrene) were not genotoxic.

An important observation was the binding of the active metabolites of PAHs to DNA, predominantly to amino groups of guanine and adenine. The major stable adduct is formed at the N2 position of desoxyguanosine. The formation of DNA adducts by electrophilic metabolites is generally regarded as one of the earliest steps in carcinogenicity of the mutagenic PAHs. However, there is a poor quantitative relationship between levels of tissue adduct and tumour formation. This indicates that other factors additional to DNA adduct formation are apparently

critical for the development of tumours caused by benzo[a]pyrene and some other PAHs, and that genotoxic end-points alone may not adequately predict the site or frequency of tumour development.

With respect to the assessment of risk of cancer, the Committee noted that the levels of benzo[c]fluorene-derived adducts were much higher than those of benzo[a]pyrene-derived adducts in the lungs of rats fed with coal tar. Although this might indicate that benzo[c]fluorene may contribute to the formation of lung tumours after oral exposure to coal tar, the Committee found no data on its occurrence in food.

Overall, the Committee concluded that the following PAHs were clearly genotoxic and carcinogenic: benz[a]anthracene, benzo[b]fluoranthene, benzo[j]-fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene.

There is limited or no evidence on the reproductive toxicity of individual PAHs, other than benzo[a]pyrene, in animals. There was no effect on reproductive capacity in a one-generation study in mice receiving diets containing benzo[a]pyrene at doses of up to 133 mg/kg bw per day. Impaired fertility was seen in the offspring of female mice given benzo[a]pyrene at doses of >10 mg/kg bw per day by gavage. Developmental toxicity has been reported after oral administration of benz[a]-anthracene, benzo[a]pyrene, dibenz[a,h]anthracene or naphthalene. A NOEL for reproductive toxicity of the latter PAH administered by the oral route has not been established.

The immunosuppressive effects of PAHs have mainly been investigated in studies using parenteral administration. It has been suggested that PAHs exert immune effects via the AhR. Observations in CYP1A1 knockout mice have indicated that CYP1A1 may protect against immunotoxic effects by benzo[a]pyrene. In a study on immunosuppressive effects in rats treated orally, the NOEL for benzo[a]pyrene was 3 mg/kg bw per day.

10.3 Observations in humans

Most data, both on the effects of PAHs in human populations and on biomarkers of exposure to PAHs, refer to occupational and environmental exposure. The available evidence regarding oral exposure to PAHs was indirect and did not include data on quantitative exposure and thus was not suitable for use in this risk assessment.

10.4 Analytical methods

HPLC with fluorescence detection has been widely used for the determination of PAHs in several food matrices. Use of a UV/diode array detector in conjunction with a fluorescence detector improved the detection limits for compounds with a low fluorescence intensity. GC-FID and GC-MS methods are also employed for their determination. Confirmation of compounds present is achieved through the

mass spectral data or by analysing on a different column when the GC-FID technique is used. Confirmation of compounds analysed by HPLC methods is achieved through GC-MS analysis in some cases. Both HPLC and GC-MS methods are suitable for routine monitoring.

The extraction and cleanup techniques used depend on the type of food matrix. Fatty foods such as meat, fish and their products are saponified with methanolic potassium hydroxide, liquid extraction into non-polar solvents, partitioning with dimethyl formamide or dimethyl sulfoxide–water followed by re-extraction with solvents like cyclohexane. Solid-phase extraction cleanup using reversed-phase cartridges has been found to give better recoveries for PAHs of high relative molecular mass and to be suitable for their cleanup in different matrices.

The limits of detection for PAHs are matrix-dependent. Limits of detection for both HPLC and GC-MS methods are adequate to detect the concentrations normally encountered in foods. The recovery of PAHs in different matrices varies, but is usually >70% for most compounds. Available CRMs cover a few selected PAHs in certain matrices only. Current proficiency testing programmes seem to be inadequate, as they cover a few matrices for selected PAHs only. Lack of CRMs and proficiency testing programmes covering a wide range of PAHs in high-risk food commodities has been a limitation in the analysis of PAHs in foods. The Committee noted that most analytical methods developed include the 16 priority pollutant PAHs listed by the United States Environmental Protection Agency and do not include most dibenzopyrenes and 5-methylchrysene. The Committee recommended that analytical methods be developed to include these compounds.

10.5 Sampling protocols

PAHs are chemically stable and highly lipophilic in nature. However, they are susceptible to photodegradation in the presence of light, and thus all sampling and analytical operations must be performed under subdued light. Little information was available on the reactions and fate of PAHs in foods. Since PAHs are chemically stable compounds, they are assumed to be stable in different food matrices, although PAHs deposited on the surface of crops may undergo photodegradation. Sampling protocols in the European Union are detailed by the SCOOP task force 3.2.12 on the occurrence of PAHs (SCOOP, 2004). Sampling protocols are available for total diet studies in the United Kingdom. Several researchers followed either routine sampling or sampling based on suspicion (e.g. edible oils, industrial pollution, oil spills).

10.6 Sources and occurrence in foods

There are two main routes of entry of PAHs into the food-chain. Foods can be contaminated by environmental PAHs that are present in air (by deposition), soil (by transfer) and water (by deposition and transfer). The PAHs that are airborne (either in the vapour phase or adhered to particulate matter) become deposited on crops, especially crops with broad leaves. Contamination of fish and marine

invertebrates occurs due to the deposition and transfer of PAHs. High concentrations of PAHs have been reported in bivalves (mussels and oysters) that feed by filtering large quantities of water. PAHs also form directly during processing (drying and smoking) or cooking (e.g. grilling, roasting, frying) of foods. High values are reported in grilled and barbecued foods. Direct smoking, especially using traditional methods, results in contamination with PAHs. Additional minor routes of contamination may include use of contaminated smoke flavouring additives and migration from contaminated packaging materials.

10.7 *Effects of processing and cooking of food*

Grilling of foods has been reported to be responsible for contamination with PAHs. Although not precisely known, it is likely that PAHs are formed from melted fat that undergoes pyrolysis when dripping onto the heat source. Higher concentrations of PAHs have been reported in foods that are cooked using horizontal grilling techniques during which fat falls directly on the hot coal than in foods cooked using the vertical technique. Contamination of vegetable oils (including olive residue oils) with PAHs usually occurs during technological processes like direct fire drying, in which combustion products may come into contact with the oil seeds or oil.

10.8 *Prevention and control of PAHs in foods*

The Committee concluded that concentrations of PAHs in foods can be reduced by avoiding contact of foods with flames from barbecuing and cooking at a lower temperature for a longer time. Broiling (heat source above) leads to lower concentrations of PAHs than does barbecuing. Fat should not drip down onto an open flame, sending up a column of smoke that coats the food with PAHs. The use of medium to low heat and placing of the meat farther from the heat source can greatly reduce contamination with PAHs. Direct contact of oil seeds or cereals with combustion products during drying processes results in contamination with PAHs. The Committee concluded that contamination of smoked foods with PAHs can be significantly reduced by replacing direct smoking (with smoke developed in the smoking chamber, traditionally in smokehouses) with indirect smoking. Washing or peeling fruit and vegetables before consumption would help to remove surface contaminants.

10.9 *Levels and pattern of food contamination*

The Committee did not receive any data on occurrence in the GEMS/Food format. However, European Union SCOOP task force 3.2.12 (SCOOP, 2004) has provided comprehensive data on occurrence in the European Union. Data available from the IPCS and Scientific Committee on Foods reports and from the literature were reviewed by the Committee. The range of concentrations of PAHs in the major food groups, from available data, is summarized in Table 52. The major foods containing higher concentrations of PAHs are meat and fish products, particularly grilled and barbecued products, oils and fats, cereals and dry foods. In

Table 52. Range of concentrations of PAHs in major food groups

PAH	PAH concentration (µg/kg)							
	Meat and meat products ^a	Fish and seafoods ^a	Vegetables	Fruits and confections ^b	Cereals and cereal products ^c	Beverages	Oils and fats	Dairy products ^d
Acenaphthene	0.05	ND–83	0.01–0.03	0.02	ND–2.3	–	0.02–45	0.01–0.08
Acenaphthylene	ND–500	<0.02–160	0.07–0.19	0.1–0.14	0.89	–	0.10–29	0.16–0.4
Anthanthrene	ND–67	–	–	–	ND–0.13	–	0.03–2.7	–
Anthracene	ND–133	ND–191	ND–17	ND–2.5	ND–9.4	ND	ND–460	ND–0.30
Benz[a]anthracene	ND–144	ND–86	0.05–15	ND–2.0	0.03–4.2	0.003–0.61	ND–79	ND–1.7
Benzo[a]fluorene ^e	ND–174	0.2–3.0	0.08–2.6	ND–1.5	–	–	–	–
Benzo[b]fluorene	ND–72	–	0.11–2.8	ND–1.0	–	–	ND–45	–
Benzo[b]fluoranthene^f	ND–197	ND–134	ND–28.7	ND–3.5	0.03–1.3	ND–0.65	ND–91	ND–0.7
Benzo[ghi]fluoranthene	ND	ND	ND	ND–0.9	ND–0.7	ND	ND–4.9	ND
Benzo[j]fluoranthene	ND–7	–	–	–	–	–	ND–5.1	–
Benzo[k]fluoranthene	ND–172	ND–55	ND–17	ND–0.2	0.02–1.4	ND–0.24	ND–99	ND–0.1
Benzo[ghi]perylene	ND–153	ND–31	ND–11	ND–6.0	ND–120	ND–0.03	ND–66	ND–1.6
Benzo[c]phenanthrene	ND–1.4	ND–280	ND–9.2	ND–0.5	ND–0.7	ND	ND	ND
Benzo[a]pyrene	ND–212	ND–173	ND–25	ND–1.5	ND–5.4	ND–0.60	ND–164	ND–1.3
Benzo[e]pyrene	ND–81	ND–50	ND–7.9	ND–1.5	0.06–5.2	ND–0.06	ND–37	ND–0.2
Chrysene^g	ND–140	ND–49	ND–62	ND–9.0	ND–2.8	ND–0.02	ND–76	ND–1.5

Table 52. (contd)

PAH	PAH concentration (µg/kg)							
	Meat and meat products ^a	Fish and seafoods ^a	Vegetables	Fruits and confections ^b	Cereals and cereal products ^c	Beverages	Oils and fats	Dairy products ^d
Coronene	–	ND–2.4	–	–	–	–	ND–7.4	–
Cyclopenta[cd]pyrene	–	–	–	–	–	–	ND–1.4	–
Dibenz[a,h]anthracene^h	ND–8.8	ND–39	ND–1.0	ND–0.05	ND–3.6	0.002–0.24	ND–43	ND–0.04
Dibenzo[a,e]pyrene	ND	ND–0.3	ND	ND	ND	ND	ND–0.04	ND
Dibenzo[a,h]pyrene	–	–	ND–0.7	–	–	–	–	–
Dibenzo[a,i]pyrene	–	–	ND–0.3	–	–	–	–	–
Dibenzo[a,l]pyrene	–	–	–	–	–	–	–	–
Fluoranthene	ND–376	ND–218	ND–117	ND–27	0.10–130	ND–8.4	ND–460	ND–8.0
Fluorene	ND–0.67	ND–252	0.03–0.06	0.03–3.5	ND–5.9	–	ND–264	0.02–0.07
Indeno[1,2,3-cd]pyrene	ND–171	ND–42	ND–7.9	ND–1.0	ND–3.2	ND	ND–81	ND–1.2
5-Methylchrysene	ND–3.7	ND–1.1	ND–2.6	ND–1.6	ND–4.9	ND–0.05	ND–3.7	ND–1.6
1-Methylphenanthrene	ND–58	ND–708	0.1–2.1	–	0.3	–	ND–190	–
Naphthalene	0.9–55	ND–156	0.06–0.5	0.18–4.3	2.6	–	ND–57	0.27–0.9
Perylene	ND–28	ND–24	0.05–1.7	–	0.1–0.7	–	ND–36	ND–0.6
Phenanthrene	ND–618	ND–334	ND–12	ND–30	ND–94	ND	ND–170	ND–1.6
Pyrene	1.2–452	ND–217	ND–70	ND–12	ND–48	ND–9.3	ND–330	ND–4.8

Table 52. (contd)

PAH	PAH concentration (µg/kg)							
	Meat and meat products ^a	Fish and seafoods ^a	Vegetables	Fruits and confections ^b	Cereals and cereal products ^c	Beverages	Oils and fats	Dairy products ^d
Triphenylene	–	–	–	–	–	–	–	–

Note: Compounds shown in bold were considered by the Committee to be genotoxic and carcinogenic.

ND, not detected

^a Includes grilled and smoked foods and smoke flavouring food additives.

^b Includes sweets and sugar.

^c Includes biscuits, bread, bran and breakfast cereals.

^d Includes butter and cheese.

^e Reported including benzo[*b*]fluorene in some publications.

^f Reported as sum with benzo[*j*+*b*+*k*]fluoranthenes in some publications.

^g Reported including triphenylene in some publications.

^h Reported as sum with dibenzo[*a,h* + *a,c*]anthracenes in some publications.

some cases, lack of information on quality control of the analytical data made it difficult for the Committee to judge the quality of the data on occurrence. It was also observed that some determinations had been carried out following episodes of contamination of a given food or incidents of environmental pollution.

For some PAHs identified by the Committee as being genotoxic and carcinogenic, there were few or no data on concentrations in the major food groups (Table 52). The Committee recommended that efforts be made to collect data for these PAHs.

10.10 Dietary intake assessment

The Committee reviewed estimates of intake for a range of PAHs from 18 countries, including data submitted by Australia, Brazil, New Zealand and the United Kingdom. The Scientific Committee on Foods review and the European Union SCOOP report submitted to the Committee also included intake estimates from a number of countries. Other intake estimates were obtained from the literature. In the studies reviewed, intakes of individual PAHs were presented, as well as intakes for "summed" PAHs and "carcinogenic" PAHs (which differed according to the authors' classification of "carcinogenic" and which may have differed from that of the Committee). For the assessment conducted by the Committee, the only intake estimates reviewed were those for the 13 PAHs that the Committee considered to be carcinogenic and genotoxic. The intake estimates were calculated in a variety of ways, including differences in the data on food consumption, derivation of concentrations, the range of foods included, the methodologies of intake calculation, the treatment of results given as "not detected" and the reporting of results.

There were no estimates of intake for 3 of the 13 PAHs assessed, namely dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene and dibenzo[*a,j*]pyrene. The ranges of intakes of the other 10 PAHs assessed from all of the studies reviewed are shown in Table 53, which incorporates data on specific foods as well as the total diet. The intakes were summarized from all of the studies reviewed, some of which reported intakes in units/person per day, and others in units/kg bw. Therefore, the range of intakes presented in µg/day may not be from the same study as those expressed in µg/kg bw per day and therefore are not directly divisible by an average body weight. Estimated intakes of benzo[*a*]pyrene ranged from <1 to 2.0 µg/day and from 0.0001 to 0.006 µg/kg bw per day. For the other nine PAHs, intakes ranged from <1 to about 12 µg/day and from 0.0001 to 0.015 µg/kg bw per day.

In order to provide a likely intake of benzo[*a*]pyrene covering the main food groups in the whole diet for the purposes of risk characterization, a separate determination of the range of intakes was conducted using only those studies that included foods from the range of major food groups (Table 54). These studies included foods that were "ready to eat" (e.g. meat was cooked) and therefore included the likely concentrations of PAHs that arise due to cooking of food. From this analysis, mean intakes of benzo[*a*]pyrene ranged from 0.0014 to 0.42 µg/day and from 0.0002 to 0.005 µg/kg bw per day. From this range, the Committee

Table 53. Summary of estimated intakes of 10 of the 13 PAHs considered by the Committee to be genotoxic and carcinogenic

PAH	No. of estimates of intake (No. of countries)	Lowest reported intake, µg/day (µg/kg bw per day)	Range of reported means, µg/day (µg/kg bw per day)	Range of reported 95th percentiles, µg/day (µg/kg bw per day)	Highest reported intake, µg/day (µg/kg bw per day)
Benz[a]anthracene	12 (9)	0.005 (0.00007)	0.0006–0.47 (0.00007–0.0018)	0.013–0.093 (0.00031–0.00132)	1.7 (0.003)
Benzo[b]fluoranthene	11 (9)	0.009 (0.0001)	0.005–0.46 (0.00045–0.0036)	0.075–0.127 (0.0081–0.0036)	1.0 (0.004)
Benzo[j]fluoranthene	1 ^a (1)	<0.030 (<0.0005) ^b	<0.030 (<0.0005) ^b	–	0.9 (0.015) ^b
Benzo[k]fluoranthene	11 (9)	0.007 (0.0002)	0.007–0.26 (0.00016–0.0032)	0.023–0.043 (0.00061–0.0015)	0.4 (0.005)
Benzo[a]pyrene	32 (18)	0.0006 (0.0001)	0.0006–2.04 (0.0001–0.005)	0.096–0.27 (0.0012–0.0047)	2.04 (0.006)
Chrysene	8 (8)	0.008 (0.0001)	0.008–5.0 (0.0001–0.0035)	0.045–0.113 (0.001–0.0021)	11.6 (0.006)
Dibenz[a,h]anthracene	10 (9)	0.005 (0.0001)	0.0046–0.76 (0.00009–0.0012)	0.016–0.028 (0.0004–0.00079)	1.5 (0.002)

Table 53. (contd)

PAH	No. of estimates of intake (No. of countries)	Lowest reported intake, µg/day (µg/kg bw per day)	Range of reported means, µg/day (µg/kg bw per day)	Range of reported 95th percentiles, µg/day (µg/kg bw per day)	Highest reported intake, µg/day (µg/kg bw per day)
Dibenzo[a,e]pyrene	1	0.010	0.01–0.63	–	0.64
	(1)	(0.00017) ^b	(0.00017–0.011) ^b		(0.011) ^b
Indeno[1,2,3-cd]pyrene	9	0.009	0.009–0.46	0.034–0.064	0.55
	(8)	(0.0001)	(0.0001–0.0034)	(0.00079–0.0017)	(0.006)
5-Methylchrysene	1	0.580	0.58–0.73	–	2.6
	(1)	(0.0097) ^b	(0.0097–0.012) ^b		(0.040) ^b

Note: The ranges of intakes presented in µg/day may not be from the same study as those expressed in µg/kg of body weight per day and therefore are not directly divisible by an average body weight.

^a Two additional estimates of intake were presented as benzo[b+j+k]fluoranthene and therefore have not been included in this summary.

^b No estimates of intake reviewed were expressed in µg/kg of body weight per day for this PAH. Therefore, intakes in µg/day were divided by 60 kg to determine a likely intake in µg/kg of body weight per day.

Table 54. Summary of estimated intakes of benzo[a]pyrene from studies covering the range of major food groups in the diet

No. of estimates of intake (No. of countries)	Range of reported means, µg/day (µg/kg bw per day)	Range of reported 95th percentiles, µg/day (µg/kg bw per day)	Highest reported intake, µg/day (µg/kg bw per day)
16	0.0014–0.42	0.07–0.27	0.77 ^b
(13) ^a	(0.0002–0.005)	(0.0012–0.0049)	(0.0062)

Note: The ranges of intakes presented in µg/day may not be from the same study as those expressed in µg/kg bw per day and therefore are not directly divisible by an average body weight.

^a Australia, Austria, Belgium, Brazil, Denmark, Finland, France, Italy, Netherlands ($n = 4$), New Zealand, Norway, Spain, United Kingdom.

^b Equates to an intake of 0.013 µg/kg bw per day, for a 60-kg person.

selected the value of 0.004 µg/kg bw per day as being representative of a mean intake for use in the present evaluation. The highest reported intake of benzo[a]pyrene from any study in µg/day was 0.77 and in µg/kg bw per day was 0.0062 (from different studies). If the intake of 0.77 µg/day were divided by an assumed body weight of 60 kg, this would result in a higher estimate on a body weight basis of 0.013 µg/kg bw per day. On the basis of these data, the Committee identified a high-level intake of 0.01 µg/kg bw per day for use in the present evaluation.

It was also noted by the Committee that there may be some regions of the world that have higher intakes than the "whole of diet" estimates presented in Table 54. In one study from northern China, intakes of PAHs were calculated from vegetables only. This area of China uses a lot of coal fuel for cooking, heating and greenhouse warming. Intake of benzo[a]pyrene in this study was estimated at about 2 µg/day. It was noted that a consumption of vegetables of about 440 g/day was used to calculate the estimated intake, although data submitted to the Committee from the Chinese National Nutrition and Health Survey indicated that actual consumption of vegetables was about 270 g/day. Therefore, actual intake of benzo[a]pyrene from vegetables alone for this part of China is likely to be about 1 µg/day. This is in the same order of magnitude as the intake of 0.77 µg/day reported as being the highest intake from the major foods in the diet.

Children generally had intakes of PAHs that were about 2–2.5 times higher than those of adults when expressed on a body weight basis.

The major contributors to intakes of PAHs were cereals and cereal products (owing to high consumption in the diets of many countries) and vegetable fats and oils (owing to higher concentrations of PAHs in this food group). Food is the major contributor to total intake of PAHs in the general population, with smaller contributions from water and inhalation. Smokers and people exposed occupationally will have additional exposures to PAHs. In developing countries, the release of PAHs during residential heating and cooking is an important cause of contamination when biomass is burnt in relatively simple stoves.

The Committee usually calculates international estimates of intake of contaminants using the GEMS/Food regional diets and extrapolating data on concentrations of the contaminant from regions in which data are collected to regions for which no data exist. In the present case, no data on concentrations of PAHs for individual samples were either submitted to the Committee or available in the literature. Therefore, no distributions of concentrations of PAHs or mean concentrations of PAHs in foods were available in the required format to be used in calculating intakes of PAHs at the regional level. Should PAHs be reassessed by the Committee in the future, the Committee recommended that raw data from individual samples be submitted to allow estimates of the regional intakes to be made.

Overall, the Committee concluded that there was considerable variation in the intake assessments, but that a representative mean intake of benzo[a]pyrene of 0.004 µg/kg bw per day and a high-level intake of benzo[a]pyrene of 0.01 µg/kg bw per day could be used in the present evaluation. However, some population groups may have higher intakes of PAHs, e.g. those with regular high consumption of food cooked over open fires or barbecues, or people habitually consuming foods from areas where the level of contamination with PAHs is higher.

