WHO FOOD ADDITIVES SERIES: 63

Safety evaluation of certain contaminants in food

FAO JECFA MONOGRAPHS 8

World Health Organization

Food and Agriculture Organization of the United Nations

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PREFACE

The monographs contained in this volume were prepared at the seventy-second meeting of the Joint Food and Agriculture Organization of the United Nations (FAO)/ World Health Organization (WHO) Expert Committee on Food Additives (JECFA), which met at FAO headquarters in Rome, Italy, on 16–25 February 2010. These monographs summarize the data on selected food contaminants reviewed by the Committee.

The seventy-second report of JECFA has been published by the World Health Organization as WHO Technical Report No. 959. Reports and other documents resulting from previous meetings of JECFA are listed in Annex 1. The participants in the meeting are listed in Annex 3 of the present publication.

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

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

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

ACRYLAMIDE (addendum)

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1. EXPLANATION

Acrylamide (CH_2=CHCONH$_2$, Chemical Abstracts Service No. 79-06-01) is a water-soluble vinyl monomer that is formed in many common foods during cooking. Acrylamide is also a component of tobacco smoke. It is readily polymerizable. Polyacrylamide has multiple applications in chemical and manufacturing industries—for example, as a flocculant for clarifying drinking-water, as a sealant for construction of dams and tunnels, as a binder in the paper and pulp industry and in dye synthesis.

The sixty-fourth meeting of the Committee (Annex 1, reference *176*) evaluated dietary acrylamide and recommended that:

- acrylamide should be re-evaluated once the results of the planned study of carcinogenicity and long-term studies of neurotoxicity become available;
- work should continue on physiologically based pharmacokinetic (PBPK) modelling to better link biomarkers in humans with dietary exposure assessments and toxicological effects in experimental animals;
- work to reduce exposure to acrylamide in food by minimizing its concentrations should continue;

- information on the occurrence of acrylamide in food consumed in developing countries would be useful to conduct a dietary exposure assessment and consider appropriate mitigation strategies to minimize acrylamide concentrations in food.

At its present meeting, the Committee reconsidered the studies described in the monograph of the sixty-fourth meeting (Annex 1, reference *177*). New information on occurrence and mitigation as well as dietary exposure was considered. Additionally, the Committee considered the recently completed toxicity studies, which included studies on metabolism, genotoxicity and neurodevelopmental effects following exposure to acrylamide as well as long-term toxicity and carcinogenicity studies on acrylamide and glycidamide. There were also many new epidemiological studies available for review.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

Recent studies in humans (Fennell et al., 2005, 2006; Boettcher et al., 2006; Fuhr et al., 2006; Kopp & Dekant, 2009) and pigs (Aureli et al., 2007) have confirmed that apart from some differences in metabolism, the absorption, distribution and excretion of acrylamide are very similar for laboratory animals and humans (Annex 1, reference *177*). Orally administered acrylamide is rapidly and extensively absorbed from the gastrointestinal tract, then metabolized and excreted in urine, mainly as metabolites. Experimental animal studies have shown that acrylamide is widely distributed to all tissues and to the fetus in pregnant animals (Annex 1, reference *177*). It has also been found in human milk (Sörgel et al., 2002). The relative internal exposure to glycidamide, the primary metabolite of acrylamide, is much higher after dietary administration than after intravenous administration, owing to extensive first-pass metabolism of acrylamide to glycidamide. Acrylamide and its metabolites are rapidly eliminated in the urine, primarily as mercapturic acid conjugates of acrylamide and glycidamide (Annex 1, reference *177*). 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 body weight (bw) administered in the diet over a period of 30 min (Annex 1, reference *177*).

(a) Effects of dietary fibre, animal age and sex on absorption

In a study designed to test the effect of dietary fibre on acrylamide-induced neurotoxicity and testicular toxicity, male Sprague-Dawley (CD(SD)IGS) rats (5 per group except for the control group, with 10) were fed a supplemented diet containing separately 2.5% sodium alginate, 5% glucomannan, 5% digestion-resistant maltodextrin, 2.5% chitin or 1% chlorophyllin. Rats were fed the modified diet for 1 week before co-treatment with either 0% or 0.02% acrylamide (0 or 200 mg/l) in the drinking-water for 4 weeks. For comparison, untreated control animals were given basal diet and tap water. Neurotoxicity was clinically assessed by the

presence of gait abnormalities and by histopathological changes in the sciatic and trigeminal nerves, as well as aberrant dot-like immunoreactivity for