10.11 Dose–response analysis

For the risk assessment for PAHs, modelling of the dose–response relationship was applied to data from two studies on the incidence of tumours in rats or mice treated by oral administration. Groups of mice and rats were given purified benzo[a]pyrene, while additional groups of mice were also given one of two mixtures of coal tar, using content of benzo[a]pyrene as a comparator. In the dose–response analysis for the study in mice, the results for animals receiving the two higher doses of coal tar mixture 1 were omitted, owing to the premature deaths of all animals at these doses.

In the dose–response analysis, eight different statistical models (Annex 5) were fitted to the experimental data. Those resulting in acceptable fits based on biological and statistical considerations were selected to derive the BMD and BMDL for a 10% extra risk of tumours. This procedure resulted in a range of BMD and BMDL values for each end-point considered (Table 55).

Taking into account the fact that mixtures of PAHs are present in food and the possibility that different PAHs may act by different mechanisms, the Committee concluded that the data on the total number of tumour-bearing mice treated with coal tar mixtures provided the most appropriate basis for the present evaluation. For this end-point, the values for the BMDL ranged from 0.10 to 0.23 mg (100 to 230 µg) of benzo[a]pyrene/kg bw per day. The Committee decided to use the more conservative lower end of this range for its evaluation. Thus, a BMDL equivalent to 100 µg of benzo[a]pyrene/kg bw per day was derived for mixtures of PAHs in food.

Table 55. BMD and BMDL values for carcinogenicity of benzo[a]pyrene and coal tar mixtures, based on a battery of statistical models

Modelled data	Range of BMD and BMDL values (mg of benzo[a]pyrene/kg bw per day)					
	Benzo[a]pyrene		Coal tar mixture 1		Coal tar mixture 2	
	BMD	BMDL	BMD	BMDL	BMD	BMDL
Mouse forestomach	0.40–0.92	0.31–0.74	0.21–0.40	0.16–0.31	0.37–0.59	0.27–0.45
Mouse lung ^a	No observed increase in incidence of tumours		0.14–0.26	0.11–0.20	0.14–0.26	0.11–0.20
Tumour-bearing mice ^a	–	–	0.13–0.29	0.10–0.23	0.13–0.29	0.10–0.23
Rat liver	3.4–4.0	2.9–3.4	–	–	–	–
Tumour-bearing rats	1.2–2.0	1.0–1.7	–	–	–	–

BMD, benchmark dose for 10% extra risk of tumours; BMDL, 95% lower confidence limit for the benchmark dose. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls.

^a The two mixtures did not produce significantly different dose–response curves, and therefore the data were combined.

10.12 Approaches for mixtures of PAHs

As a variety of PAHs are found together, it is necessary to evaluate the combined toxicity of mixtures of PAHs. There are two general approaches to this problem. The first technique is based on the assumption of dose additivity, where the effective dose of the mixture is assumed to be equal to the sum of the effective doses of each individual compound. Because different compounds differ in their ability to produce a toxic effect, adjustment factors (TEFs) are used to scale the effect of each compound relative to that of a standard compound, which is typically chosen because it has a high relative potency and/or has been best characterized with respect to its effects and dose–response relationship. The Committee noted that the TEFs that have been proposed for PAHs are derived from studies involving parenteral administration or in vitro approaches and that no data on oral administration were available that were suitable for this purpose.

The second option is the use of the surrogate approach. This method uses a single component as the measure of concentration in relation to the response of the whole mixture. For PAHs, benzo[a]pyrene is used as a marker of exposure and of the effects of the mixture.

The Committee compared the PAH profiles in the coal tar mixtures used in the study of carcinogenicity in mice with those profiles typically reported in food. The concentrations of the genotoxic and carcinogenic PAHs relative to that of benzo[a]pyrene were generally within a factor of 2, but some of the non-genotoxic PAHs of lower relative molecular mass (e.g. phenanthrene, pyrene, fluoranthene)

were present at much higher concentrations relative to benzo[a]pyrene in food than in the coal tar mixtures. The Committee concluded that a surrogate approach should be used in the present evaluation, with benzo[a]pyrene being used as a marker of exposure to the genotoxic and carcinogenic PAHs, because this approach is based on data from a study of carcinogenicity with a relevant mixture of PAHs administered by the oral route. Furthermore, the surrogate approach is much simpler to apply and is generally as accurate as the TEF approach for most purposes.

Since benzo[a]pyrene is not a good marker for some of the PAHs of lower relative molecular mass and because some of these PAHs are tumour promoters when administered by the dermal route, further information was needed to establish whether these substances may act as promoters after administration by the oral route. However, because tumour promotion is more likely to occur at higher doses than carcinogenicity arising from genotoxic effects, a margin of exposure (MOE) approach for genotoxic and carcinogenic substances is also likely to adequately allow for the effects of PAHs of lower relative molecular mass.

11. EVALUATION

The Committee concluded that the critical effect of PAHs is carcinogenicity. As some PAHs are genotoxic, it is not possible to assume a threshold mechanism, and a provisional tolerable weekly intake (PTWI) could not be established. The present evaluation focused on 13 PAHs that the Committee identified as being genotoxic and carcinogenic: benz[a]anthracene, benzo[b]fluoranthene, benzo[j]-fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene.

The Committee decided to apply a surrogate approach to the evaluation, in which benzo[a]pyrene was used as a marker of exposure to, and effect of, the 13 genotoxic and carcinogenic PAHs.

A BMDL equivalent to 100 µg of benzo[a]pyrene/kg bw per day was derived for PAHs in food on the basis of a study of carcinogenicity in mice treated orally with mixtures of PAHs representative of the genotoxic and carcinogenic PAHs present in food.

A wide range of estimates of intake were available for benzo[a]pyrene and, to a lesser extent, for nine of the other genotoxic and carcinogenic PAHs. While these may not completely reflect the levels of PAHs generated during cooking of food over barbecues and open fires, the Committee concluded that a representative mean intake of benzo[a]pyrene of 0.004 µg/kg bw per day and an estimated high-level intake of benzo[a]pyrene of 0.01 µg/kg bw per day could be used in the present evaluation as a marker for PAHs in food. Comparison of these mean and high-level intakes with the BMDL indicates MOEs of 25 000 and 10 000, respectively. Based on these MOEs, the Committee concluded that the estimated intakes of PAHs were of low concern for human health.

Measures to reduce intake of PAHs could include avoiding contact of foods with flames and cooking with the heat source above rather than below the food. Efforts should be made to reduce contamination with PAHs during drying and smoking processes, e.g. by replacing direct smoking (with smoke developed in the smoking chamber, traditionally in smokehouses) with indirect smoking. Washing or peeling fruit and vegetables before consumption would help to remove surface contaminants.

11.1 Recommendations

- The Committee recommended that future monitoring should include, but not be restricted to, analysis of the 13 PAHs identified as being genotoxic and carcinogenic, i.e. benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,j]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene. In addition, analysis of benzo[c]fluorene in food may help to inform future evaluations.
- The Committee recommended that analytical methods be developed to include most dibenzopyrenes and 5-methylchrysene.
- The Committee recommended that efforts be made to collect data on concentrations in the major food groups for PAHs identified by the Committee as being genotoxic and carcinogenic.
- The Committee recommended that, should PAHs be reassessed by the Committee in the future, raw data from individual samples be submitted to allow estimates of the regional intakes to be made.

12. REFERENCES

- Alavanja, M.C., Field, R.W., Sinha, R., Brus, C.P., Shavers, V.L., Fisher, E.L., Curtain, J. & Lynch, C.F. (2001) Lung cancer risk and red meat consumption among Iowa women. *Lung Cancer*, **34**, 37–46.
- Ali, M.Y. & Cole, R.B. (2002) One-step SFE-plus-C18 selective extraction of low polarity compounds, with lipid removal, from smoked fish and bovine milk. *Anal. Bioanal. Chem.*, **374**, 923–931.
- Al-Yakoob, S., Saeed, T. & Al-Hashash, H. (1993) Polycyclic aromatic hydrocarbons in edible tissue of fish from the Gulf after 1991 oil spill. *Mar. Pollut. Bull.*, **27**, 297–301.
- Armstrong, B., Hutchinson, E., Unwin, J. & Fletcher, T. (2004) Lung cancer risk after exposure to polycyclic aromatic hydrocarbons: a review and meta-analysis. *Environ. Health Perspect.*, **112**, 970–978.
- Ates, I., Yilmazer-Musa, M., Yucelsoy, B. & Karakaya, A. (2004) Determination of exposure to polycyclic aromatic hydrocarbons in some work groups in Turkey by measurement of 1-hydroxypyrene levels in urine. *Bull. Environ. Contam. Toxicol.*, **73**, 242–248.
- ATSDR (1995) *Toxicological Profile for Polycyclic Aromatic Hydrocarbons*. Atlanta, Georgia: United States Department of Health and Human Services, Agency for Toxic Substances and Disease Registry.

- Autrup, H. (1986) Host factors in carcinogenesis: carcinogen metabolism and DNA damage. In: Harris, C.C., ed., *Biochemical and Molecular Epidemiology of Cancer*. New York: Alan R. Liss, pp. 359–372. As cited in EC (2002).
- Awogi, T. & Sato, T. (1989) Micronucleus test with benzo[a]pyrene using a single peroral administration and intraperitoneal injection in males of the MS/Ae and CD1-1 mouse strains. *Mutat. Res.*, **223**, 353–356.
- Barcelo, D., Oubina, A., Salau, J.S. & Perez, S. (1998) Determination of PAHs in river water sample by ELISA. *Anal. Chim. Acta*, **376**, 49–53.
- Barranco, A., Alonso-Salces, R.M., Bakkali, A., Burrueta, L.A., Gallo, B., Vicenta, F. & Sarobe, M. (2003) Solid-phase clean-up in the liquid chromatographic determination of polycyclic aromatic hydrocarbons from edible oils. *J. Chromatogr. A*, **988**, 33–40.
- Barranco, A., Alonso-Salces, R.M., Corta, E., Burrueta, L.A., Gallo, B., Vicenta, F. & Sarobe, M. (2004) Comparison of donor–acceptor and alumina column for clean-up of polycyclic aromatic hydrocarbons from edible oils. *Food Chem.*, **86**, 465–474.
- Bartle, K.D. (1985) Recent advances in the analysis of polycyclic aromatic compounds by gas chromatography. In: Bjorseth, A. & Ramdahl, T., eds., *Handbook of Polycyclic Aromatic Hydrocarbons. Vol. 2. Emission Sources and Recent Progress in Analytical Chemistry*. New York: Marcel Dekker, pp. 193–236. As cited in IPCS (1998).
- Bartosek, I., Guaitani, A., Modica, R., Fiume, M. & Urso, R. (1984) Comparative kinetics of oral benzo[a]pyrene, chrysene and triphenylene in rats: study with hydrocarbon mixtures. *Toxicol. Lett.*, **23**, 333–339. As cited in EC (2002).
- BCL (1980a) Unpublished subchronic toxicity study: Naphthalene (C52904), B6C3F1 mouse. Prepared by Battelle Columbus Laboratories. As cited in US EPA (1998).
- BCL (1980b) Unpublished subchronic toxicity study: Naphthalene (C52904), Fischer 344 rats. Prepared by Battelle Columbus Laboratories. As cited in US EPA (1998) and EC (2002).
- Beliaeff, B., O'Connor, T.P., Munsch, C., Raffin, B. & Claisse, D. (2002) Comparison of polycyclic aromatic hydrocarbon levels in mussels and oysters in France and the United States. *Environ. Toxicol. Chem.*, **21**, 1783–1787.
- Bergard, A., Colmsjo, A. & Lundmark, B.-O. (1993) Gas chromatographic analysis of high molecular mass polycyclic aromatic hydrocarbons. *J. Chromatogr. A*, **630**, 287–295.
- Bender, M.E. & Huggett, R.J. (1988) Polynuclear aromatic hydrocarbon residues in shellfish: Species variations and apparent intraspecific differences. In: Kaiser, H.E., ed., *Cancer Growth and Progression. Vol. 5. Comparative Aspects of Tumor Development*. Dordrecht: Kluwer Academic Publishers, pp. 226–234. As cited in IPCS (1998).
- Berenblum, I. & Haran, H. (1955) The influence of croton oil and of polyethylene glycol-400 on carcinogenesis in the forestomach of the mouse. *Cancer Res.*, **15**, 510–516.
- Black, J.J., Hart, T.F., Jr., & Evans, E. (1981) HPLC studies of PAH pollution in a Michigan trout stream. In: Cooke, M. & Dennis, A., eds., *Polynuclear Aromatic Hydrocarbons: Chemical Analysis and Biological Fate*. Columbus, Ohio: Battelle Press, pp. 343–355. As cited in IPCS (1998).
- Bláha, L., Kapplová, P., Vondráček, K., Upham, B. & Machala, M. (2002) Inhibition of gap-junctional intercellular communication by environmentally occurring polycyclic aromatic hydrocarbons. *Toxicol. Sci.*, **65**, 43–51.
- Bock, F.G. & King, D.W. (1959) A study of the sensitivity of the mouse forestomach toward certain polycyclic aromatic hydrocarbons. *J. Natl. Cancer Inst.*, **23**, 833–838.
- Bolling, H. (1964) [Carcinogenic substances in cereals dried by combustion gas.] *Tech. Monit. Pinerolo*, **15**, 137–142 (in Italian). As cited in IPCS (1998).

- Bolonova, L.N. (1967) [Action of naphthalene and its methyl derivatives on the ammonia content in rat brain.] *Farmakol. Toksikol.*, **30**, 484–486 (in Russian).
- Boom, M.M. (1987) The determination of polycyclic aromatic hydrocarbons in indigenous and transplanted mussels (*Mytilus edulis* L.) along the Dutch coast. *Int. J. Environ. Anal. Chem.*, **31**, 251–261. As cited in IPCS (1998).
- Borneff, J. & Kunte, H. (1979) Method 1. Analysis of polycyclic aromatic hydrocarbons in water using thin layer chromatography and spectrofluorometry. In: Egan, H., Castegnaro, M., Bogovski, P., Kunte, H. & Walker, E.A., eds., *Environmental Carcinogens: Selected Methods of Analysis. Vol. 3. Analysis of Polycyclic Aromatic Hydrocarbons in Environmental Samples*. Lyon: International Agency for Research on Cancer, pp. 129–139 (IARC Scientific Publications No. 29). As cited in IPCS (1998).
- Boroujerdi, M., Kung, H.C., Wilson, A.G.E. & Anderson, M.W. (1981) Metabolism and DNA binding of benzo[a]pyrene *in vivo* in rats. *Cancer Res.*, **41**, 951–957. As cited in IPCS (1998).
- Bourcart, J. & Mallet, L. (1965) [Coastal marine pollution in the central region of the Tyrrhenian Sea (Bay of Naples) by polyaromatic hydrocarbons of the benzo-3,4-pyrene type.] *C. R. Acad. Sci. Paris*, **260**, 3729–3734 (in French). As cited in IPCS (1998).
- Burgaz, S., Demircigil, G.C., Karahalil, B. & Karakaya, A.E. (2002) Chromosomal damage in peripheral blood lymphocytes of traffic policemen and taxi drivers exposed to urban air pollution. *Chemosphere*, **47**, 57–64.
- Burstyn, I., Boffetta, P., Heederik, D., Partanen, T., Kromhout, H., Svane, O., Langard, S., Frentzel-Beyme, R., Kauppinen, T., Stucker, I., Shaham, J., Ahrens, W., Cenee, S., Ferro, G., Heikkilä, P., Hooiveld, M., Johansen, C., Randem, B.G. & Schill, W. (2003) Mortality from obstructive lung diseases and exposure to polycyclic aromatic hydrocarbons among asphalt workers. *Am. J. Epidemiol.*, **158**, 468–478.
- Buss, D.H. & Lindsay, D.G. (1978) Reorganisation of the UK total diet study for monitoring minor constituents of food. *Food Cosmet. Toxicol.*, **16**, 597. As cited in Dennis et al. (1983).
- Buters, J.T.M., Mahadevan, B., Quintanilla-Martinez, L., Gonzalez, F.J., Greim, H., Baird, W.M. & Luch, A. (2002) Cytochrome P450 1B1 determines susceptibility to dibenzo[a,h]pyrene-induced tumor formation *Chem. Res. Toxicol.*, **15**, 1127–1135.
- Butler, J.P., Post, G.B., Lioy, P.J., Waldman, J.M. & Greenberg, A. (1993) Assessment of carcinogenic risk from personal exposure to benzo[a]pyrene in the total human environmental exposure study (THEES). *J. Air Waste Manage. Assoc.*, **43**, 970–977. As cited in EC (2002).
- CAC (2003) *Report of the Thirty-fifth Session of the Codex Committee on Food Additives and Contaminants, Arusha, Tanzania, 17–21 March 2003*. Rome: Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ALINORM 03/12A; <http://www.codexalimentarius.net/web/archives.jsp?year=03>).
- Camargo, M.C.R. & Toledo, M.C.F. (1999) Effect of processing on the contamination of refined corn oil by benzo[a]pyrene. *Braz. J. Food Technol.*, **1**, 97–106.
- Camargo, M.C.R. & Toledo, M.C.F. (2000) Polycyclic aromatic hydrocarbons in margarine, vegetable cream and mayonnaise. *Cienc. Tecnol. Aliment., Campinas*, **20**, 51–55.
- Camargo, M.C.R. & Toledo, M.C. (2001) Dietary polycyclic aromatic hydrocarbon intakes in some Brazilian metropolitan areas. *Rev. Bras. Toxicol.*, **14** (2), 23–30.
- Camargo, M.C.R. & Toledo, M.C.F. (2002a) Coffee and mate tea as a dietary source of polycyclic aromatic hydrocarbon (PAHs) in Campinas. *Cienc. Tecnol. Aliment., Campinas*, **22**, 49–53.

- Camargo, M.C.R. & Toledo, M.C.F. (2002b) Polycyclic aromatic hydrocarbon contamination in different commodity groups. *Braz. J. Food Technol.*, **5**, 19–26.
- Camargo, M.C.R. & Toledo, M.C.F. (2002c) Polycyclic aromatic hydrocarbons — A review. *Bol. SBCTA (Soc. Bras. Cienc. Tecnol. Aliment.)*, Campinas, **36**, 69–78.
- Camargo, M.C.R. & Toledo, M.C.F. (2003) Polycyclic aromatic hydrocarbons in Brazilian vegetables and fruits. *Food Control*, **14**, 49–53.
- Camargo, M.C.R. & Toledo, M.C.F. (2004) Submission to 64th JECFA: Polycyclic aromatic hydrocarbons in the Brazilian diet. Unpublished work.
- Camargo, M.C.R. & Toledo, M.C.F. (no date) *Polycyclic Aromatic Hydrocarbons in the Brazilian Diet* [Thesis]. Campinas: Univeridade Estadual de Campinas.
- Carretero, M.V., Latasa, M.U., Garcia-Trevijano, E.R., Corrales, F.J., Wagner, C., Mato, J.M. & Avila, M.A. (2001) Inhibition of liver methionine adenosyltransferase gene expression by 3-methylcholanthrene: protective effect of S-adenosylmethionine. *Biochem. Pharmacol.*, **61**, 1119–1128. As cited in EC (2002).
- Castano-Vinyals, G., D'Errico, A., Malats, N. & Kogevinas, M. (2004) Biomarkers of exposure to polycyclic aromatic hydrocarbons from environmental air pollution. *Occup. Environ. Med.*, **61**, e12.
- CEC (2002) *Proposal for a European Parliament and Council Regulation on Smoke Flavorings Used or Intended for Use in or on Foods (Presented by the Commission)*. Commission of the European Communities (COM/2002/0400 final – COD 2002/0163).
- Charles, G.D., Bartels, M.J., Zacharewski, T.R., Gollapudi, B.B., Freshour, N.L. & Carney, E.W. (2000) Activity of benzo[a]pyrene and its hydroxylated metabolites in an estrogen receptor- α reporter gene assay. *Toxicol. Sci.*, **55**, 320–326.
- Chen, B.H. (1997) Analysis, formation and inhibition of polycyclic aromatic hydrocarbons in foods: An overview. *J. Food Drug Anal.*, **5**, 25–42.
- Chen, B.H. & Lin, Y.S. (1997) Formation of polycyclic aromatic hydrocarbons during processing of duck meat. *J. Agric. Food Chem.*, **45**, 1394–1403.
- Chen, B.H., Wang, C.Y. & Chiu, C.P. (1996) Evaluation of analysis of polycyclic aromatic hydrocarbons in meat products by liquid chromatography. *J. Agric. Food Chem.*, **44**, 2244–2251.
- Chen, J. & Chen, S. (2005) Removal of polycyclic aromatic hydrocarbons by low density polyethylene from liquid model and roasted meat. *Food Chem.*, **90**, 461–469.
- Chen, M.L., Lee, B.C., Lu, P.L., Mao, I.F. & Liu, T.Y. (2003) Polycyclic aromatic hydrocarbon–deoxyribonucleic acid (PAH–DNA) adduct levels and exposure to coke oven emissions among workers in Taiwan. *Arch. Environ. Health*, **58**, 298–305.
- Chinese National Nutrition and Health Survey (2005) Submission to 64th JECFA: Chinese food consumption data.
- Chipman, J.K. (1982) Bile as a source of potential reactive metabolites. *Toxicology*, **25**, 99–111. As cited in IPCS (1998).
- Chipman, J.K., Hirom, P.C., Frost, G.S. & Millburn, P. (1981) The biliary excretion of entero-hepatic circulation of benzo[a]pyrene and its metabolites in the rat. *Biochem. Pharmacol.*, **30**, 937–944. As cited in IPCS (1998).
- Chu, E.W. & Malmgren, R.A. (1965) An inhibitory effect of vitamin A on the induction of tumours of forestomach and cervix in the Syrian hamster by carcinogenic polycyclic aromatic hydrocarbons. *Cancer Res.*, **25**, 884–895.
- Committee on Pyrene and Selected Analogues (1983) *Polycyclic Aromatic Hydrocarbons: Evaluation of Sources and Effects*. Washington, D.C.: National Academy Press.

- Compaan, H. & Laane, R.W. (1992) *Polycyclic Aromatic Hydrocarbons (PAH) in the North Sea: An Inventory*. Delft: TNO Institute of Environmental Sciences, 130 pp. (TNO Report IMW-R 92/392). As cited in IPCS (1998).
- COT (2002) *Polycyclic Aromatic Hydrocarbons in the 2000 Total Diet Study*. London: Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (Report TOX/2002/26; <http://www.food.gov.uk/multimedia/pdfs/TOX-2002-26.PDF>). As cited in UKFSA (2002) and EC (2002).
- Crosby, N.T., Hunt, D.C., Phillip, L.A. & Patel, I. (1981) Determination of polynuclear aromatic hydrocarbons in food, water and smoke using high-performance liquid chromatography. *Analyst*, **106**, 135–145.
- Crössmann, G. & Wüstemann, M. (1992) [Loadings in Domestic Gardens and Allotments by Inorganic and Organic Substances with Damaging Potential: Actual Documentation. Part I: Soils and Garden Wastes and Part II: Vegetables and Fruits.] Berlin: Ministry of Environment, pp. 40–42, 108–124 (in German). As cited in IPCS (1998).
- Culp, S.J., Gaylor, D.W., Sheldon, W.G., Goldstein, L.S. & Beland, F.A. (1998) A comparison of the tumors induced by coal tar and benzo[a]pyrene in a 2-year bioassay. *Carcinogenesis*, **19**, 117–124.
- Curfs, D.M.J., Lutgens, E., Gijbels, M.J.J., Kockx, M.M., Daemon, M.J.A.P. & van Schooten, F.J. (2004) Chronic exposure to the carcinogenic compound benzo[a]pyrene induces larger and phenotypically different atherosclerotic plaques in ApoE-knockout mice. *Am. J. Pathol.*, **164**, 101–108.
- Daisey, J.M. (1983) Analysis of polycyclic aromatic hydrocarbons by thin-layer chromatography. In: Bjorseth, A., ed., *Handbook of Polycyclic Aromatic Hydrocarbons*. New York: Marcel Dekker, pp. 397–437. As cited in IPCS (1998).
- de Boer, J. & Law, R.J. (2003) Developments in the use of chromatographic techniques in marine laboratories for the determination of halogenated contaminants and polycyclic aromatic hydrocarbons. *J. Chromatogr. A*, **1000**, 223–251.
- De Jong, W.H., Kroese, E.D., Vos, J.G. & Van Loveren, H. (1999) Detection of immunotoxicity of benzo[a]pyrene in a subacute toxicity study after oral exposure in rats. *Toxicol. Sci.*, **50**, 214–220. As cited in EC (2002).
- de Kok, T.M. & van Maanen, J.M. (2000) Evaluation of fecal mutagenicity and colorectal cancer risk. *Mutat. Res.*, **463**, 53–101.
- de Kruijf, N., Schoten, T. & van der Stegen, G.H.D. (1987) Rapid determination of benzo[a]pyrene in roasted coffee and coffee-brew by high-performance liquid chromatography with fluorescence detection. *J. Agric. Food Chem.*, **35**, 545–549.
- Dennis, M.J., Massey, R.C., McWeeny, D.J., Knowles, M.E. & Watson, D. (1983) Analysis of polycyclic aromatic hydrocarbons in UK total diets. *Food Chem. Toxicol.*, **21** (5), 569–574. As cited in EC (2002).
- Dennis, M.J., Massey, R.C., Cripps, G., Venn, I., Howarth, N. & Lee, G. (1991) Factors affecting the polycyclic aromatic hydrocarbons content of cereals, fats and other food products. *Food Addit. Contam.*, **8**, 517–530.
- de Vos, R.H., van Dokkum, W., Schouten, A. & de Jong-Berkhout, P. (1990) Polycyclic aromatic hydrocarbons in Dutch total diet samples (1984–1986). *Food Chem. Toxicol.*, **28** (4), 263–268. As cited in IPCS (1998) and EC (2002).
- Diletti, G., Scortichini, G., Scarpone, R., Gatti, G., Torreti, L. & Migliorati, G. (2005) Isotope dilution determination of polycyclic aromatic hydrocarbons in olive pomace oil by gas chromatography–mass spectrometry. *J. Chromatogr. A*, **1062**, 247–254.

- Dong, M.W. & Greenberg, A. (1988) Liquid chromatographic analysis of polynuclear aromatic hydrocarbons with diode array detection. *J. Liq. Chromatogr.*, **11**, 1887–1905.
- Doremire, M.E., Harmon, G.E. & Pratt, D.E. (1979) Benzopyrene in charcoal grilled meats. *J. Food Sci.*, **44**, 622–623.
- DouAbdul, A.A.Z., Abaychi, J.K., Al-Edanee, T.E., Ghani, A.A. & Al-Saad, H.T. (1987) Polynuclear aromatic hydrocarbons (PAHs) in fish from the Arabian Gulf. *Bull. Environ. Contam. Toxicol.*, **38**, 546–552. As cited in IPCS (1998).
- Draudt, H.N. (1963) The meat smoking process: A review. *Food Technol.*, **17**, 85–90. As cited in IPCS (1998).
- Dugay, A., Herrenknecht, C., Czok, M., Guyon, F. & Pages, N. (2002) New procedure for selective extraction of polycyclic aromatic hydrocarbons in plants for gas chromatographic–mass spectrometric analysis. *J. Chromatogr. A*, **958**, 1–7.
- EC (2002) *Opinion of the Scientific Committee on Foods on the Risks to Human Health of Polycyclic Aromatic Hydrocarbons in Food, expressed on 4th December 2002*. Brussels: European Commission, Health and Consumer Protection Directorate-General, Scientific Committee on Foods (SCF/CS/CNTM/PAH/29 Final; http://europa.eu.int/comm/food/fs/sc/scf/outcome_en.html).
- Edwards, N.T. (1983) Polycyclic aromatic hydrocarbons (PAHs) in the terrestrial environment. A review. *J. Environ. Qual.*, **12**, 427–441. As cited in IPCS (1998).
- EEC (1988) Council Directive 88/388/EEC of 21 June 1988 on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production. European Economic Community. *Off. J. Eur. Commun.*, **L184**, 61–67.
- Environment Canada (1994) *Canadian Environmental Protection Act. Priority Substances List Assessment Report: Polycyclic Aromatic Hydrocarbons*. Ottawa, Ontario: Ministry of Supply and Services, 61 pp.
- EPIC (2002) *Food Content of Potential Carcinogens, Nitrates, Nitrites, Nitrosamines, Heterocyclic Amines and Polycyclic Aromatic Hydrocarbons*. European Prospective Investigation of Cancer (EPIC) (<http://www.epic-spain.com/libro.html>).
- Falco, G., Domingo, J.L., Llobet, J.M., Teixido, A., Casas, C. & Muller, L. (2003) Polycyclic aromatic hydrocarbons in foods: Human exposure through the diet in Catalonia, Spain. *J. Food Prot.*, **66** (12), 2325–2331.
- FAO (2001) *Compendium of Food Additive Specifications*. Rome: Food and Agriculture Organization of the United Nations (Food and Nutrition Paper 52, Addendum 9).
- Fazio, T. (1990) Polycyclic aromatic hydrocarbons and benzo[a]pyrene in food. Spectrophotometric method (No. 973.30). In: Helrich, K., ed., *Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC)*, 15th Ed., Vol. 2. Arlington, Virginia: Association of Official Analytical Chemists, pp. 1176–1178.
- Fretheim, K. (1983) Polycyclic aromatic hydrocarbons in grilled meat products — A review. *Food Chem.*, **10**, 129–139.
- Gaines, T.B. (1969) Acute toxicity of pesticides. *Toxicol. Pharmacol.*, **14**, 515–534.
- Gammon, M.D., Santella, R.M., Neugut, A.I., Eng, S.M., Teitelbaum, S.L., Paykin, A., Levin, B., Terry, M.B., Young, T.L., Wang, L.W., Wang, Q., Britton, J.A., Wolff, M.S., Stellman, S.D., Hatch, M., Kabat, G.C., Senie, R., Garbowski, G., Maffeo, C., Montalvan, P., Berkowitz, G., Kemeny, M., Citron, M., Schnabel, F., Schuss, A., Hajdu, S. & Vinciguerra, V. (2002) Environmental toxins and breast cancer on Long Island. I. Polycyclic aromatic hydrocarbon DNA adducts. *Cancer Epidemiol. Biomarkers Prev.*, **11**, 677–685.

- Garcia-Falcon, M.S., Gonzalez Amigo, S., Lage Yusty, M.A., Lopez de Alda Villaizan, M.J. and Simal Lozano, J. (1996) Enrichment of benzo[a]pyrene in smoked food products and determination by high-performance liquid chromatography–fluorescence detection. *J. Chromatogr. A*, **753**, 207–215.
- Garcia-Falcon, M.S., Amigo, S.G., Yusty, M.A.L. & Lozano, J.S. (1999) Determination of benzo[a]pyrene in some Spanish commercial smoked products by HPLC-FL. *Food Addit. Contam.*, **16**, 9–14.
- Garcia-Falcon, M.S., Perez-Lamela, M. & Simal-Gandara, J. (2004) Comparison of strategies for extraction of high molecular weight polycyclic aromatic hydrocarbons from drinking waters. *J. Agric. Food Chem.*, **52**, 6897–6903.
- Garcia-Falcon, M.S., Cancho-Grande, B. & Simal-Gandara, J. (2005) Minimal clean-up and rapid determination of polycyclic aromatic hydrocarbons in instant coffee. *Food Chem.*, **90**, 643–647.
- Garner, R.C. (1998) The role of DNA adducts in chemical carcinogenesis. *Mutat. Res.*, **402**, 67–75.
- Gaspari, L., Chang, S.S., Santella, R.M., Garte, S., Pedotti, P. & Taioli, E. (2003) Polycyclic aromatic hydrocarbon–DNA adducts in human sperm as a marker of DNA damage and infertility. *Mutat. Res.*, **535**, 155–160.
- Gerarde, H.W. (1960) Toxicology and biochemistry of aromatic hydrocarbons. In: Browning, E., ed., *Elsevier Monographs on Toxic Agents*. Amsterdam: Elsevier, pp. 240–321. As cited in EC (2002).
- German Ministry of Environment (1979) [*Air Quality Criteria for Selected Polycyclic Aromatic Hydrocarbons. PAH as an Environmental Carcinogen.*] Berlin: Erich Schmidt Verlag, 270 pp. (Report No. UBA-1/79) (in German):
- Godschalk, R.W., Moonen, E.J., Schilderman, P.A., Broekmans, W.M., Kleinjans, J.C. & Van Schooten, F.J. (2000) Exposure-route-dependent DNA adduct formation by polycyclic aromatic hydrocarbons. *Carcinogenesis*, **21**, 87–92. As cited in EC (2002).
- Goldstein, L.S. (2001) To BaP or not to BaP? That is the question. *Environ. Health Perspect.* **A**, **109** (8), 356–357.
- Goldstein, L.S., Weyand, E.H.S., Steinberg, M., Culp, S.J., Gaylor, D.W., Beland, F.A. & Rodriguez, L.V. (1998) Tumors and DNA adducts in mice exposed to benzo[a]pyrene and coal tars: Implications for risk assessment. *Environ. Health Perspect.*, **106** (Suppl. 6), 1325–1330.
- Gomaa, E.A., Gray, J.I., Rabie, S., Lopez-Bote, C. & Booren, A.M. (1993) Polycyclic aromatic hydrocarbons in smoked food products and commercial liquid smoke flavourings. *Food Addit. Contam.*, **10** (5), 503–521.
- Gräf, W. (1970) Levels of 3,4-benzopyrene in human organs of different ages. Second communication. *Arch. Hyg.*, **154**, 331–335. As cited in IPCS (1998).
- Gräf, W., Eff, H. & Schormair, S. (1975) Levels of carcinogen, polycyclic aromatic hydrocarbons in human and animal tissues. Third communication. *Zentralbl. Bakteriol. Hyg. Abt. Orig. B*, **161**, 85–103. As cited in IPCS (1998).
- Grimmer, G. & Böhnke, H. (1975) Polycyclic aromatic hydrocarbon profile analysis of high-protein foods, oils, and fats by gas chromatography. *J. Assoc. Off. Anal. Chem.*, **58**, 725–733.
- Grimmer, G. & Böhnke, H. (1979a) Method 4: Gas chromatographic profile analysis of polycyclic aromatic hydrocarbons in (i) high protein foods, (ii) fats and vegetable oils and (iii) plants, soils and sewage sludge. In: Egan, H., Castegnaro, M., Bogovski, P., Kunte, H. & Walker, E.A., eds., *Environmental Carcinogens: Selected Methods of*

- Analysis. Vol. 3. Analysis of Polycyclic Aromatic Hydrocarbons in Environmental Samples.* Lyon: International Agency for Research on Cancer, pp. 163–173 (IARC Scientific Publications No. 29). As cited in IPCS (1998).
- Grimmer, G. & Böhnke, H. (1979b) Method 3: Gas chromatographic profile analysis of polycyclic aromatic hydrocarbons in lubricating oil, cutting oil and fuel. In: Egan, H., Castegnaro, M., Bogovski, P., Kunte, H. & Walker, E.A., eds., *Environmental Carcinogens: Selected Methods of Analysis. Vol. 3. Analysis of Polycyclic Aromatic Hydrocarbons in Environmental Samples.* Lyon: International Agency for Research on Cancer, pp. 155–162 (IARC Scientific Publications No. 29). As cited in IPCS (1998).
- Grimmer, G. & Hildebrandt, A. (1965) [The content of polyaromatic hydrocarbons in various vegetables.] *Dtsch. Lebensm.-Rundsch.*, **61**, 237–239 (in German). As cited in IPCS (1998).
- Grova, M., Feidt, C., Laurent, C. & Rychen, G. (2002) [¹⁴C] Milk, urine and faeces excretion kinetics in lactating goats after an oral administration of [¹⁴C]polycyclic aromatic hydrocarbons. *Int. Dairy J.*, **12**, 1025–1031.
- Guillen, M.D., Sopelana, P. & Partearroyo, M.A. (1997) Food as a source of polycyclic aromatic carcinogens. *Rev. Environ. Health*, **12**, 133–146.
- Guillen, M.D., Sopelana, P. & Partearroyo, M.A. (2000) Study of several aspects of a general method for the determination of polycyclic aromatic hydrocarbons in liquid smoke flavourings by gas chromatography–mass spectrometry. *Food Addit. Contam.*, **17**, 27–44.
- Guillen, M.D., Sopelana, P. & Gemma, P. (2004a) Occurrence of polycyclic aromatic hydrocarbons in smoked cheese. *J. Dairy Sci.*, **87**, 556–564.
- Guillen, M.D., Sopelana, P. & Gemma, P. (2004b) Polycyclic aromatic hydrocarbons and olive pomace oil. *J. Agric. Food Chem.*, **52**, 2123–2132.
- Hansen, A.M., Wallin, H., Binderup, M.L., Dybdahl, M., Autrup, H., Loft, S. & Knudsen, L.E. (2004) Urinary 1-hydroxypyrene and mutagenicity in bus drivers and mail carriers exposed to urban air pollution in Denmark. *Mutat. Res.*, **557**, 7–17.
- Hardin, B.D., Bond, G.O., Sikov, M.R., Andrew, F.D., Beliles, R.P. & Niemeier, R.W. (1981) Testing of selected workplace chemicals for teratogenic potential. *Scand. J. Work Environ. Health*, **7**, 66–75.
- Hecht, S.S., Chen, M., Yagi, H., Jerina, D.M. & Carmella, S.G. (2003) r-1,t-2,3,c-4-Tetrahydroxy-1,2,3,4-tetrahydrophenanthrene in human urine: a potential biomarker for assessing polycyclic aromatic hydrocarbon metabolic activation. *Cancer Epidemiol. Biomarkers Prev.*, **12**, 1501–1508.
- Heeschen, W.H. (1985) [Scientific working group on chemicals in human milk.] *Arch. Gynkol.*, **238**, 199–216 (in German). As cited in IPCS (1998).
- Hites, R.A. (1989) Mass spectrometry of polycyclic aromatic compounds. In: Vo-Dinh, T., ed., *Chemical Analysis of Polycyclic Aromatic Compounds.* New York: John Wiley & Sons, pp. 219–261.
- Hoogervorst, E.M., De Vries, A., Beems, R.B., Van Oostrom, C.T., Wester, P.W., Vos, J.G., Bruins, W., Roodbergen, M., Cassee, F.R., Vijg, J., Van Schooten, F.J. & Van Steeg, H. (2003) Combined oral benzo[a]pyrene and inhalatory ozone exposure have no effect on lung tumor development in DNA-repair deficient Xpa mice. *Carcinogenesis*, **24** (3), 613–619.
- Hopia, A., Pyysalo, H. & Wickström, K. (1986) Margarines, butter and vegetable oils as sources of polycyclic aromatic hydrocarbons. *J. Am. Oil Chem. Soc.*, **63**, 889–893.

- Howard, J.W. & Fazio, T. (1980) Review of polycyclic aromatic hydrocarbons in foods. Analytical methodology and reported findings of polycyclic aromatic hydrocarbons in foods. *J. Assoc. Off. Anal. Chem.*, **63**, 1077–1104. As cited in IPCS (1998).
- Howard, P.H., Boethling, R.S., Jarvis, W.F., Meylan, W.M. & Michalenko, E.M. (1991) *Handbook of Environmental Degradation Rates*. Chelsea, Michigan: Lewis Publishers.
- Huggins, C. & Yang, N.C. (1962) Induction and extinction of mammary cancer. A striking effect of hydrocarbons permits analysis of mechanisms of causes and cure of breast cancer. *Science*, **137**, 257–262.
- Hungspreugs, M., Silpipat, S., Tonapong, C., Lee, R.F., Windom, H.L. & Tenore, K.R. (1984) Heavy metals and polycyclic hydrocarbon compounds in benthic organisms of the upper Gulf of Thailand. *Mar. Pollut. Bull.*, **15**, 213–218. As cited in IPCS (1998).
- Husain, A., Naeemi, E., Dashti, B., Al-Omirah, H. & Al-Zenki, S. (1997) Polycyclic aromatic hydrocarbons in food products originating from locally reared animals in Kuwait. *Food Addit. Contam.*, **14** (3), 295–299.
- IARC (1984a) *Polynuclear Aromatic Hydrocarbons. Part 2: Carbon Blacks, Mineral Oils (Lubricant Base Oils and Derived Products) and Some Nitroarenes*. Lyon: International Agency for Research on Cancer (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 33).
- IARC (1984b) *Polynuclear Aromatic Compounds. Part 3: Industrial Exposures in Aluminium Production, Coal Gasification, Coke Production, and Iron and Steel Founding*. Lyon: International Agency for Research on Cancer (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 34).
- IARC (1985) *Polynuclear Aromatic Compounds. Part 4: Bitumens, Coal-tars and Derived Products, Shale-oils and Soots*. Lyon: International Agency for Research on Cancer (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 35).
- IARC (2002) *Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene*. Lyon: International Agency for Research on Cancer (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 82).
- Iman, A. & Inaam, A. (2002) Gas chromatography–mass spectrometric determination of polycyclic aromatic hydrocarbons in five species of fish from three sites in the Arabian Gulf. *Int. J. Environ. Health Res.*, **12**, 193–200.
- IPCS (1998) *Selected Non-heterocyclic Polycyclic Aromatic Hydrocarbons*. Geneva: World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 202).
- IUPAC (1987) Recommended method for a thin-layer-chromatographic screening method for the determination of benzo[a]pyrene in smoked food. International Union of Pure and Applied Chemistry. *Pure Appl. Chem.*, **59**, 1735–1738.
- Jacob, J. & Seidel, A. (2004) *Verwendung eines auf Wirkungsäquivalenten basierenden Summenparameters für die Abschätzung der PAH-Exposition und ihres Krebs erregenden Potenzials. Polyklinische aromatische Kohlenwasserstoffe*. Deutsche Forschungsgemeinschaft. Weinheim: Wiley VCH, pp. 8–26.
- Jakszyn, P., Agudo, A., Ibanaz, R., Garcia-Closas, R., Pera, G., Amiano, P. & Gonzalez, A. (2004) Development of a food data base of nitrosamines, heterocyclic amines and polycyclic aromatic hydrocarbons. *J. Nutr.*, **134**, 2011–2014.
- Janini, G.M., Johnston, K. & Zielinski, W.L., Jr. (1975) Use of nematic liquid crystal for gas–liquid chromatographic separation of polyaromatic hydrocarbons. *Anal. Chem.*, **47**, 670–674. As cited in IPCS (1998).

- Janini, G.M., Muschik, G.M., Schroer, J.A. & Zielinski, W.L., Jr. (1976) Gas-liquid chromatographic evaluation and gas-chromatography/mass spectrometric application of new high-temperature liquid crystal stationary phases for polycyclic aromatic hydrocarbon separations. *Anal. Chem.*, **48**, 1879–1883. As cited in IPCS (1998).
- Järvenpää, E., Huopalahti, R. & Tapanainen, P. (1996) Use of supercritical fluid extraction–high performance liquid chromatography in the determination of polynuclear aromatic hydrocarbons from smoked and broiled fish. *J. Liq. Chromatogr. Relat. Technol.*, **19** (9), 1473–1482.
- Joe, F.L., Jr., Salemm, J. & Fazio, T. (1984) Liquid chromatographic determination of trace residues of polynuclear aromatic hydrocarbons in smoked foods. *J. Assoc. Off. Anal. Chem.*, **67**, 1076–1082.
- Jones, K.C., Grimmer, G., Jacob, J. & Johnston, A.E. (1989) Changes in the polynuclear aromatic hydrocarbon content of wheat grain and pasture grassland over the last century from one site in the UK. *Sci. Total Environ.*, **78**, 117–130.
- Jones, S.H., Chase, M., Sowles, J., Hennigar, P., Landry, N., Wells, P.G., Harding, C.H., Krahforst, C. & Brun, G.L. (2001) Monitoring for toxic contaminants in *Mytilus edulis* from New Hampshire and the Gulf of Maine. *J. Shellfish Res.*, **20**, 1203–1214.
- Jongeneelen, F.J., van Leeuwen, F.E., Oosterink, S., Anzion, R.B., van der Loop, F., Bos, R.P. & van Veen, H.G. (1990) Ambient and biological monitoring of cokeoven workers: determinants of the internal dose of polycyclic aromatic hydrocarbons. *Br. J. Ind. Med.*, **47**, 454–461. As cited in IPCS (1998).
- Kagi, R., Alexander, R. & Cumbers, M. (1985) Polycyclic aromatic hydrocarbons in rock oysters: A baseline study. *Int. J. Environ. Anal. Chem.*, **22**, 135–153. As cited in IPCS (1998).
- Karl, H. & Leinemann, M. (1996) Determination of polycyclic aromatic hydrocarbons in smoked fishery products from different smoking kilns. *Z. Lebensm.-Unters. Forsch.*, **202**, 458–464.
- Kato, M., Loomis, D., Brooks, L.M., Gattas, G.F., Gomes, L., Carvalho, A.B., Rego, M.A. & DeMarini, D.M. (2004) Urinary biomarkers in charcoal workers exposed to wood smoke in Bahia State, Brazil. *Cancer Epidemiol. Biomarkers Prev.*, **13**, 1005–1012.
- Kaupp, H. & Sklorz, M. (1996) A method for analyzing polycyclic aromatic hydrocarbons in plant samples. *Chemosphere*, **32**, 849–854.
- Kawai, M. (1979) [Review of toxicological and occupational aspects of naphthalene.] *Aromatics*, **31**, 168–181 (in Japanese). As cited in IPCS (1998).
- Kawamura, Y., Kamata, E., Ogawa, Y., Kaneko, T., Uchiyama, S. & Saito, Y. (1988) The effect of various foods on the intestinal absorption of benzo[a]pyrene in rats. *J. Food Hyg. Soc. Jpn.*, **29**, 21–25. As cited in EC (2002).
- Kayali-Sayadi, M.N., Rubio-Barroso, S., Cuesta-Jimenez, M.P. & Polo-Diez, L.M. (1998) Rapid determination of polycyclic aromatic hydrocarbons in tea infusion samples by high-performance liquid chromatography and fluorimetric detection based on solid-phase extraction. *Analyst*, **123**, 2145–2148.
- Kayali-Sayadi, M.N., Rubio-Barroso, S., Cuesta-Jimenez, M.P. & Polo-Diez, L.M. (1999) A new method for the determination of selected PAH in coffee brew samples by HPLC with fluorimetric detection and solid-phase extraction. *J. Liq. Chromatogr. Relat. Technol.*, **22**, 615–627.
- Kazerouni, N., Sinha, R., Hsu, C.-H., Greenberg, A. & Rothman, N. (2001) Analysis of 200 food items for benzo[a]pyrene and estimation of its intake in an epidemiologic study. *Food Chem. Toxicol.*, **39**, 423–436. As cited in EC (2002).

- Kicinski, H.G., Adamek, S. & Kettrup, A. (1989) Trace enrichment and HPLC analysis of polycyclic aromatic hydrocarbons in environmental samples, using solid phase extraction in connection with UV/VIS diode-array and fluorescence detection. *Chromatographia*, **28**, 203–208.
- King, A.J., Readman, J.W. & Zhou, J.L. (2004) Determination of polycyclic aromatic hydrocarbons in water by solid-phase microextraction–gas chromatography–mass spectrometry. *Anal. Chim. Acta*, **523**, 259–267.
- Kishikawa, N., Wada, M., Kuroda, N., Akiyama, S. & Nakashima, K. (2003) Determination of polycyclic aromatic hydrocarbons in milk samples by high performance liquid chromatography with fluorescence detection. *J. Chromatogr. B*, **789**, 257–264.
- Kiss, G., Verga-Puchony, Z. & Hlavay, J. (1996) Determination of polycyclic aromatic hydrocarbons in precipitation using solid-phase extraction and column chromatography. *J. Chromatogr. A*, **725**, 261–272.
- Klein, M. (1963) Susceptibility of strain B6AF1/J hybrid infant mice to tumourigenesis with 1,2-benzanthracene, deoxycholic acid and 3-methylcholanthrene. II. Tumours called forth by painting the skin with dibenzpyrene. *Cancer Res.*, **23**, 1701–1707.
- Knize, M.G., Salmon, C.P., Pais, P. & Felton, J.S. (1999) Food heating and the formation of heterocyclic aromatic amine and polycyclic aromatic hydrocarbon mutagens/carcinogens. *Adv. Exp. Med. Biol.*, **459**, 179–193.
- Knuckles, M.E., Inyang, F. & Ramesh, A. (2001) Acute and subacute oral toxicities of benz[a]pyrene in F-344 rats. *Toxicol. Sci.*, **61**, 382–388.
- Kolarovic, L. & Traittler, H. (1982) Determination of polycyclic aromatic hydrocarbons in vegetable oils by caffeine complexation and glass capillary gas chromatography. *J. Chromatogr.*, **237**, 263–272.
- Kondraganti, S.R., Fernandez-Salugero, P., Gonzalez, F.J., Ramos, K.S., Jiang, W. & Noorthy, B. (2003) Polycyclic aromatic hydrocarbon-inducible DNA adducts: evidence by ³²P-postlabelling and use of knockout mice for Ah receptor-independent mechanisms of metabolic activation in vivo. *Int. J. Cancer*, **103**, 5–11.
- Kotin, P., Falk, H.L. & Busser, R. (1959) Distribution, retention and elimination of C¹⁴-3,4-benzpyrene after administration to mice and rats. *J. Natl. Cancer Inst.*, **23**, 541–555. As cited in IPCS (1998).
- Krewski, D., Thorslund, T. & Withey, J. (1989) Carcinogenic risk assessment of complex mixtures. *Toxicol. Ind. Health*, **5**, 851–867.
- Kroese, E.D., Muller, J.J.A., Mohn, G.R., Dortant, P.M. & Wester, P.W. (2001) *Tumourigenic Effects in Wistar Rats Orally Administered Benzo[a]pyrene for Two Years (Gavage Studies). Implications for Human Cancer Risks Associated with Oral Exposure to Polycyclic Aromatic Hydrocarbons*. Bilthoven: National Institute of Public Health and the Environment, November (RIVM Report No. 658603 010). As cited in EC (2002).
- Kuusimäki, L., Peltonen, Y., Mutanen, P., Peltonen, K. & Savela, K. (2004) Urinary hydroxy-metabolites of naphthalene, phenanthrene and pyrene as markers of exposure to diesel exhaust. *Int. Arch. Occup. Environ. Health*, **77**, 23–30.
- Larsson, B.K. (1982) Polycyclic aromatic hydrocarbons in smoked fish. *Z. Lebensm.-Unters. Forsch.*, **174**, 101–107. As cited in IPCS (1998).
- Larsson, B. (1986) *Polycyclic Aromatic Hydrocarbons in Swedish Foods: Aspects on Analysis, Occurrence and Intake* [Doctoral Thesis]. Uppsala: Swedish University of Agricultural Sciences, 60 pp. As cited in EC (2002).
- Larsson, B. & Sahlberg, G. (1982) Polycyclic aromatic hydrocarbons in lettuce. Influence of a highway and an aluminium smelter. In: Cooke, M. & Fisher, A.J., eds., *Polynuclear*

Aromatic Hydrocarbons: Physical and Biological Chemistry. Columbus, Ohio: Battelle Press, pp. 417–426.

- Larsson, B.K., Sahlberg, G.P., Eriksson, A.T. & Busk, L.A. (1983) Polycyclic aromatic hydrocarbons in grilled food. *J. Agric. Food Chem.*, **31**, 867–873.
- Larsson, B.K., Eriksson, A.T. & Cervenka, M. (1987) Polycyclic aromatic hydrocarbons in crude and deodorized vegetable oils. *J. Am. Oil Chem. Soc.*, **64**, 365–370.
- Laurent, C., Feidt, C., Lichtfouse, E., Grova, N., Laurent, F. & Rychen, G. (2001) Milk–blood transfer of ^{14}C -tagged polycyclic aromatic hydrocarbons (PAH) in pigs. *J. Agric. Food Chem.*, **49**, 2493–2496. As cited in EC (2002).
- Laurent, C., Feidt, C., Grova, N., Mpassi, D., Lichtfouse, E., Laurent, F. & Rychen, G. (2002) Portal absorption of ^{14}C after ingestion of spiked milk with ^{14}C -phenanthrene, ^{14}C -benzo[a]pyrene or ^{14}C -TCDD in growing pigs. *Chemosphere*, **48**, 843–848.
- Lawrence, J.F. & Weber, D.F. (1984a) Determination of polycyclic aromatic hydrocarbons in some Canadian commercial fish, shellfish and meat products by liquid chromatography with confirmation by capillary gas chromatography–mass spectrometry. *J. Agric. Food Chem.*, **32** (4), 789–794.
- Lawrence, J.F. & Weber, D.F. (1984b) Determination of polycyclic aromatic hydrocarbons in Canadian samples of processed vegetable and dairy products by liquid chromatography with fluorescence detection. *J. Agric. Food Chem.*, **32**, 794–797.
- Lechner, W., Huber, M., Bonn, G.K. & Daxenbichler, G. (1991) Analysis of polycyclic aromatic hydrocarbons in lipid-containing biological matrices. Study of contamination of breast milk by benzo[a]pyrene along transit routes through Tyrol. *Wien. Klin. Wochenschr.*, **103**, 88–90.
- Lee, J., Kang, D., Lee, K.H., Ichiba, M., Zhang, J., Tomokuni, K., Hwang, E.S., Park, C.G., Ha, M., Kim, S., Han, S.B., Choi, J.W., Lee, E., Jang, J.Y., Strickland, P.T., Hirvonen, A. & Cho, S.H. (2002) Influence of GSTM1 genotype on association between aromatic DNA adducts and urinary PAH metabolites in incineration workers. *Mutat. Res.*, **514**, 213–221. As cited in EC (2002).
- Lee, M.L., Novotny, M.V. & Bartle, K.D. (1981) *Analytical Chemistry of Polycyclic Aromatic Compounds*. New York: Academic Press.
- Legraverend, C., Harrison, D.E., Ruscetti, F.W. & Nebert, D.W. (1983) Bone marrow toxicity induced by oral benzo[a]pyrene: protection resides at the level of the intestine and liver. *Toxicol. Appl. Pharmacol.*, **70**, 390–401. As cited in EC (2002).
- Le Marchand, L., Hankin, J.H., Pierce, L.M., Sinha, R., Nerurkar, P.V., Franke, A.A., Wilkens, L.R., Kolonel, L.N., Donlon, T., Seifried, A., Custer, L.J., Lum-Jones, A. & Chang, W. (2002) Well-done red meat, metabolic phenotypes and colorectal cancer in Hawaii. *Mutat. Res.*, **506–507**, 205–214.
- Lenin, K. (1994) *Contamination with Polycyclic Aromatic Hydrocarbons through Sewage Irrigation* [Doctoral thesis]. Delhi: Jawaharlal Nehru University, 22 pp.
- Lijinsky, W. & Ross, A.E. (1967) Production of carcinogenic polynuclear hydrocarbons in the cooking of food. *Food Cosmet. Toxicol.*, **5**, 343–347. As cited in IPCS (1998).
- Lijinsky, W. & Shubik, P. (1965a) Benzo[a]pyrene and other polynuclear hydrocarbons in charcoal-broiled meat. *Science*, **145**, 53–55.
- Lijinsky, W. & Shubik, P. (1965b) Polynuclear hydrocarbon carcinogens in cooked meat and smoked foods. *Ind. Med. Surg.*, **34**, 152–154.
- Lin, D., Tu, Y. & Zhu, L. (2005) Concentrations and health risk of polycyclic aromatic hydrocarbons in tea. *Food Chem. Toxicol.*, **43**, 42–48.

- Lintas, C., De Matthaëis, M.C. & Merli, F. (1979) Determination of benzo[a]pyrene in smoked, cooked and toasted food products. *Food Cosmet. Toxicol.*, **17**, 325–328. As cited in IPCS (1998).
- Lioy, P.L., Walman, J.M., Greenburg, A., Harkov, R. & Pietarinen, C. (1988) The Total Human Environmental Exposure Study (THEES) to benzo[a]pyrene: comparison of the inhalation and food pathways. *Arch. Environ. Health*, **43**, 304–312.
- Lo, M.T. & Sandi, E. (1978) Polycyclic aromatic hydrocarbons (polynuclear) in foods. *Res. Rev.*, **69**, 35–85. As cited in IPCS (1998).
- Lodovici, M., Dolara, P., Casalini, C., Ciappellano, S. & Testolin, G. (1995) Polycyclic aromatic hydrocarbon contamination in the Italian diet. *Food Addit. Contam.*, **12** (5), 703–713.
- Lopez-Abente, G., Sanz-Anquela, J.M. & Gonzalez, C.A. (2001) Consumption of wine stored in leather wine bottles and incidence of gastric cancer. *Arch. Environ. Health*, **56**, 559–561. As cited in EC (2002).
- Lu, P.L., Chen, M.L. & Mao, I.F. (2002) Urinary 1-hydroxypyrene levels in workers exposed to coke oven emissions at various locations in a coke oven plant. *Arch. Environ. Health*, **57**, 255–261.
- Luch, A. & Glatt, H.R. (2004) *Die Bedeutung der Entstehung von DNA-Addukten polycyclischer aromatischer Kohlenwasserstoffe für die Krebsentstehung. Polyklinische aromatische Kohlenwasserstoffe*. Deutsche Forschungsgemeinschaft. Weinheim: Wiley VCH, pp. 118–124.
- Luch, A. & Jacob, J. (2004) *Dibenzo[a,h]pyren, ein polycyclischer aromatischer Kohlenwasserstoff mit aussergewöhnlichen biologischen Wirkungen: Analytik, Umweltrelevanz und Bioaktivierung. Polyklinische aromatische Kohlenwasserstoffe*. Deutsche Forschungsgemeinschaft. Weinheim: Wiley VCH, pp. 27–41.
- Lutz, W.K. & Schlatter, J. (1992) Chemical carcinogens and overnutrition in diet-related cancer. *Carcinogenesis*, **13**, 2211–2216. As cited in IPCS (1998).
- Machala, M., Vondracek, J., Bláha, L., Ciganek, M. & Neca, J. (2001) Aryl hydrocarbon receptor-mediated activity of mutagenic polycyclic aromatic hydrocarbons determined using in vitro reporter gene assay. *Mutat. Res.*, **497**, 49–62. As cited in EC (2002).
- MacKenzie, K.M. & Angevine, D.M. (1981) Infertility in mice exposed in utero to benzo[a]pyrene. *Biol. Reprod.*, **24**, 183–191. As cited in EC (2002).
- Madhavan, N.D. & Naidu, K.A. (1995) Polycyclic aromatic hydrocarbons in placenta, maternal blood, umbilical cord blood and milk of Indian women. *Human Exp. Toxicol.*, **14**, 503–506. As cited in IPCS (1998).
- Maga, J.A. (1986) Polycyclic aromatic hydrocarbon composition of mesquite (*Prosopis fuliflora*) smoke and grilled beef. *J. Agric. Food Chem.*, **34**, 249–251.
- Malcolm, H.M. & Dobson, S. (1994) *The Calculation of an Environmental Assessment Level (EAL) for Atmospheric PAH Using Relative Potencies*. London: Department of the Environment, 34 pp. (Report No. DoE/HMIP/RR/94/041).
- Malins, D.C., Krahn, M.M., Brown, D.W., Rhodes, L.D., Myers, M.S., McCain, B.B. & Chan, S.L. (1985) Toxic chemicals in marine sediment and biota from Mukilteo, Washington: Relationships with hepatic neoplasms and other hepatic lesions in English sole (*Parophrys vetulus*). *J. Natl. Cancer Inst.*, **74**, 487–494. As cited in IPCS (1998).
- Manoli, E. & Samara, C. (1999) Polycyclic aromatic hydrocarbons in natural waters: sources, occurrence and analysis. *Trends Anal. Chem.*, **18**, 417–428.

- Mao, C. & Tucker, S.A. (2002) High performance liquid chromatographic separation of polycyclic aromatic hydrocarbons using pyridinium chloride as a selective fluorescence quencher to aid detection. *J. Chromatogr. A*, **966**, 53–61.
- Marcus, J.M. & Stokes, T.P. (1985) Polynuclear aromatic hydrocarbons in oyster tissue around three coastal marinas. *Bull. Environ. Contam. Toxicol.*, **35**, 835–844. As cited in IPCS (1998).
- McCormick, D.L., Burns, F.J. & Albert, R.E. (1981) Inhibition of benzo[a]pyrene-induced mammary carcinogenesis by retinyl acetate. *J. Natl. Cancer Inst.*, **66**, 559–564.
- McFall, J.A., Antoine, S.R. & DeLeon, I.R. (1985) Base-neutral extractable organic pollutants in biota and sediments from Lake Pontchartrain. *Chemosphere*, **14**, 1561–1569. As cited in IPCS (1998).
- McGill, A.S., Mackie, P.R., Parsons, E., Bruce, C. & Hardy, R. (1982) The polynuclear aromatic hydrocarbon content of smoked foods in the United Kingdom. In: Cooke, M., Dennis, A.J. & Fisher, G.L., eds., *Polynuclear Aromatic Hydrocarbons: Physical and Biological Chemistry*. Columbus, Ohio: Battelle Press, pp. 491–499.
- Menichini, E. (1992) Urban air pollution by polycyclic aromatic hydrocarbons: Levels and sources of variability. *Sci. Total Environ.*, **116**, 109–135. As cited in IPCS (1998).
- Menichini, E., Bocca, A., Merli, F., Ianni, D. & Monfredini, F. (1991) Polycyclic aromatic hydrocarbons in olive oils on the Italian market. *Food Addit. Contam.*, **8** (3), 363–369.
- Milano, J.C., Fache, B. & Vernet, J.L. (1986) [Pollution of the sediment, fauna and flora by polyaromatic hydrocarbons at Cap Sicié at the emission site of the city of Toulon (French Mediterranean coast).] *J. Rech. Oceanogr.*, **11**, 93–96 (in French). As cited in IPCS (1998).
- Miller, J.S. (1999) Determination of polycyclic aromatic hydrocarbons by spectrofluorimetry. *Anal. Chim. Acta*, **388**, 27–34.
- Mix, M.C. & Schaffer, R.L. (1983) Concentrations of unsubstituted polycyclic aromatic hydrocarbons in softshell clams from Coos Bay, Oregon, USA. *Mar. Pollut. Bull.*, **14**, 94–97. As cited in IPCS (1998).
- Montizaan, G.K., Kramers, P.G.N., Janus, J.A. & Posthumus, R. (1989) *Integrated Criteria Document Polynuclear Aromatic Hydrocarbons (PAH): Effects of 10 Selected Compounds*. Bilthoven: National Institute of Public Health and Environmental Protection (Appendix to RIVM Report No. 758474007).
- Moret, S. & Conte, L.S. (2000) Polycyclic aromatic hydrocarbons in edible fats and oils: occurrence and analytical methods. *J. Chromatogr. A*, **882**, 245–253.
- Morris, H.P., Velat, C.A., Wagner, B.P., Dahlgard, M. & Ray, F.E. (1960) Studies of carcinogenicity in the rat of derivatives of aromatic amines related to *N*-2-fluorenyl-acetamide. *J. Natl. Cancer Inst.*, **24**, 149–180.
- Mostafa, G.A. (2002) Monitoring of polycyclic aromatic hydrocarbons in sea foods from lake Timsah. *Int. J. Environ. Health Res.*, **12**, 83–91.
- Mottier, P., Parisod, V. & Turesky, R.J. (2000) Quantitative determination of polycyclic aromatic hydrocarbons in barbecued meat sausages by gas chromatography coupled to mass spectrometry. *J. Agric. Food Chem.*, **48**, 1160–1166.
- Muller, P., Leece, B. & Raha, D. (1995a) Estimated risk of cancer from exposure to PAH fractions of complex mixtures. In: *15th International Symposium on Polycyclic Aromatic Compounds: Chemistry, Biology and Environmental Impact*, Belgirate, Italy, 19–22 September 1995. Ispra: European Commission, Joint Research Centre, pp. 159–160.
- Muller, P., Leece, B. & Raha, D. (1995b) *Dose-Response Assessment: PAH*. Ottawa, Ontario: Ontario Ministry of Environment and Energy, 197 pp.

- Muller, P., Leece, B. & Raha, D. (1996) *Scientific Criteria Document for Multimedia Environmental Standards Development: Polycyclic Aromatic Hydrocarbons (PAH). Part 1. Dose Response Assessment*. Ottawa, Ontario: Ontario Ministry of Environment and Energy, 203 pp.
- Nathalie, G., Cyril, F., Cecile, C., Claire, L., Eroc, L.P., Adam, H. & Guido, R. (2002) Detection of polycyclic aromatic hydrocarbon levels in milk collected near potential contaminated sources. *J. Agric. Food Chem.*, **16**, 4640–4642.
- Neal, J. & Rigdon, R.H. (1967) Gastric tumours in mice fed benzo[a]pyrene: a quantitative study. *Tex. Rep. Biol. Med.*, **25**, 553–557.
- Neubert, D. & Tapken, S. (1988) Transfer of benzo[a]pyrene into mouse embryos and fetuses. *Arch. Toxicol.*, **62**, 236–239. As cited in IPCS (1998).
- Nielsen, T., Jørgensen, H.E., Larsen, J.C. & Poulsen, M. (1996) City air pollution of polycyclic aromatic hydrocarbons and other mutagens: occurrence, sources and health effects. *Sci. Total Environ.*, **189/190**, 41–49.
- Nilsson, R., Nordlinder, R., Moen, B.E., Ovrebo, S., Bleie, K., Skorve, A.H., Hollund, B.E. & Tagesson, C. (2004) Increased urinary excretion of 8-hydroxydeoxyguanosine in engine room personnel exposed to polycyclic aromatic hydrocarbons. *Occup. Environ. Med.*, **61**, 692–696.
- Nirmaier, H.P., Fisher, E., Meyer, A. & Henze, G. (1996) Determination of polycyclic aromatic hydrocarbons in water samples using high performance liquid chromatography with amperometric detection. *J. Chromatogr. A*, **730**, 169–175.
- Nisbet, I.C.T. & LaGoy, P.K. (1992) Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAH). *Regul. Toxicol. Pharmacol.*, **16**, 290–300.
- Noll, I.B. & Toledo, M.C. (1995) Polycyclic aromatic hydrocarbons in charcoal-broiled meat in Brazil. *Rev. Esp. Cienc. Tecnol. Aliment.*, **35**, 209–216.
- Nousiainen, U., Törrönen, R. & Hänninen, O. (1984) Differential induction of various carboxylases by certain polycyclic aromatic hydrocarbons in the rat. *Toxicology*, **32**, 243–251. As cited in EC (2002).
- NTP (2000) *Toxicology and Carcinogenesis Studies of Naphthalene (CAS No. 91-20-3) in F344/N Rats (Inhalation Studies)*. Research Triangle Park, North Carolina: United States Department of Health and Human Services, Public Health Service, National Institutes of Health, National Toxicology Program (Technical Report Series No. 91-20-3; NIH Publication No. 01-4434).
- Obana, H., Hori, S., Kashimoto, T. & Kunita, N. (1981) Polycyclic aromatic hydrocarbons in human fat and liver. *Bull. Environ. Contam. Toxicol.*, **27**, 23–27. As cited in EC (2002).
- O'Connor, T.P. (2002) National distribution of chemical concentrations in mussels and oysters in the USA. *Mar. Environ. Res.*, **53**, 117–143.
- Olufsen, S.B. & Bjørseth, A. (1983) Analysis of polycyclic aromatic hydrocarbons by gas chromatography. In: Bjørseth, A. ed., *Handbook of Polycyclic Aromatic Hydrocarbons*. New York: Marcel Dekker, pp. 257–300.
- Osborne, M.R. & Crosby, N.T. (1987) Binding to proteins and nucleic acids. In: *Benzo-pyrenes*. Cambridge: Cambridge University Press, pp. 137–176 (Cambridge Monographs on Cancer Research). As cited in IPCS (1998).
- Palackal, N.T., Burczynski, M.E., Harvey, R.G. & Penning, T.M. (2001) The ubiquitous aldehyde reductase (AKR1A1) oxidises proximate carcinogen *trans*-dihydrodiols to o-quinones: potential role in polycyclic aromatic hydrocarbon activation. *Biochemistry*, **40**, 10901–10910.

- Palackal, N.T., Lee, S.H., Harvey, R.G., Blair, I.A. & Penning, T.M. (2002) Activation of polycyclic aromatic hydrocarbon *trans*-dihydrodiol proximate carcinogens by human aldo-keto reductase (AKR1C) enzymes and their functional overexpression in human lung carcinoma (A549) cells. *J. Biol. Chem.*, **277**, 24799–24808.
- Palli, D., Vineis, P., Russo, A., Berrino, F., Krogh, V., Masala, G., Panico, S., Taioli, E., Tumino, R., Garte, S. & Peluso, M. (2000) Diet, metabolic polymorphisms and DNA adducts: the EPIC-Italy cross-sectional study. *Int. J. Cancer*, **87**, 444–451. As cited in EC (2002).
- Pastorelli, R., Guanci, M., Restano, J., Berri, A., Micoli, G., Minoia, C., Alcini, D., Carrer, P., Negri, E., La Vecchia, C., Fanelli, R. & Airolidi, L. (1999) Seasonal effect on airborne pyrene, urinary 1-hydroxypyrene, and benzo[a]pyrene diol epoxide-hemoglobin adducts in the general population. *Cancer Epidemiol. Biomarkers Prev.*, **8**, 561–565.
- Peluso, M., Ceppi, M., Munia, A., Puntoni, R. & Parodi, R. (2001) Analysis of 13 ³²P-DNA postlabeling studies on occupational cohorts exposed to air pollution. *Am. J. Epidemiol.*, **153**, 546–558.
- Penning, T.M. (2004) Aldo-keto reductases and formation of polycyclic aromatic hydrocarbon *o*-quinones. *Meth. Enzymol.*, **378**, 31–67.
- Perera, F.P., Rauh, V., Tsai, W.Y., Kinney, P., Camann, D., Barr, D., Bernert, T., Garfinkel, R., Tu, Y.H., Diaz, D., Dietrich, J. & Whyatt, R.M. (2003) Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population. *Environ. Health Perspect.*, **111**, 201–205.
- Perfetti, G.A., Nyman, P.J., Fisher, S., Joe, F.L., Jr., & Diachenko, G.W. (1992) Determination of polynuclear aromatic hydrocarbons in seafood by liquid chromatography with fluorescence detection. *J. Assoc. Off. Anal. Chem.*, **75**, 872–877.
- Pfannhauser, W. (1991) [Polycyclic aromatic hydrocarbons (PAH) in foods and selected vegetables in Austria.] *Mitt. Geb. Lebensmittelunters. Hyg.*, **82**, 66–79 (in German). As cited in IPCS (1998) and EC (2002).
- Phillips, D.H. (1999) Polycyclic aromatic hydrocarbons in the diet. *Mutat. Res.*, **443**, 139–147.
- Pisani, P. & Mitton, N. (2002) Cooking methods, metabolic polymorphisms and colorectal cancer. *Eur. J. Cancer Prev.*, **11**, 75–84.
- Plasterer, M.R., Bradshaw, W.S., Booth, G.M., Carter, M.W., Schuler, R.L. & Hardin, B.D. (1985) Developmental toxicity of nine selected compounds following prenatal exposure in the mouse: naphthalene, *p*-nitrophenol, sodium selenite, dimethylphthalate, ethylene-thiourea and four glycol ether derivatives. *J. Toxicol. Environ. Health*, **15**, 25–38.
- Plopper, C.G., Suverkropp, C., Morin, D., Nishio, S. & Buckpitt, A. (1992) Relationship of cytochrome P-450 activity to Clara cell cytotoxicity. I. Histopathologic comparison of the respiratory tract of mice, rats and hamsters after parenteral administration of naphthalene. *J. Pharmacol. Exp. Ther.*, **261**, 353–363.
- Prinsen, A.J. & Kennedy, W.B.H. (1977) [Quantity of alpha-benzopyrene in smoked foods.] In: [Aroma and Flavouring Substances.] Bilthoven: National Institute of Public Health and Environmental Protection, pp. 1–11 (Report No. 11) (in Dutch). As cited in IPCS (1998).
- Prinsen, A.J. & Kennedy, W.B.H. (1978) [Quantity of alpha benzopyrene in various tea samples.] In: [Aroma and Flavouring Substances.] Bilthoven: National Institute of Public Health and Environmental Protection, pp. 1–5 (Report No. 12) (in Dutch). As cited in IPCS (1998).

- Pupin, A.M. & Toledo, M.C.F. (1996a) Benzo[a]pyrene in Brazilian vegetable oils. *Food Addit. Contam.*, **13** (6), 639–646.
- Pupin, A.M. & Toledo, M.C.F. (1996b) Benzo[a]pyrene in olive oil on the Brazilian market. *Food Chem.*, **55** (2), 185–188.
- Rahman, A., Barrowman, J.A. & Rahimtula, A. (1986) The influence of bile on the bio-availability of polynuclear aromatic hydrocarbons from the rat intestine. *Can. J. Physiol.*, **64**, 1214–1218. As cited in IPCS (1998).
- Rahman, M.H., Arslan, M.I., Chen, Y., Ali, S., Parvin, T., Wang, L.W., Santella, R.M. & Ahsan, H. (2003) Polycyclic aromatic hydrocarbon–DNA adducts among rickshaw drivers in Dhaka City, Bangladesh. *Int. Arch. Occup. Environ. Health*, **76**, 533–538.
- Rainio, K., Linko, R.R. & Ruotsila, L. (1986) Polycyclic aromatic hydrocarbons in mussel and fish from the Finnish archipelago sea. *Bull. Environ. Contam. Toxicol.*, **37**, 337–343. As cited in IPCS (1998).
- Ramesh, A., Walker, S.A., Hood, D.B., Guillen, M.D., Schneider, K. & Weyand, E.H. (2004) Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. *Int. J. Toxicol.*, **23**, 301–333.
- Rees, E.D., Mandelstam, P., Lowry, J.Q. & Lipscomb, H. (1971) A study of intestinal absorption of benzo[a]pyrene. *Biochem. Biophys. Acta*, **225**, 96–107. As cited in IPCS (1998).
- Renwick, A.G. & Drasar, B.S. (1976) Environmental carcinogens and large bowel cancer. *Nature*, **263**, 234–235. As cited in IPCS (1998).
- Rigdon, R.H. & Neal, J. (1965) Effects of feeding benzo[a]pyrene on fertility, embryos and young mice. *J. Natl. Cancer Inst.*, **34**, 297–305. As cited in EC (2002).
- Rigdon, R.H. & Neal, J. (1966) Gastric adenomas and pulmonary adenomas in mice fed benzo[a]pyrene. *Texas Rep. Biol. Med.*, **24**, 195–207.
- Rigdon, R.H. & Rennels, E.G. (1964) Effect of feeding benzo[a]pyrene on reproduction in the rat. *Experientia*, **20**, 224–226. As cited in EC (2002).
- Rivera, L., Curto, M.J.C., Pais, P., Galceran, M.T. & Puignou, L. (1996) Solid phase extraction for the selective isolation of polycyclic aromatic hydrocarbons, azaarenes and heterocyclic aromatic amines in charcoal grilled meat. *J. Chromatogr. A*, **731**, 85–94.
- Robinson, J.R., Felton, J.S., Levitt, R.C., Thorgeirsson, S.S. & Nebert, D.W. (1975) Relationship between aromatic hydrocarbon responsiveness and the survival times in mice treated with various drugs and environmental compounds. *Mol. Pharmacol.*, **11**, 850–865. As cited in EC (2002).
- Rostad, C.E. & Pereira, W.E. (1987) Creosote compounds in snails obtained from Pensacola Bay, Florida, near an onshore hazardous-waste site. *Chemosphere*, **16**, 2397–2404. As cited in IPCS (1998).
- Ruchirawat, M., Mahidol, C., Tangjarukij, C., Pui-ock, S., Jensen, O., Kampeerawipakorn, O., Tuntaviron, J., Aramphongphan, A. & Autrup, H. (2002) Exposure to genotoxins present in ambient air in Bangkok, Thailand — particle associated polycyclic aromatic hydrocarbons and biomarkers. *Sci. Total Environ.*, **287**, 121–132.
- Saeed, T., Al-Yakoob, S., Al-Hashash, H. & Al-Bahlou, M. (1995) Preliminary exposure assessment for Kuwaiti consumers to polycyclic aromatic hydrocarbons in seafood. *Environ. Int.*, **21**, 255–263.
- Sagredos, A.N., Sinha-Roy, D. & Thomas, A. (1988). Determination, sources and composition of polycyclic aromatic hydrocarbons in oils and fats. *Fat Sci. Technol.*, **90**, 76–81.

- Saint-Aubert, B., Cooper, J.F., Astre, C., Spiliotis, J. & Joyeux, H. (1992) Evaluation of the induction of polycyclic aromatic hydrocarbons (PAH) by cooking on two geometrically different types of barbecue. *J. Food Compos. Anal.*, **5**, 257–263.
- Salamone, M. (1981) Toxicity of 41 carcinogens and noncarcinogenic analogues, In: De Serres, F.J. & Ashby, J., eds., *Evaluation of Short-term Tests for Carcinogens. Report of the International Collaborative Programme*. Amsterdam: Elsevier North Holland, pp. 682–685 (Progress in Mutation Research, Vol. 1).
- Sandmeyer, E.E. (1981) Aromatic hydrocarbons. In: Clayton, G.D. & Clayton, F.E., eds., *Patty's Industrial Hygiene and Toxicology*, 3rd Revised Ed. New York: John Wiley & Sons, pp. 3333–3339.
- Santodonato, J., Basu, D. & Howard, P.H. (1980) Multimedia human exposure and carcinogenic risk assessment for environmental PAH. In: Bjørseth, A. & Dennis, A.J., eds., *Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects*. Columbus, Ohio: Battelle Press, pp. 435–454. As cited in IPCS (1998).
- Santodonato, J., Howard, P. & Basu, D. (1981) Health and ecological assessment of polynuclear aromatic hydrocarbons. *J. Environ. Pathol. Toxicol.*, **5**, 1–364.
- Saunders, C.R., Shockley, D.C. & Knuckles, M.E. (2001) Behavioral effects induced by acute exposure to benzo[a]pyrene in F-344 rats. *Neurotoxicity Res.*, **3**, 557–579.
- Saunders, C.R., Shockley, D.C. & Knuckles, M.E. (2003) Fluoranthene-induced neuro-behavioral toxicity in F-344 rats. *Int. J. Toxicol.*, **22**, 263–276.
- Sax, N.I. & Lewis, J.R., Sr. (1984) *Dangerous Properties of Industrial Materials*, 7th Ed. New York: Van Nostrand Reinhold Co., pp. 2451–2452.
- Saxton, W.L., Newton, R.T., Rohrborg, J., Sutton, J. & Johnson, L.J. (1993) Polycyclic aromatic hydrocarbons in seafood from the Gulf of Alaska following a major crude oil spill. *Bull. Environ. Contam. Toxicol.*, **51**, 515–522.
- Scherer, G., Conze, C., Von Meyerinck, L., Sorsa, M. & Adlkofer, F. (1990) Importance of exposure to gaseous and particulate phase components of tobacco smoke in active and passive smokers. *Int. Arch. Occup. Environ. Health*, **62**, 459–466. As cited in IPCS (1998).
- Scherer, G., Frank, S., Riedel, K., Meger-Kossien, I. & Renner, T. (2000) Biomonitoring of exposure to polycyclic aromatic hydrocarbons of non-occupationally exposed persons. *Cancer Epidemiol. Biomarkers Prev.*, **9** (4), 373–380.
- Schlede, E., Kuntzman, R., Haber, S. & Conney, A.H. (1970a) Effect of enzyme induction on the metabolism and tissue distribution of benzo[a]pyrene. *Cancer Res.*, **30**, 2893–2897. As cited in IPCS (1998).
- Schlede, E., Kuntzman, R. & Conney, A.H. (1970b) Stimulatory effect of benzo[a]pyrene metabolites in the rat. *Cancer Res.*, **30**, 2898–2904. As cited in IPCS (1998).
- Schmahl, D. (1955) Testing of naphthalene and anthracene for carcinogenic effects in rats. *Z. Krebsforsch.*, **60**, 697–710 (in German).
- Schneider, K., Roller, M., Kalberlah, F. & Schuhmacher-Wolz, U. (2002) Cancer risk assessment for oral exposure to PAH mixtures. *J. Appl. Toxicol.*, **22**, 73–83.
- SCOOP (2004) *Reports on Tasks for Scientific Cooperation. Report of Experts Participating in Task 3.2.12: Collection of Occurrence Data on Polycyclic Aromatic Hydrocarbons in Food*. Maisons-Alfort: Agence française de Sécurité Sanitaire des Aliments (AFSSA), Directorate-General Health and Consumer Protection, Scientific Cooperation on Food (http://europa.eu.int/comm/food/food/chemicalsafety/contaminants/index_en.htm).

- Seidel, A. & Angerer, J. (2004) *Nukleobasen-Addukte von PAH im Humanurin. Polyklinische aromatische Kohlenwasserstoffe*. Deutsche Forschungsgemeinschaft. Weinheim: Wiley VCH, pp. 70–76.
- Serdar, B., Egeghy, P.P., Waidyanatha, S., Gibson, R. & Rappaport, S.M. (2003) Urinary biomarkers of exposure to jet fuel (JP-8). *Environ. Health Perspect.*, **111**, 1760–1764.
- Shendrikova, I.A. & Aleksandrov, V.A. (1974) Comparative characteristics of penetration of polycyclic hydrocarbons through the placenta in the fetus in rats. *Bull. Exp. Biol. Med.*, **77**, 77–79. As cited in IPCS (1998).
- Shendrikova, I.A., Ivanov-Golitsyn, M.M., Anisimov, Y.N. & Likhachev, A.Ya. (1973) [Dynamics of transplacental penetration of 7,12-dimethylbenz[a]anthracene in mice.] *Vopr. Onkol.*, **19**, 75–79 (in Russian with English translation). As cited in IPCS (1998).
- Shendrikova, I.A., Ivanov-Golitsyn, M.N. & Likhachev, A.Ya. (1974) [The transplacental penetration of benzo[a]pyrene in mice.] *Vopr. Onkol.*, **20**, 53–56 (in Russian with English abstract). As cited in IPCS (1998).
- Shopp, G.M., White, K.L., Holsapple, M.P., Barnes, D.W., Duke, S.S., Anderson, A.C., Condie, L.W., Jr., Hayes, J.R. & Borzelleca, J.F. (1984) Naphthalene toxicity in CD-1 mice: general toxicity and immunotoxicology. *Fundam. Appl. Toxicol.*, **4**, 406–419. As cited in EC (2002).
- Simko, P. (2002) Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. *J. Chromatogr. B*, **770**, 3–18.
- Simmon, V.F., Rosenkranz, H.S., Zeiger, E. & Poirier, L.A. (1979) Mutagenic activity of chemical carcinogens and related compounds in the intraperitoneal host-mediated assay. *J. Natl. Cancer Inst.*, **62**, 911–918.
- Sirota, G.R. & Uthe, J.F. (1981) Polynuclear aromatic hydrocarbon contamination in marine shellfish. In: Cooke, M. & Dennis, A.J., eds., *Polynuclear Aromatic Hydrocarbons: Chemical Analysis and Biological Fate*. Columbus, Ohio: Battelle Press, pp. 329–341. As cited in IPCS (1998).
- Sirota, R., Uthe, J.F., Sreedharan, A., Matheson, R., Musial, J. & Hamilton, K. (1983) Polynuclear aromatic hydrocarbons in lobster (*Homarus americanus*) and sediments in the vicinity of a coking facility. In: Cooke, M. & Dennis, A.J., eds., *Polynuclear Aromatic Hydrocarbons: Chemical Analysis and Biological Fate*. Columbus, Ohio: Battelle Press, pp. 1123–1136. As cited in IPCS (1998).
- Siwinska, E., Mielzynska, D. & Kapka, L. (2004) Association between urinary 1-hydroxypyrene and genotoxic effects in coke oven workers. *Occup. Environ. Med.*, **61**, e10.
- Smith, J.D., Bagg, J. & Bycroft, B.M. (1984) Polycyclic aromatic hydrocarbons in the clam *Tridacna maxima* from the Great Barrier Reef, Australia. *Environ. Sci. Technol.*, **18**, 353–358. As cited in IPCS (1998).
- Smith, J.D., Bagg, J. & Sin, Y.O. (1987) Aromatic hydrocarbons in seawater, sediments and clams from Green Island, Great Barrier Reef, Australia. *Aust. J. Mar. Freshwater Res.*, **38**, 501–510.
- Smyth, H.F., Carpenter, C.P., Weil, C.S., Pozzani, U.C. & Streigel, J.A. (1962) Range-finding toxicity data: List VI. *Ind. Hyg. J.*, **23**, 95–107.
- Snell, K.C. & Stewart, H.L. (1962) Pulmonary adenomatosis induced in DBA/2 mice by oral administration of dibenz[a,h]anthracene. *J. Natl. Cancer Inst.*, **28**, 1043–1049.
- Sparnins, V.L., Mott, A.W., Baraney, G. & Wattenberg, L.W. (1986) Effects of allyl methyl trisulfide on glutathione-S-transferase activity and BP-induced neoplasia in the mouse. *Nutr. Cancer*, **8**, 211–215.

- Speer, K., Steeg, E., Horstmann, P., Kühn, T.H. & Montag, A. (1990) Determination and distribution of PAH in native vegetable oils, smoked fish products, mussels and oysters, and bream from the River Elbe. *J. High Resol. Chromatogr.*, **13**, 104–111.
- Stall, W. & Eisenbrand, G. (1988) Determination of polynuclear aromatic hydrocarbons and nitrosamines. In: Macrae, R., ed., *HPLC in Food Analysis*. New York: Academic Press, pp. 377–408.
- State Chemical Analysis Institute (1995) *Food Control and the Environment, Annual Report 1994*. Freiburg: State Chemical Analysis Institute.
- State Committee for Air Pollution Control (1992) [*Cancer Risk from Air Pollution. Development of Evaluation Criteria for Carcinogenic Pollutants.*] Düsseldorf: Minister for Environment and Agriculture for Northrhine-Westfalia, 71 pp. (in German).
- Steinig, J. (1976) 3,4-Benzopyrene contents in smoked fish depending on smoking procedure. *Z. Lebensmittel.-Unters. Forsch.*, **162**, 235–242. As cited in IPCS (1998).
- Stolyhwo, A. & Sikorski, Z.E. (2005) Polycyclic aromatic hydrocarbons in smoked fish — a critical review. *Food Chem.*, **91**, 303–311.
- Storelli, M.M. & Marcotrigiano, G.O. (2001) Polycyclic aromatic hydrocarbons in mussels (*Mytilus galloprovincialis*) from the Ionian Sea, Italy. *J. Food Prot.*, **64**, 405–409.
- Stout, P. & Mamantov, G. (1989) *Recent Advances in Infrared Analysis of Polycyclic Aromatic Compounds. Chemical Analysis of Polycyclic Aromatic Compounds*. New York: John Wiley & Sons.
- Strunk, P., Ortlepp, K., Heinz, H., Rossbach, B. & Angerer, J. (2002) Ambient and biological monitoring of coke plant workers — determination of exposure to polycyclic aromatic hydrocarbons. *Int. Arch. Occup. Environ. Health*, **75**, 354–358.
- Sul, D., Oh, E., Im, H., Yang, M., Kim, C.W. & Lee, E. (2003) DNA damage in T- and B-lymphocytes and granulocytes in emission inspection and incineration workers exposed to polycyclic aromatic hydrocarbons. *Mutat. Res.*, **538**, 109–119.
- Swetman, T., Head, S. & Evans, D. (1999) Contamination of coconut oil by PAH. *INFORM*, **10**, 706–712.
- Takahashi, G. (1978) Distribution and excretion of the hydrocarbon 3-methylcholanthrene in the animal body. In: Gelboin, H.V. & Ts'o, P.O.P., eds., *Polycyclic Hydrocarbons and Cancer. Vol. 1*. New York: Academic Press, pp. 233–246. As cited in IPCS (1998).
- Takahashi, G. & Yasuhira, K. (1973) Macroautoradiographic and radiometric studies on the distribution of 3-methylcholanthrene in mice and their fetuses. *Cancer Res.*, **33**, 710–715. As cited in IPCS (1998).
- Takatsuki, K., Suzuki, S., Sato, N. & Ushizawa, I. (1985) Liquid chromatographic determination of polycyclic aromatic hydrocarbons in fish and shellfish. *J. Assoc. Off. Anal. Chem.*, **68**, 945–949. As cited in IPCS (1998).
- Takino, M., Daishima, S., Yamaguchi, K. & Nakahara, T. (2001) Determination of polycyclic aromatic hydrocarbons by liquid chromatography electrospray ionization mass spectrometry using silver nitrate as a post column reagent. *J. Chromatogr. A*, **928**, 53–61.
- Tang, D., Phillips, D.H., Stampfer, M., Mooney, L.A., Hsu, Y., Cho, S., Tsai, W.Y., Ma, J., Cole, K.J., Shé, M.N. & Perera, F.P. (2001) Association between carcinogen–DNA adducts in white blood cells and lung cancer risk in the Physicians Health Study. *Cancer Res.*, **61**, 6708–6712.
- Thomson, B.M., Lake, R.J. & Lill, R.E. (1996) The contribution of margarine to cancer risk from polycyclic aromatic hydrocarbons in the New Zealand diet. *Polycyclic Aromat. Compd.*, **11**, 177–184.

- Tilgner, D.J. (1968) Carcinogens in food. *Food Manuf.*, **43**, 37–39, 42. As cited in IPCS (1998).
- Toledo, M.C.F. & Camargo, M.S.F.O. (1998) Benzo[a]pyrene in corn oils produced and commercialized in Brazil. *Cienc. Tecnol. Aliment.*, **18**, 73–76.
- Törrönen, R., Nousianen, U. & Hänninen, O. (1981) Induction of aldehyde dehydrogenase by polycyclic aromatic hydrocarbons in rats. *Chem.-Biol. Interact.*, **36**, 33–44. As cited in EC (2002).
- Troche, S.V., Garcia-Falcon, M.S., Amigo, S.G., Yusty, M.A.L. & Lozano, J.S. (2000) Enrichment of benzo[a]pyrene in vegetable oils and determination by HPLC-FL. *Talanta*, **51**, 1069–1076.
- Tsai, P.J., Shieh, H.Y., Lee, W.J., Chen, H.L. & Shih, T.S. (2002) Urinary 1-hydroxypyrene as a biomarker of internal dose of polycyclic aromatic hydrocarbons in carbon black workers. *Ann. Occup. Hyg.*, **46**, 229–235.
- Tsai, P.J., Shih, T.S., Chen, H.L., Lee, W.J., Lai, C.H. & Liou, S.H. (2004) Urinary 1-hydroxypyrene as an indicator for assessing the exposures of booth attendants of a highway toll station to polycyclic aromatic hydrocarbons. *Environ. Sci. Technol.*, **38**, 56–61.
- Tuominen, J.P., Pyysalo, H.S. & Sauri, M. (1988) Cereal products as a source of polycyclic aromatic hydrocarbons. *J. Agric. Food Chem.*, **36**, 118–120.
- Turrio-Baldassarri, L., di Domenico, A., La Rocca, C., Iacovella, N. & Rodriguez, F. (1996) Polycyclic aromatic hydrocarbons in Italian national and regional diets. *Polycyclic Aromat. Compd.*, **10**, 343–349. As cited in EC (2002) and SCOOP (2004).
- UKFSA (2002) *PAHs in the UK Diet: 2000 Total Diet Study Samples*. London: United Kingdom Food Standards Agency (Food Survey Information Sheet No. 31/02; <http://www.food.gov.uk/science/surveillance/fsis-2002/31pah>).
- Uno, S., Dalton, T.P., Derkenne, S., Curran, C.P., Miller, M.L., Shertzer, H.G. & Nebert, D.W. (2004) Oral exposure to benzo[a]pyrene in the mouse: detoxication by inducible cytochrome P450 is more important than metabolic activation. *Mol. Pharmacol.*, **65**, 1225–1237.
- Urso, P. & Johnson, R.A. (1987) Early changes in T lymphocytes and subsets of mouse progeny defective as adults in controlling growth of a syngeneic tumor after in utero insult with benzo[a]pyrene. *Immunopharmacology*, **14**, 1–10. As cited in EC (2002).
- Urso, P., Zhang, W. & Cobb, J.R. (1992) Immunological consequences from exposure to benzo[a]pyrene during pregnancy. *Scand. J. Immunol.*, **36** (Suppl. 11), 203–206. As cited in EC (2002).
- Urso, P., Wirsy, Y., Zhang, W., Rodriguez, J. & Moolenaar, P. (1994) Persistent defective T-cell differentiation in mice exposed to benzo[a]pyrene in utero. *FASEB J.*, **8**, A482. As cited in EC (2002).
- US EPA (1978) *Ambient Water Quality Criteria: Naphthalene*. Washington, D.C.: United States Environmental Protection Agency (PB-296 786).
- US EPA (1988) *13-Week Mouse Oral Subchronic Toxicity Study (Fluoranthene)*. Washington, D.C.: United States Environmental Protection Agency, 104 pp. (TRL Study No. 042-008; <http://www.epa.gov/iris/subst/0444.htm>). As cited in EC (2002).
- US EPA (1989a) *Mouse Oral Subchronic Toxicity Study with Acenaphthene*. Washington, D.C.: United States Environmental Protection Agency, 89 pp. (<http://www.epa.gov/iris/subst/0442.htm>). As cited in EC (2002).

- US EPA (1989b) *Subchronic Toxicity in Mice with Anthracene: Final Report*. Washington, D.C.: United States Environmental Protection Agency, 465 pp. (HLA Study No. 2399-131). As cited in EC (2002).
- US EPA (1989c) *Mouse Oral Subchronic Toxicity Study (Fluorene)*. Washington, D.C.: United States Environmental Protection Agency, 38 pp. (TRL Study No. 042010). As cited in EC (2002).
- US EPA (1989d) *Mouse Oral Subchronic Toxicity of Pyrene*. Washington, D.C.: United States Environmental Protection Agency, 102 pp. (TRL Study No. 042-012). As cited in EC (2002).
- US EPA (1998) *Toxicological Review of Naphthalene (CAS 91-20-3). In Support of Summary Information on the Integrated Risk Information System (IRIS)*. Washington, D.C.: United States Environmental Protection Agency, August.
- Uthe, J.F. & Musial, C.J. (1986) Polycyclic aromatic hydrocarbon contamination of American lobster, *Homarus americanus*, in the proximity of a coal-coking plant. *Bull. Environ. Contam. Toxicol.*, **37**, 730–738. As cited in IPCS (1998).
- Vaananen, V., Hameila, M., Kontsas, H., Peltonen, K. & Heikkilä, P. (2003) Air concentrations and urinary metabolites of polycyclic aromatic hydrocarbons among paving and remixing workers. *J. Environ. Monit.*, **5**, 739–746.
- Vaessen, H.A.M.G., Schuller, P.L., Jekel, A.A. & Wilbers, A.A.M.M. (1984) Polycyclic aromatic hydrocarbons in selected foods. Analysis and occurrence. *Toxicol. Environ. Chem.*, **7**, 297–324. As cited in IPCS (1998).
- Vaessen, H.A.M.G., Jekel, A.A. & Wilbers, A.A.M.M. (1988) Dietary intake of polycyclic aromatic hydrocarbons. *Toxicol. Environ. Chem.*, **16**, 281–294.
- van de Wiele, T., Vanhaecke, L., Beckaert, C., Peru, K., Headley, J., Verstraete, W. & Siciliano, S. (2005) Human colon microbiota transform polycyclic aromatic hydrocarbons to estrogenic metabolites. *Environ. Health Perspect.*, **113** (1) (online at <http://ehp.niehs.nih.gov/docs/2004/7259/abstract.html>).
- van Schooten, F.J., Moonen, E.J.C., Rhijnsburger, E., van Algen, B., Thijssen, H.H.W. & Kleinjans, J.C.S. (1994) Dermal uptake of polycyclic aromatic hydrocarbons after hairwash with coal-tar containing shampoo. *Lancet*, **344**, 1505–1506.
- van Stijn, F., Kerkhoff, M.A.T. & Vandeginste, B.G.M. (1996) Determination of polycyclic aromatic hydrocarbons in edible oils and fats by on-line donor-acceptor complex chromatography and high performance liquid chromatography with fluorescence detection. *J. Chromatogr. A*, **750**, 263–273.
- Vassilaros, D.L., Stoker, P.W., Booth, G.M. & Lee, M.L. (1982) Capillary gas chromatographic determination of polycyclic aromatic compounds in vertebrate fish tissue. *Anal. Chem.*, **54**, 106–112. As cited in IPCS (1998).
- Viau, C., Diakite, A., Ruzgyte, A., Tuchweber, B., Blais, C., Bouchard, M. & Vyskocil, A. (2002) Is 1-hydroxypyrene a reliable bioindicator of measured dietary polycyclic aromatic hydrocarbon under normal conditions? *J. Chromatogr. B*, **778**, 165–177.
- Villeneuve, J.-P., de Mora, S. & Cattini, C. (2004) Determination of organochlorinated compounds and petroleum hydrocarbons in fish homogenate sample IAEA-406: results from a worldwide interlaboratory study. *Trends Anal. Chem.*, **23**, 469–478.
- Voutsas, D. & Samara, C. (1998) Dietary intake of trace elements and polycyclic aromatic hydrocarbons via vegetables grown in an industrial Greek area. *Sci. Total Environ.*, **218** (2–3), 203–216.
- Vreuls, J.J., De Jong, G.J. & Brinkman, U.A.T. (1991) On-line coupling of liquid chromatography, capillary gas chromatography and mass spectrometry for the determination

- and identification of polycyclic aromatic hydrocarbons in vegetable oils. *Chromatographia*, **31**, 113–118.
- Waidyanatha, S., Zheng, Y. & Rappaport, S.M. (2003) Determination of polycyclic aromatic hydrocarbons in urine of coke oven workers by headspace solid phase microextraction and gas chromatography–mass spectrometry. *Chem.-Biol. Interact.*, **145**, 165–174.
- Warren, D.L., Brown, D.R., Jr., & Buckpitt, A.R. (1982) Evidence for cytochrome P450 mediated metabolism in the bronchiolar damage by naphthalene. *Chem.-Biol. Interact.*, **40**, 287–303.
- Wattenberg, L.W. & Leong, J.L. (1970) Inhibition of the carcinogenic action of benzo[a]pyrene by flavones. *Cancer Res.*, **30**, 1922–1925.
- Welch, R.M., Gommi, B., Alvares, A.P. & Conney, A.H. (1972) Effect of enzyme induction on the metabolism of benzo[a]pyrene and 3-methyl-4-monomethylamino-azobenzene in the pregnant and fetal rat. *Cancer Res.*, **32**, 973–978.
- Weyand, E.H. & Bevan, D.R. (1986) Benzo[a]pyrene disposition and metabolism following intratracheal instillation. *Cancer Res.*, **46**, 5655–5661. As cited in IPCS (1998).
- Weyand, E.H., Modi, N., Parimoo, B., Mauro, D.M. & Craig, D.R. (2002) Evaluation of PAH:DNA adduct formation in rats fed coal-tar contaminated diets. *Polycyclic Aromat. Compd.*, **22**, 911–921.
- White, P.A. (2002) The genotoxicity of priority polycyclic aromatic hydrocarbons in complex mixtures. *Mutat. Res.*, **515**, 85–98.
- WHO (2003) *GEMS/Food Regional Diets Revision September 2003*. Geneva: World Health Organization.
- WHO (2004) *Guidelines for Drinking-water Quality*, 3rd Ed. Vol. 1. *Recommendations*. Geneva: World Health Organization.
- WHO. *SIGHT: Summary Information and Global Health Trends*. Geneva: World Health Organization (<http://sight.who.int/>).
- WHO Regional Office for Europe (2000) Polycyclic aromatic hydrocarbons (PAH). In: *Air Quality Guidelines for Europe*, 2nd Ed. Copenhagen: World Health Organization.
- WHO Regional Office for Europe (2003) *Health Risks of Persistent Organic Pollutants from Long-Range Transboundary Air Pollution*. Copenhagen: World Health Organization.
- Wickström, K., Pyysalo, H., Plaami-Heikkilä, S. & Tuominen, J. (1986) Polycyclic aromatic compounds (PAC) in leaf lettuce. *Z. Lebensm.-Unters. Forsch.*, **183**, 182–185.
- Wise, S.A., Sander, L.C. & May, W.E. (1993) Determination of polycyclic aromatic hydrocarbons by liquid chromatography. *J. Chromatogr. A*, **642**, 329–349.
- Withey, J.R., Law, F.C.P. & Endrenyi, L. (1991) Pharmacokinetics and bioavailability of pyrene in the rat. *J. Toxicol. Environ. Health*, **32**, 429–447. As cited in IPCS (1998).
- Withey, J.R., Shedden, J., Law, F.C.P. & Abedini, S. (1992) Distribution to the fetus and major organs of the rat following inhalation exposure to pyrene. *J. Appl. Toxicol.*, **12**, 223–231. As cited in IPCS (1998).
- Wright, B.W. & Smith, R.D. (1989) Capillary supercritical fluid chromatography methods. In: Vo-Dinh, T, ed., *Chemical Analysis of Polycyclic Aromatic Compounds*. New York: John Wiley & Sons.
- Wu, M.T., Simpson, C.D., Christiani, D.C. & Hecht, S.S. (2002) Relationship of exposure to coke-oven emissions and urinary metabolites of benzo[a]pyrene and pyrene in coke-oven workers. *Cancer Epidemiol. Biomarkers Prev.*, **11**, 311–314.
- Wu, M.T., Pan, C.H., Huang, Y.L., Tsai, P.J., Chen, C.J. & Wu, T.N. (2003a) Urinary excretion of 8-hydroxy-2-deoxyguanosine and 1-hydroxypyrene in coke-oven workers. *Environ. Mol. Mutagen.*, **42**, 98–105.

- Wu, M.T., Pan, C.H., Wu, T.N., Huang, Y.L., Chen, C.Y., Huang, L.H. & Ho, C.K. (2003b) Immunological findings in a group of coke-oven workers exposed to polycyclic aromatic hydrocarbons. *J. Occup. Environ. Med.*, **45**, 1034–1039.
- Yusty, M.A.L. & Davina, J.L.C. (2005) Supercritical fluid extraction and high performance liquid chromatography–fluorescence detection method for polycyclic aromatic hydrocarbons investigation in vegetable oil. *Food Control*, **16**, 59–64.
- Zhang, J., Ichiba, M., Hanaoka, T., Pan, G., Yamano, Y., Hara, K., Takahashi, K. & Tomokuni, K. (2003) Leukocyte 8-hydroxydeoxyguanosine and aromatic DNA adduct in coke-oven workers with polycyclic aromatic hydrocarbon exposure. *Int. Arch. Occup. Environ. Health*, **76**, 499–504.
- Zhong, W. & Wang, M. (2002) Some polycyclic aromatic hydrocarbons in vegetables from northern China. *J. Environ. Sci. Health*, **A37** (2), 287–296.
- Zougagh, M., Redigolo, H., Rios, A. & Valcarcel, M. (2004) Screening and confirmation of PAHs in vegetable oil samples by use of supercritical fluid extraction in conjunction with liquid chromatography and fluorimetric detection. *Anal. Chim. Acta*, **525**, 265–271.
- Zuelzer, W.W. & Apt, L. (1949) Acute hemolytic anemia due to naphthalene poisoning. *J. Am. Med. Assoc.*, **141**, 185–190. As cited in EC (2002).

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. **Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents** (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings 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, diethylpyrocabamate, 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 anti-oxidants, 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.
87. **Residues of some veterinary drugs in animals and foods**. FAO Food and Nutrition Paper, No. 41/2, 1990.
88. **Evaluation of certain food additives and contaminants** (Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 789, 1990, and corrigenda.
89. **Toxicological evaluation of certain food additives and contaminants**. WHO Food Additives Series, No. 26, 1990.
90. **Specifications for identity and purity of certain food additives**. FAO Food and Nutrition Paper, No. 49, 1990.
91. **Evaluation of certain veterinary drug residues in food** (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.

92. **Toxicological evaluation of certain veterinary drug residues in food.** WHO Food Additives Series, No. 27, 1991.
93. **Residues of some veterinary drugs in animals and foods.** FAO Food and Nutrition Paper, No. 41/3, 1991.
94. **Evaluation of certain food additives and contaminants** (Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 806, 1991, and corrigenda.
95. **Toxicological evaluation of certain food additives and contaminants.** WHO Food Additives Series, No. 28, 1991.
96. **Compendium of food additive specifications** (Joint FAO/WHO Expert Committee on Food Additives (JECFA)). Combined specifications from 1st through the 37th meetings, 1956–1990. Rome, Food and Agricultural Organization of the United Nations, 1992 (2 volumes).
97. **Evaluation of certain veterinary drug residues in food** (Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 815, 1991.
98. **Toxicological evaluation of certain veterinary residues in food.** WHO Food Additives Series, No. 29, 1991.
99. **Residues of some veterinary drugs in animals and foods.** FAO Food and Nutrition Paper, No. 41/4, 1991.
100. **Guide to specifications—General notices, general analytical techniques, identification tests, test solutions, and other reference materials.** FAO Food and Nutrition Paper, No. 5, Ref. 2, 1991.
101. **Evaluation of certain food additives and naturally occurring toxicants** (Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 828, 1992.
102. **Toxicological evaluation of certain food additives and naturally occurring toxicants.** WHO Food Additive Series, No. 30, 1993.
103. **Compendium of food additive specifications: addendum 1.** FAO Food and Nutrition Paper, No. 52, 1992.
104. **Evaluation of certain veterinary drug residues in food** (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 832, 1993.
105. **Toxicological evaluation of certain veterinary drug residues in food.** WHO Food Additives Series, No. 31, 1993.
106. **Residues of some veterinary drugs in animals and food.** FAO Food and Nutrition Paper, No. 41/5, 1993.
107. **Evaluation of certain food additives and contaminants** (Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 837, 1993.
108. **Toxicological evaluation of certain food additives and contaminants.** WHO Food Additives Series, No. 32, 1993.
109. **Compendium of food additive specifications: addendum 2.** FAO Food and Nutrition Paper, No. 52, Add. 2, 1993.
110. **Evaluation of certain veterinary drug residues in food** (Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 851, 1995.
111. **Toxicological evaluation of certain veterinary drug residues in food.** WHO Food Additives Series, No. 33, 1994.
112. **Residues of some veterinary drugs in animals and foods.** FAO Food and Nutrition Paper, No. 41/6, 1994.
113. **Evaluation of certain veterinary drug residues in food** (Forty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 855, 1995, and corrigendum.

114. **Toxicological evaluation of certain veterinary drug residues in food.** WHO Food Additives Series, No. 34, 1995.
115. **Residues of some veterinary drugs in animals and foods.** FAO Food and Nutrition Paper, No. 41/7, 1995.
116. **Evaluation of certain food additives and contaminants** (Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 859, 1995.
117. **Toxicological evaluation of certain food additives and contaminants.** WHO Food Additives Series, No. 35, 1996.
118. **Compendium of food additive specifications: addendum 3.** FAO Food and Nutrition Paper, No. 52, Add. 3, 1995.
119. **Evaluation of certain veterinary drug residues in food** (Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 864, 1996.
120. **Toxicological evaluation of certain veterinary drug residues in food.** WHO Food Additives Series, No. 36, 1996.
121. **Residues of some veterinary drugs in animals and foods.** FAO Food and Nutrition Paper, No. 41/8, 1996.
122. **Evaluation of certain food additives and contaminants** (Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 868, 1997.
123. **Toxicological evaluation of certain food additives.** WHO Food Additives Series, No. 37, 1996.
124. **Compendium of food additive specifications: addendum 4.** FAO Food and Nutrition Paper, No. 52, Add. 4, 1996.
125. **Evaluation of certain veterinary drug residues in food** (Forty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 876, 1998.
126. **Toxicological evaluation of certain veterinary drug residues in food.** WHO Food Additives Series, No. 38, 1996.
127. **Residues of some veterinary drugs in animals and foods.** FAO Food and Nutrition Paper, No. 41/9, 1997.
128. **Evaluation of certain veterinary drug residues in food** (Forty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 879, 1998.
129. **Toxicological evaluation of certain veterinary drug residues in food.** WHO Food Additives Series, No. 39, 1997.
130. **Residues of some veterinary drugs in animals and foods.** FAO Food and Nutrition Paper, No. 41/10, 1998.
131. **Evaluation of certain food additives and contaminants** (Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 884, 1999.
132. **Safety evaluation of certain food additives and contaminants.** WHO Food Additives Series, No. 40, 1998.
133. **Compendium of food additive specifications: addendum 5.** FAO Food and Nutrition Paper, No. 52, Add. 5, 1997.
134. **Evaluation of certain veterinary drug residues in food** (Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 888, 1999.
135. **Toxicological evaluation of certain veterinary drug residues in food.** WHO Food Additives Series, No. 41, 1998.
136. **Residues of some veterinary drugs in animals and foods.** FAO Food and Nutrition Paper, No. 41/11, 1999.
137. **Evaluation of certain food additives** (Fifty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 891, 2000.

138. **Safety evaluation of certain food additives.** WHO Food Additives Series, No. 42, 1999.
139. **Compendium of food additive specifications: addendum 6.** FAO Food and Nutrition Paper, No. 52, Add. 6, 1998.
140. **Evaluation of certain veterinary drug residues in food** (Fifty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 893, 2000.
141. **Toxicological evaluation of certain veterinary drug residues in food.** WHO Food Additives Series, No. 43, 2000.
142. **Residues of some veterinary drugs in animals and foods.** FAO Food and Nutrition Paper, No. 41/12, 2000.
143. **Evaluation of certain food additives and contaminants** (Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 896, 2000.
144. **Safety evaluation of certain food additives and contaminants.** WHO Food Additives Series, No. 44, 2000.
145. **Compendium of food additive specifications: addendum 7.** FAO Food and Nutrition Paper, No. 52, Add. 7, 1999.
146. **Evaluation of certain veterinary drug residues in food** (Fifty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 900, 2001.
147. **Toxicological evaluation of certain veterinary drug residues in food.** WHO Food Additives Series, No. 45, 2000.
148. **Residues of some veterinary drugs in animals and foods.** FAO Food and Nutrition Paper, No. 41/13, 2000.
149. **Evaluation of certain food additives and contaminants** (Fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 901, 2001.
150. **Safety evaluation of certain food additives and contaminants.** WHO Food Additives Series, No. 46, 2001.
151. **Compendium of food additive specifications: addendum 8.** FAO Food and Nutrition Paper, No. 52, Add. 8, 2000.
152. **Evaluation of certain mycotoxins in food** (Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 906, 2002.
153. **Safety evaluation of certain mycotoxins in food.** WHO Food Additives Series, No. 47/FAO Food and Nutrition Paper 74, 2001.
154. **Evaluation of certain food additives and contaminants** (Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 909, 2002.
155. **Safety evaluation of certain food additives and contaminants.** WHO Food Additives Series, No. 48, 2002.
156. **Compendium of food additive specifications: addendum 9.** FAO Food and Nutrition Paper, No. 52, Add. 9, 2001.
157. **Evaluation of certain veterinary drug residues in food** (Fifty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 911, 2002.
158. **Toxicological evaluation of certain veterinary drug residues in food.** WHO Food Additives Series, No. 49, 2002.
159. **Residues of some veterinary drugs in animals and foods.** FAO Food and Nutrition Paper, No. 41/14, 2000.
160. **Evaluation of certain food additives and contaminants** (Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 913, 2002.
161. **Safety evaluation of certain food additives and contaminants.** WHO Food Additives Series, No. 50, 2003.

162. **Compendium of food additive specifications: addendum 10.** FAO Food and Nutrition Paper No. 52, Add. 10, 2002.
163. **Evaluation of certain veterinary drug residues in food** (Sixtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 918, 2003.
164. **Toxicological evaluation of certain veterinary drug residues in food.** WHO Food Additives Series, No. 51, 2003.
165. **Residues of some veterinary drugs in animals and foods.** FAO Food and Nutrition Paper, No. 41/15, 2003.
166. **Evaluation of certain food additives and contaminants** (Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 922, 2004.
167. **Safety evaluation of certain food additives and contaminants.** WHO Food Additives Series, No. 52, 2004.
168. **Compendium of food additive specifications: addendum 11.** FAO Food and Nutrition Paper, No. 52, Add. 11, 2003.
169. **Evaluation of certain veterinary drug residues in food** (Sixty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 925, 2004.
170. **Residues of some veterinary drugs in animals and foods.** FAO Food and Nutrition Paper, No. 41/16, 2004.
171. **Toxicological evaluation of certain veterinary drug residues in food.** WHO Food Additives Series, No. 53, 2005.
172. **Compendium of food additive specifications: addendum 12.** FAO Food and Nutrition Paper, No. 52, Add. 12, 2004.
173. **Evaluation of certain food additives** (Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 928, 2005.
174. **Evaluation of certain food contaminants** (Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 930, 2005 (in preparation).
175. **Safety evaluation of certain contaminants in food.** WHO Food Additives Series, No. 55/FAO Food and Nutrition Paper, No. 82, 2006.

ANNEX 2

ABBREVIATIONS USED IN THE MONOGRAPHS

3-APA	3-aminopropionamide
8-OH-dG	8-hydroxy-2'-deoxyguanosine
AA	acrylamide
ABS	acrylonitrile–butadiene–styrene
ADI	acceptable daily intake
AED	atomic emission detection
Ah	aryl hydrocarbon
AhR	aryl hydrocarbon receptor
ALARA	as low as reasonably achievable
ALT	alanine transferase
AR	androgen receptor
ARA	arachidonic acid
ARfD	acute reference dose
ATDS	Australian Total Diet Survey
AUC	area under the curve
BCG	Bacillus Calmette-Guérin
BDE	brominated diphenyl ether
BMD	benchmark dose
BMDL	lower confidence limit on the benchmark dose
BMR	benchmark response
Br-GC-MS	bromination–gas chromatography–mass spectrometry
BROD	benzyloxyresorufin O-deethylase
bw	body weight
CAS	Chemical Abstracts Service
CCFAC	Codex Committee on Food Additives and Contaminants
cGMP	cyclic guanosine monophosphate
CI	confidence interval
CIAA	Confederation of Food and Drink Industries of the European Union
COX	prostaglandin H synthase
CRM	certified reference material
CV	coefficient of variation
CYP	cytochrome P450
Cys	cysteine
dA	deoxyadenosine
dC	deoxycytidine
DDT	dichlorodiphenyltrichloroethane
dG	deoxyguanosine
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
EA	early antigen

EC ₅₀	median effective concentration
ECD	electron capture detection
ECNI	electron capture negative ionization
ED ₅₀	median effective dose
EI	electron impact
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency (USA)
ER	estrogen receptor
EROD	7-ethoxyresorufin O-deethylase
ESI	electrospray ionization
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FCA	Freund's complete adjuvant
FD	fluorescence detection
FID	flame ionization detection
FOB	functional observational (test) battery
FSH	follicle stimulating hormone
FT3	free triiodothyronine
FT4	free thyroxine
GA	glycidamide
GC	gas chromatography
GD	gestation day
GEMS/Food	Global Environment Monitoring System Food Contamination Monitoring and Assessment Programme
GM-CSF	granulocyte-macrophage colony stimulating factor
GSD	geometric standard deviation
GST	glutathione-S-transferase
GSTM1	glutathione-S-transferase M1
GSTT1	glutathione-S-transferase T1
hAR	human androgen receptor
HPLC	high-performance liquid chromatography
HRGC	high-resolution gas chromatography
HRMS	high-resolution mass spectrometry
IARC	International Agency for Research on Cancer
IC ₅₀	median inhibitory concentration
IEF	induction equivalency factor
Ig	immunoglobulin
IGF	insulin-like growth factor
i.m.	intramuscular
i.p.	intraperitoneal
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
IU	international units
IUPAC	International Union for Pure and Applied Chemistry
JECFA	Joint FAO/WHO Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
K _i	inhibition constant
K _m	Michaelis-Menten constant

K_{ow}	octanol–water partition coefficient
LC	liquid chromatography
LD	lactational day
LD ₅₀	median lethal dose
LDH	lactate dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOEL	lowest-observed-effect level
LOQ	limit of quantification
LOR	limit of reporting
LRMS	low-resolution mass spectrometry
LTP	long-term potentiation
MAE	microwave-assisted extraction
MAP	mitogen-activated protein
MEK	mitogen-activated protein kinase
MeO	methoxy
ML	maximum level
MOE	margin of exposure
MPO	medial preoptic area
mRNA	messenger ribonucleic acid
MROD	7-methoxyresorufin O-deethylase
MS	mass spectrometry
m/z	mass to charge ratio
N1-GA-dA	N1-(2-carboxy-2-hydroxyethyl)-2'-deoxyadenosine
N3-GA-Ade	N3-(2-carbamoyl-2-hydroxyethyl)adenine
N ⁶ -GA-dA	N ⁶ -(2-carboxy-2-hydroxyethyl)-2'-deoxyadenosine
N7-GA-Gua	N7-(2-carbamoyl-2-hydroxyethyl)guanine
NA	not available; not analysed
NADH	nicotinamide adenine dinucleotide, reduced form
NADPH	nicotinamide adenine dinucleotide phosphate, reduced form
NAT	N-acetyltransferase
NCV	nerve conduction velocity
ND	not detected
NHL	non-Hodgkin lymphoma
NIH	National Institutes of Health (USA)
NK	natural killer
NMA	N-methylolacrylamide
NMDA	N-methyl-D-aspartate
nNOS	neural nitric oxide synthase
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NPD	nitrogen–phosphorus detection
NQ	not quantified
NTP	National Toxicology Program (USA)
OECD	Organisation for Economic Co-operation and Development
OH	hydroxy
OR	odds ratio

PAH	polycyclic aromatic hydrocarbon
PBB	polybrominated biphenyl
PBDD	polybrominated dibenzo- <i>p</i> -dioxin
PBDE	polybrominated diphenyl ether
PBDF	polybrominated dibenzofuran
PBPK	physiologically based pharmacokinetic
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo- <i>p</i> -dioxin
PCDE	polychlorinated diphenyl ether
PCDF	polychlorinated dibenzofuran
PCE	polychromatic erythrocyte
PCNA	proliferating cell nuclear antigen
PFC	plaque-forming cell
PKC	protein kinase C
PLA ₂	phospholipase A ₂
PMTDI	provisional maximum tolerable daily intake
PND	postnatal day
ppm	part per million
ppt	part per trillion
PR	progesterone receptor
PROD	7-pentoxoresorufin O-depentylase
PTWI	provisional tolerable weekly intake
PTWI _d	daily equivalent of the provisional tolerable weekly intake
QA	quality assurance
QC	quality control
QSAR	quantitative structure–activity relationship
RIVM	National Institute of Public Health and the Environment (Netherlands)
RIVO	Netherlands Institute of Fisheries Research
RNA	ribonucleic acid
RR	relative risk
SAR	Special Administrative Region
s.c.	subcutaneous
SCOOP	Scientific Cooperation on Food
SD	standard deviation
SFE	supercritical fluid extraction
SIM	selected ion monitoring
SMR	standardized mortality ratio
SPE	solid-phase extraction
SRM	selective-reaction monitoring
T3	triiodothyronine
T4	thyroxine
TBG	thyroxine binding globulin
TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
TD ₅₀	chronic dose that results in one half of animals developing tumours
TDS	Total Diet Study
TEF	toxic equivalency factor

TGF	transforming growth factor
TLC	thin-layer chromatography
TPA	tetradecanoyl-phorbol acetate
TSH	thyroid stimulating hormone
TT3	total triiodothyronine
TT4	total thyroxine
TTC	threshold of toxicological concern
TTR	transthyretin
UDPGT	uridine diphosphate glucuronosyltransferase
USA	United States of America
US EPA	United States Environmental Protection Agency
UV	ultraviolet
Val	valine
V_d	volume of distribution
V_{max}	maximum rate of metabolism
VMH	ventromedial hypothalamic nucleus
v/v	volume by volume
WHO	World Health Organization
w/w	weight by weight

ANNEX 3
JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES
Rome, 8–17 February 2005

Members

- Dr L. Barraj, Senior Managing Scientist, Exponent, Washington, DC, USA
- Dr D.C. Bellinger, Professor of Neurology, Harvard Medical School and Professor in the Department of Environmental Health, Harvard School of Public Health, Children's Hospital Boston, Boston, MA, USA
- Dr M. Bolger, Chief, Risk Assessment Staff, Division of Risk Assessment, Food and Drug Administration, College Park, MD, USA
- Dr L. Castle, Principal Scientist, Central Science Laboratory, Sand Hutton, York, England
- Professor J. Chen, Senior Research Professor, Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, Beijing, China
- Dr M.C. de Figueiredo Toledo, Professor of Food Toxicology, State University of Campinas, Faculty of Food Engineering — Unicamp, Campinas São Paulo, Brazil (*Rapporteur*)
- Mrs T. Hambridge, Team Manager — Dietary Modelling, Modelling, Evaluation and Surveillance Section, Food Standards Australia New Zealand, Canberra BC, ACT, Australia
- Dr J.C. Larsen, Senior Consultant, Division of Toxicology and Risk Assessment, Danish Institute of Food and Veterinary Research, Søborg, Denmark (*Chairperson*)
- Mrs I. Meyland, Senior Scientific Adviser, Danish Institute of Food and Veterinary Research, Søborg, Denmark (*Vice-Chairperson*)
- Dr L.V. Moreno, Centro de Investigación en Alimentación y Desarrollo, Sonora CP, Mexico (*Unable to attend*)
- Dr M.V. Rao, Director, Central Laboratories Unit, United Arab Emirates University, Al Ain, United Arab Emirates
- Professor A.G. Renwick, Emeritus Professor, University of Southampton, School of Medicine, Southampton, England
- Dr J. Schlatter, Head of Food Toxicology Section, Swiss Federal Office of Public Health, Zurich, Switzerland

Professor C. Tohyama, Professor of Environmental Toxicology, Division of Environmental Health, Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, University of Tokyo, Bunkyo-ku, Tokyo, Japan

Dr P. Verger, Director of INRA Unit 1204 — Food Risk Analysis Methodologies, National Institute for Agricultural Research, Paris, France

Professor R. Walker, Emeritus Professor of Food Science, Ash, Aldershot, Hampshire, England

Secretariat

Dr A. Agudo, Catalan Institute of Oncology (ICO), Unit of Epidemiology and Cancer Registration (SERC), L'Hospitalet de Llobregat, Spain (*WHO Temporary Adviser*)

Dr S. Barlow, Brighton, East Sussex, England (*WHO Temporary Adviser*)

Dr D. Benford, Food Standards Agency, London, England (*WHO Temporary Adviser*)

Dr C. Carrington, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*WHO Temporary Adviser*)

Ms R. Charrondiere, Nutrition Officer, Nutrition Planning, Assessment and Evaluation Service, Food and Nutrition Division, FAO, Rome, Italy (*FAO Staff Member*)

Ms M.L. Costarrica, Food Quality and Standards Service, Food and Nutrition Division, FAO, Rome, Italy (*FAO Staff Member*)

Dr P.O. Darnerud, Toxicology Division, National Food Administration, Uppsala, Sweden (*WHO Temporary Adviser*)

Ms A. de Veer, Chairman of the Codex Committee on Food Additives and Contaminants, Ministry of Agriculture, Nature and Food Quality, The Hague, Netherlands (*WHO Temporary Adviser*)

Dr M. DiNovi, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*WHO Temporary Adviser*)

Dr D.R. Doerge, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AZ, USA (*WHO Temporary Adviser*)

Ms S.K. Egan, Division of Risk Assessment, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*WHO Temporary Adviser; unable to attend*)

Ms B. Engeli, Swiss Federal Office of Public Health, Food Toxicology Section, Zurich, Switzerland (*WHO Temporary Adviser*)

- Mr M. Feeley, Bureau of Chemical Safety, Food Directorate, Health Canada, Tunney's Pasture, Ottawa, Ontario, Canada (*WHO Temporary Adviser*)
- Dr K.-E. Hellenäs, National Food Administration, Chemistry Division 1, Uppsala, Sweden (*FAO Consultant*)
- Dr S.H. Henry, Office of Plant and Dairy Foods and Beverages, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*WHO Temporary Adviser*)
- Dr A. Hirose, Division of Risk Assessment, National Institute of Health Sciences, Setagaya-ku, Tokyo, Japan (*WHO Temporary Adviser*)
- Mr J. Howlett, Wembley Park, Middlesex, England (*FAO Consultant*)
- Professor F. Kayama, Division of Environmental Medicine, Center for Community Medicine, Jichi Medical School, Tochigi, Japan (*WHO Temporary Adviser*)
- Professor R. Kroes, Institute for Risk Assessment Sciences, Utrecht University, Soest, Netherlands (*WHO Temporary Adviser*)
- Dr J.-C. Leblanc, Food Risk Analysis Methodologies, National Institute for Agricultural Research, Paris, France (*FAO Consultant*)
- Dr H. Lilienthal, BGFA — Research Institute for Occupational Medicine of the Institutions for Statutory Accident Insurance and Prevention, Ruhr University of Bochum, Bochum, Germany (*WHO Temporary Adviser*)
- Dr G. Moy, Food Safety Department, WHO, Geneva, Switzerland (*WHO Staff Member*)
- Dr M. Olsen, Food and Nutrition Division, FAO, Rome, Italy (*Joint Secretary*)
- Dr S. Page, International Programme on Chemical Safety, WHO, Geneva, Switzerland (*WHO Staff Member*)
- Mr O. Pöpke, Ergo Research, Hamburg, Germany (*FAO Consultant; Rapporteur*)
- Professor W. Slob, National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands (*WHO Temporary Adviser*)
- Dr A. Tritscher, International Programme on Chemical Safety, WHO, Geneva, Switzerland (*Joint Secretary*)
- Dr F.X.R. van Leeuwen, National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands (*WHO Temporary Adviser*)
- Ms E. Vavasour, Pre-Market Toxicology Assessment Section, Food Directorate, Health Canada, Ottawa, Ontario, Canada (*WHO Temporary Adviser*)

ANNEX 4

THE FORMULATION OF ADVICE ON COMPOUNDS THAT ARE BOTH GENOTOXIC AND CARCINOGENIC¹

The Committee has established procedures for determining health-based guidance values, such as the acceptable daily intake (ADI) or provisional tolerable weekly intake (PTWI), for chemicals that produce adverse effects that are thought to show a threshold in their dose–response relationships. Compounds that are both genotoxic and carcinogenic may show non-linear dose–response relationships, but the no-observed-effect level (NOEL) in a study of carcinogenicity represents the limit of detection in that bioassay, rather than an estimate of a possible threshold. Therefore, the Committee does not establish health-based guidance values for compounds that are genotoxic and carcinogenic using the NOEL and safety (uncertainty) factors. In the absence of evidence on the influence of non-linearity on the incidence of cancer at low levels of exposure, the advice given previously by the Committee for compounds that are both genotoxic and carcinogenic has been that intakes should be reduced to as low as reasonably achievable (ALARA). Such advice is of limited value, because it does not take into account either human exposure or carcinogenic potency and has not allowed risk managers to prioritize different contaminants or to target risk management actions. In addition, ever-increasing analytical sensitivity means that the number of chemicals with both genotoxic and carcinogenic potential detected in food will increase.

The Committee at its present meeting considered a number of compounds for which genotoxicity and carcinogenicity are important issues. The Committee was aware of a number of recent developments relevant to the risk assessment of such compounds, including:

- a WHO workshop that developed a strategy for dose–response assessment and the formulation of advice (1);
- discussions within the European Food Safety Authority about a margin of exposure (MOE) that would indicate the level of priority for risk management action (2); and
- Australian recommendations for genotoxic and carcinogenic soil contaminants regarding a guideline dose that would be protective of human health based on a modified benchmark dose and the application of uncertainty factors to allow for interspecies differences, intraspecies variability, quality of the database and the seriousness of the carcinogenic response (3).

¹ Taken from section 2.1 of the Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives (see Annex 1, reference 174).

The Committee discussed approaches to the formulation of advice on contaminants that are both genotoxic¹ and carcinogenic, which would inform risk managers about the possible magnitude of health concerns at different levels of intake in humans.

Hazard identification would normally be based on data from studies on genotoxicity and from cancer bioassays. Some chemicals increase the incidence of cancer in experimental animals by non-genotoxic mechanisms, and establishing a health-based guidance value, such as a PTWI, would be appropriate. The present guidance relates to chemicals that are both genotoxic and carcinogenic.

Hazard characterization (dose–response assessment) would be based on the available dose–response data for cancer, which would mostly be derived from studies in rodents given daily doses many orders of magnitude greater than the estimated intakes in humans. Dose–response data from studies of epidemiology may also be used for hazard characterization and would avoid interspecies comparisons and extrapolation over many orders of magnitude. The recent WHO workshop recommended the use of the benchmark dose lower confidence limit (BMDL) as a starting point for hazard characterization based on data from a bioassay for cancer in animals when the data are suitable for dose–response modelling. The BMDL is the lower one-sided confidence limit of the benchmark dose (BMD) for a predetermined level of response, called the benchmark response (BMR), such as a 5% or 10% incidence. The BMD in most cases shows less variation than the BMDL for different mathematical models and may be more suitable for ranking different compounds in terms of their potency, while the BMDL may be more appropriate for risk characterization purposes because it reflects the quality of the data. The derivation and interpretation of a BMDL require considerable statistical and biological expertise.

A number of aspects of the database need to be considered in dose–response modelling, including data selection, model selection, statistical linkage, parameter estimation, implementation and evaluation (1). The dose metric used for modelling could be a biomarker, providing that it was critically related to the process by which cancer arises and had been validated in relation to the external dose or intake. For carcinogenesis, selection of the dose–response data for modelling will need to consider both site-specific incidences of tumours, especially for the site showing the greatest sensitivity, and combined data (e.g. numbers of tumour-bearing animals) for compounds that do not show clear organ specificity. Analyses based on the numbers of tumour-bearing animals may also be

¹ The present guidance does not address the situation where a compound shows genotoxicity, or has structural alerts for genotoxicity, but where a bioassay for cancer has not been performed. The Committee is aware of developments, such as the threshold of toxicological concern (TTC) for compounds with structural alerts for genotoxicity, that may allow the formulation of limited advice to risk managers, and would welcome a critical evaluation of such approaches.

appropriate under other circumstances, for example in the assessment of complex mixtures of compounds that are both genotoxic and carcinogenic. Dose–response characterization should aim to define the BMDL for the carcinogenic response(s) of relevance to human health, at the lowest level of response (the BMR) that reliably defines the lower end of the observed experimental dose–response relationship. A BMR of a 10% incidence is likely to be the most appropriate for modelling of data from bioassays for cancer, because the values for different mathematical models show wider divergence at incidences below 10%. The consistent use of the same benchmark response, i.e. 10%, will facilitate comparisons of the risks associated with different compounds that are both genotoxic and carcinogenic. Non-cancer effects produced by compounds that are both genotoxic and carcinogenic may be analysed using the same approach, and comparison of the derived BMDL values and their associated slopes can help to identify the adverse effect that is critical to risk assessment of the compound.

The intake (exposure) assessment for a compound that is both genotoxic and carcinogenic is no different from that for other types of contaminants.

Risk characterization involves comparison of the estimated exposure with the identified BMDL. In principle, this can take different forms:

- *Calculation of the margin of exposure (MOE, the ratio of the BMDL to the estimated intake in humans).* The MOE can be used to prioritize different contaminants, providing that a consistent approach has been adopted. The acceptability of an MOE depends on its magnitude and is ultimately a risk management decision (1). To aid that decision, the risk assessor should provide information on the nature and magnitude of uncertainties in both the toxicological and exposure data. Although the risk assessor should not provide an assessment of the acceptability of the MOE, guidance should be given on its adequacy taking into account the inherent uncertainties and variability.
- *Dose–response analysis outside the observed dose range.* Quantitative dose–response analysis could be used to calculate the incidence of cancer that is theoretically associated with the estimated exposure for humans, or the exposure associated with a predetermined incidence (e.g. 1 in 1 million). In order to provide realistic estimates of the possible carcinogenic effect at the estimated exposure for humans, mathematical modelling would need to take into account the shape of the dose–response relationship for the high doses used in the bioassay for cancer and for the much lower intakes by humans. Such information cannot be derived from the available data on cancer incidence from studies in animals. In the future, it may be possible to incorporate data on dose–response or concentration–response relationships for the critical biological activities involved in the generation of cancer (e.g. metabolic bioactivation and detoxication processes, DNA binding, DNA repair, rates of cell proliferation and apoptosis) into a biologically based dose–response model for cancer that would also incorporate data on species differences in these processes. However, such data are not currently

available. At present, any estimate of the possible incidence of cancer in experimental animals at intakes equal to those for humans has to be based on empirical mathematical equations that may not reflect the complexity of the underlying biology. A number of mathematical equations have been proposed for extrapolation to low doses. The resulting risk estimates are dependent on the mathematical model used; the divergence increases as the dose decreases, and the output by different equations can differ by orders of magnitude at very low incidences.

- *Linear extrapolation from a point of departure.* Because the estimated risks at low doses are model-dependent, linear extrapolation from the BMDL, which is conservative and simple to apply, has been used as a matter of policy by some agencies in order to calculate levels of exposure associated with different theoretical incidences of cancer. The incidence used is regarded as an upper-bound estimate for lifetime risk of cancer, and the actual risk may lie anywhere between zero and the calculated upper-bound estimate. Calculation of the intake associated with an incidence of 1 in 1 million from the BMDL for a 10% incidence using linear extrapolation is simply equivalent to dividing the BMDL by 100 000, and this approach is therefore no more informative than calculation of an MOE.

Of the three options given above, the MOE and linear extrapolation from a point of departure are the most pragmatic and usable at the present time. Linear extrapolation from a point of departure offers no advantages over an MOE, and the results are open to misinterpretation because the numerical estimates may be regarded as quantification of the actual risk.

The Committee at its present meeting decided that advice on compounds that are both genotoxic and carcinogenic should be based on estimated MOEs. The strengths and weaknesses inherent in the data used to calculate the MOE should be given as part of the advice to risk managers, together with advice on its interpretation.

References

1. *Principles for modelling low-dose response for risk assessment of chemicals.* International Programme on Chemical Safety Workshop, Geneva, World Health Organization, 2004 (http://www.who.int/ipcs/methods/harmonization/en/draft_document_for_comment.pdf).
2. *EFSA Scientific Committee Draft Opinion on a harmonised approach for risk assessment of compounds which are both genotoxic and carcinogenic.* Brussels, European Food Safety Authority, 2005 (Request No. EFSA-Q-2004-20 ; http://www.efsa.eu.int/science/sc_committee/sc_consultations/882/sc_consultation_genocar_draft_opinion_en1.pdf).
3. *Toxicity assessment for carcinogenic soil contaminants.* Commonwealth of Australia, Canberra, National Health and Medical Research Council, 1999.

ANNEX 5

APPROACH TO DOSE-RESPONSE MODELLING¹

At the present meeting, cancer dose-response data were analysed by dose-response modelling, in accordance with the International Programme on Chemical Safety (IPCS) document *Principles for modelling dose-response for the risk assessment of chemicals* (1). The statistical methods of dose-response modelling as applied at this meeting are briefly described below.

For each tumour end-point considered relevant, the quantal dose-response models given in Table 1 were fitted to the dose-incidence data:

Table 1. Dose-response models used

Model	Model equation	Constraints
One-stage	$R = a + (1-a)(1-\exp(-x/b))$	$0 \leq a \leq 1$,
Two-stage	$R = a + (1-a)(1-\exp(-(x/b)-c(x/b)^2))$	$0 \leq a \leq 1$
Log-logistic	$R = a + (1-a)/(1+\exp(c \log_{10}(b/x)))$	$0 \leq a \leq 1$, $c \geq \ln(10)$
Log-probit	$R = a + (1-a) \Phi(c \log_{10}(x/b))$	$0 \leq a \leq 1$
Weibull	$R = a + (1-a)(1-\exp(-(x/b)^c))$	$0 \leq a \leq 1$, $c > 1$
Proast M2	$y = \exp(bx)$, th1	
Proast M3	$y = \exp(b x^d)$, th1	$d \geq 1$
Proast M4	$y = c - (c-1)\exp(-bx)$, th1	

Φ denotes the (cumulative) standard normal distribution function.

The first five of these models directly relate the incidence (R , expressed as a fraction) to the dose (x). In these models, the parameter a (also expressed as a fraction) reflects the incidence in the controls, the parameter b denotes the slope and parameter c can be considered as a shape parameter. The last three models (Proast M2-M4) are a specific family of models that assume an underlying continuous response (indicated by y), which is translated into a binary response

¹ Taken from Annex 3 of the Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives (see Annex 1, reference 174).

(incidence) by incorporating a cut-off point (*th1*) in the normal distribution around *y*, below which an animal does not respond, and above which it does respond.

Some of the models are nested members of a larger family of models. Two models are nested when the one model can be seen as an extension of the other (simpler) model by incorporating one or more parameters. For instance, the two-stage model is an extension of the one-stage model by including parameter *c*. Also, the Proast models are a nested family of models (2). Nested models can be formally compared with each other as follows. Inclusion of an extra model parameter should result in a higher log-likelihood value, and if this increase is >1.92, inclusion of the parameter has resulted in a significantly better fit (log-likelihood ratio test). If the increase is <1.92, the fit is not significantly better, and the parameter is omitted.

When dose-response data are available from more than one study, or for both sexes, these models are fitted simultaneously to both such subgroups. This was done either by assuming all parameters in the model being the same for all subgroups or by assuming only the background response parameter (*a*) being different, or only the slope (*b*). When all parameters are assumed to be the same, a single curve results, otherwise different curves for the subgroups will result. A model in which a parameter is assumed to be different represents a model that is nested to the same model with the parameter assumed the same for the subgroups. Hence, the log-likelihood ratio test can be used for testing if an additional background or slope parameter results in a significantly better fit.

Selection of models

In general, those models that do not result in a significantly worse fit than the saturated model (one parameter per data point) are considered to be acceptable. For instance, when the saturated model has eight parameters (i.e. eight observed incidences available), a fitted dose-response model with three parameters should result in a log-likelihood that is no more than 5.54 lower than the log-likelihood associated with the saturated model. Table 2 summarizes the critical differences in log-likelihood values for various numbers of degrees of freedom (= difference in number of parameters between the models to be compared).

For those models that were considered acceptable according to the criteria mentioned, the benchmark dose (BMD) values as well as the benchmark dose lower confidence limit (BMDL) values were calculated. All BMD and BMDL values were calculated for a 10% extra risk, defined as:

$$\text{extra risk} = \frac{R(\text{BMD}) - R(0)}{1 - R(0)}$$

This represents the additional-response fraction divided by the tumour-free fraction in the controls.

Table 2. Critical differences in log-likelihood values making an increase by a number of parameters (= number of degrees of freedom) to result in a significantly better fit

Number of degrees of freedom	Critical difference in log-likelihood ($\alpha = 0.05$)
1	1.92
2	3.00
3	3.91
4	4.74
5	5.54
6	6.30
7	7.03
8	7.75

The BMD and BMDL values were estimated by the bootstrap method, usually performing 500 bootstrap runs. These values therefore contain some random error, but usually no more than about 10% for the BMDL.

The calculations were performed using the dose–response software package PROAST, version V07 (developed at the National Institute of Public Health and the Environment [RIVM], Bilthoven, The Netherlands), which is freely available.

References

1. IPCS (2004) *Principles for modelling dose–response for the risk assessment of chemicals*. Geneva: World Health Organization, International Programme on Chemical Safety Workshop (http://www.who.int/ipcs/methods/harmonization/en/draft_document_for_comment.pdf).
2. Slob, W. (2002) Dose–response modeling of continuous endpoints. *Toxicol. Sci.*, **66**, 298–312.

FAO TECHNICAL PAPERS REPORTS
FOOD AND NUTRITION PAPERS

1. 1/1 Review of food consumption surveys 1977 – Vol. 1. Europe, North America, Oceania, 1977 (E)
1/2 Review of food consumption surveys 1997 – Vol. 2. Africa, Latin America, Near East, Far East, 1979 (E)
2. Report of the joint FAO/WHO/UNEP conference on mycotoxins, 1977 (E F S)
3. Report of a joint FAO/WHO expert consultation on dietary fats and oils in human nutrition, 1977 (E F S)
4. JECFA Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers, 1978 (E).
5. JECFA – guide to Specifications, 1978 (E F)
5. Rev. 1 JECFA – guide to Specifications, 1983 (E F)
5. Rev. 2 JECFA – guide to Specifications, 1991 (E)
6. The feeding of workers in developing countries, 1976 (E S)
7. JECFA Specifications for identity and purity of food colours, enzyme preparations and other food additives, 1978 (E F)
8. Women in food production, food handling and nutrition, 1979 (E F S)
9. Arsenic and tin in foods: review of commonly used methods of analysis 1979 (E)
10. Prevention of mycotoxins, 1979 (E F S)
11. The economic value of breast-feeding, 1979 (E F)
12. JECFA Specifications for identity and purity of food colours, flavouring agents and other food additives, 1979 (E F)
13. Perspective on mycotoxins, 1979 (E F S)
14. Manuals on food quality control
14/1 Food control laboratory, 1979 (Ar E)
14/1 Rev. 1 The food control laboratory, 1986 (E)
14/2 Additives, contaminants, techniques, 1980 (E)
14/3 Commodities, 1979 (E)
14/4 Microbiological analysis, 1979 (E F S)
14/5 Food inspection, 1981 (Ar E (Rev. 1984, E S))
14/6 Food for Export, 1979 (E S)
14/7 Rev. 1 Food for export, 1990 (E S)
14/8 Food analysis: general techniques, additives, contaminants and composition, 1986 (C E)
14/9 Introduction to food sampling, 1988 (Ar C E F S)

- 14/10 Training in mycotoxin analysis, 1990 (E S)
- 14/11 Management of food control programmes, 1991 (E)
- 14/12 Quality assurance in the food control microbiological laboratory, 1992 (E F S)
- 14/13 Pesticide residue analysis in the food control laboratory, 1993 (E F)
- 14/14 Quality assurance in the food control chemical laboratory, 1993 (E)
- 14/15 Imported food inspection, 1993 (E F)
- 14/16 Radionuclides in food, 1994 (E)
- 14/17 Unacceptable visible can defects – a pictory manual, 1998 (E F S)
- 15. Carbohydrates in human nutrition, 1980 (E F S)
- 16. Analysis of food consumption survey data for developing countries, 1980, (E F S)
- 17. JECFA specifications for the identity and purity of sweetening agents, emulsifying agents, flavouring agents and other food additives, 1980 (E F).
- 18. Bibliography of food consumption surveys, 1981 (E)
 - 18. Rev. 1 Bibliography of food consumption surveys, 1984 (E)
 - 18. Rev. 2 Bibliography of food consumption surveys, 1987 (E)
 - 18. Rev. 3 Bibliography of food consumption surveys, 1990 (E)
- 19. JECFA specifications for the identity and purity of carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents and food additives, 1981 (E F)
- 20. Legumes in human nutrition, 1982 (E F S)
- 21. Mycotoxin surveillance – a guideline, 1982 (E)
- 22. Guidelines for agricultural training curricula in Africa, 1982 (E F)
- 23. Management of group feeding programmes, 1982 (E F)
 - 23. Rev. 1 Food and nutrition in the management of group feeding programmes, 1993 (E F S)
- 24. Evaluation of nutrition interventions, 1982 (E).
- 25. JECFA specifications for the identity and purity of buffering agents, salts; emulsifiers, thickening agents, stabilizers; flavouring agents, food colours, sweetening agents and miscellaneous food additives, 1982 (E F)
- 26. Food composition tables for the Near East, 1983 (E)
- 27. Review of food composition surveys, 1981, 1983 (E)
- 28. JECFA specifications for the identity and purity of buffering agents, salts, emulsifiers, stabilizers, thickening agents, extraction solvents, flavouring agents, sweetening agents and miscellaneous food additives, 1983 (E F)
- 29. Post-harvest losses in quality food grains, 1983 (E F)

30. FAO/WHO food additives data system, 1984 (E)
30. Rev. 1 FAO/WHO food additives data system, 1985 (E)
31. 31/1 JECFA specifications for the identity and purity of food colours, 1984 (E F)
31/2 JECFA specifications for the identity and purity of food additives, 1984 (E F)
32. Residues of veterinary drugs in foods, 1985 (E/F/S)
33. Nutritional implications of food aid: an annotated bibliography, 1985 (E)
34. JECFA specifications for the identity and purity of certain food additives, 1986 (E F)
35. Review of food composition surveys, 1985, 1986 (E)
36. Guidelines for can manufacturers and food canners, 1986 (E)
37. JECFA specifications for the identity and purity of certain food additives, 1986 (E F)
38. JECFA specifications for the identity and purity of certain food additives, 1988 (E)
39. Quality control in fruit and vegetable processing, 1988 (E F S)
40. Directory of food and nutrition institutions in the Near East, 1987 (E)
41. Residues of some veterinary drugs in animals and foods, 1988 (E)
41/2 Residues of some veterinary drugs in animals and foods. Thirty-fourth meeting of the joint FAO/WHO Expert Committee on Food Additives, 1990 (E)
41/3 Residues of some veterinary drugs in animals and foods. Thirty-sixth meeting of the joint FAO/WHO Expert Committee on Food Additives, 1991 (E)
41/4 Residues of some veterinary drugs in animals and foods. Thirty-eighth meeting of the joint FAO/WHO Expert Committee on Food Additives, 1991 (E)
41/5 Residues of some veterinary drugs in animals and foods. Fortieth meeting of the joint FAO/WHO Expert Committee on Food Additives, 1993 (E)
41/6 Residues of some veterinary drugs in animals and foods. Forty-second meeting of the joint FAO/WHO Expert Committee on Food Additives, 1994 (E)
41/7 Residues of some veterinary drugs in animals and foods. Forty-third meeting of the joint FAO/WHO Expert Committee on Food Additives, 1994 (E)
41/8 Residues of some veterinary drugs in animals and foods. Forty-fifth meeting of the joint FAO/WHO Expert Committee on Food Additives, 1996 (E)
41/9 Residues of some veterinary drugs in animals and foods. Forty-seventh meeting of the joint FAO/WHO Expert Committee on Food Additives, 1997 (E)
41/10 Residues of some veterinary drugs in animals and foods. Forty-eighth meeting of the joint FAO/WHO Expert Committee on Food Additives, 1998 (E)
41/11 Residues of some veterinary drugs in animals and foods. Fiftieth meeting of the joint FAO/WHO Expert Committee on Food Additives, 1999 (E)

- 41/12 Residues of some veterinary drugs in animals and foods. Fifty-second meeting of the joint FAO/WHO Expert Committee on Food Additives, 2000 (E)
- 41/13 Residues of some veterinary drugs in animals and foods. Fifty-fourth meeting of the joint FAO/WHO Expert Committee on Food Additives, 2000 (E)
- 41/14 Residues of some veterinary drugs in animals and foods. Fifty-eighth meeting of the joint FAO/WHO Expert Committee on Food Additives, 2002 (E)
- 41/15 Residues of some veterinary drugs in animals and foods. Sixtieth meeting of the joint FAO/WHO Expert Committee on Food Additives, 2003 (E)
- 41/16 Residues of some veterinary drugs in animals and foods. Sixty-second meeting of the joint FAO/WHO Expert Committee on Food Additives, 2004 (E)
- 42. Traditional food plants. 1988 (E)
 - 42/1 Edible plants of Uganda. The value of wild and cultivated plants as food, 198 (E)
- 43. Guidelines for agricultural training curricula in Arab countries, 1988 (Ar)
- 44. Review of food consumption surveys, 1988 (E)
- 45. Exposure of infants and children to lead, 1989 (E)
- 46. Street foods, 1990 (E/F/S)
- 47. 47/1 Utilization of tropical foods: cereals, 1989 (E F S)
 - 47/2 Utilization of tropical foods: roots and tubers, 1989 (E F S)
 - 47/3 Utilization of tropical foods: trees, 1989 (E F S)
 - 47/4 Utilization of tropical foods: tropical beans, 1989 (E F S)
 - 47/5 Utilization of tropical foods: tropical oil seeds, 1989 (E F S)
 - 47/6 Utilization of tropical foods: sugars, spices and stimulants, 1989 (E F S)
 - 47/7 Utilization of tropical foods: fruits and leaves, 1989 (E F S)
 - 47/8 Utilization of tropical foods: animal products, 1989 (E F S)
- 48. Number not assigned
- 49. JECFA specifications for the identity and purity of certain food additives, 1990 (E)
- 50. Traditional foods in the Near East, 1991 (E)
- 51. Protein quality evaluation, Report of the Joint FAO/WHO Expert Consultation, 1991 (E F)
- 52. 52/1 Compendium of food additive specifications – Vol. 1, 1992 (E)
 - 52/2 Compendium of food additive specifications – Vol. 2, 1992 (E)
 - 52 Add. 1 Compendium of food additive specifications – Addendum 1, 1992 (E)
 - 52 Add. 2 Compendium of food additive specifications – Addendum 2, 1993 (E)
 - 52 Add. 3 Compendium of food additive specifications – Addendum 3, 1995 (E)
 - 52 Add. 4 Compendium of food additive specifications – Addendum 4, 1996 (E)

- 52 Add. 5 Compendium of food additive specifications – Addendum 5, 1997 (E)
- 52 Add. 6 Compendium of food additive specifications – Addendum 6, 1998 (E)
- 52 Add. 7 Compendium of food additive specifications – Addendum 7, 1999 (E)
- 52 Add. 8 Compendium of food additive specifications – Addendum 8, 2000 (E)
- 52 Add. 9 Compendium of food additive specifications – Addendum 9, 2001 (E)
- 52 Add. 10 Compendium of food additive specifications – Addendum 10, 2002 (E)
- 52 Add. 11 Compendium of food additive specifications – Addendum 11, 2003 (E)
- 52 Add. 12 Compendium of food additive specifications – Addendum 12, 2004 (E)
- 52 Add. 13 Compendium of food additive specifications – Addendum 13, 2005 (E)
- 53. Meat and meat products in human nutrition in developing countries, 1992 (E)
- 54. Number not assigned
- 55. Sampling plans for aflatoxin analysis in peanuts and corn, 1993 (E)
- 56. Body mass index – A measure of chronic energy deficiency in adults, 1994 (E F S)
- 57. Fat and oils in human nutrition, 1995 (Ar E F S)
- 58. The use of hazard analysis critical control point (HACCP) principles in food control, 1995 (E F S)
- 59. Nutrition education for the public, 1995 (E F S)
- 60. Food fortification: technology and quality control, 1996 (E)
- 61. Biotechnology and food safety, 1996 (E)
- 62. Nutrition education for the public – Discussion papers of the FAO/WHO Expert consultation, 1996 (E)
- 63. Street foods, 1997 (E/F/S)
- 64. Worldwide regulations for mycotoxins 1995 – A compendium, 1997 (E)
- 65. Risk management and food safety, 1998 (E S)
- 66. Carbohydrates in human nutrition, 1998 (E S)
- 67. Les activités nutritionnelles au niveau communautaire – Expériences dans les pays du Sahel, 1998 (F)
- 68. Validation of analytical methods for food control, 1998 (E)
- 69. Animal feeding and food safety, 1998 (E)
- 70. The application of risk communication to food standards and safety matters, 1999 (Ar C E F S)

71. Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods, 2004 (E F S)
72. Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods - Risk characterization of *Salmonella* spp. in eggs and broiler chickens and *Listeria monocytogenes* in ready-to-eat foods, 2001 (E F S)
73. Manual on the application of the HACCP system in mycotoxin prevention and control, 2001 (E F S)
74. Safety assessment of certain mycotoxins in food, 2001 (E)
75. Risk assessment of *Campylobacter* spp. in broiler chicken and *Vibrio* spp. in seafood, 2003 (E)
76. Assuring food safety and quality – Guidelines for strengthening of national food control systems, 2003 (E F S)
77. Food energy – Methods of analysis and conversion factors, 2003 (E)
78. Energy in human nutrition. Report of a Joint FAO/WHO/UNU Expert Consultation, 2003 (E). Issued as No. 1 in the FAO Food and Nutrition Technical Report Series entitled Human energy Requirements, Report of a Joint FAO/WHO/UNU Expert Consultation, 2004 (E)
79. Safety assesment of foods derived from genetically modified animals, including fish, 2004 (E)
80. Marine biotoxins, 2004 (E)
81. Worldwide regulations for mycotoxins in food and feed in 2003, 2004 (C E F S)
82. Safety evaluation of certain contaminants in food, 2005 (E) .
83. Globalization of food systems in developing countries: impact on food security and nutrition, 2004 (E)

Availability: December 2005

Ar – Arabic C – Chinese E – English F – French S - Spanish

The FAO Technical papers are available through the authorized FAO Sales Agents or directly from Sales and Marketing Group, FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy.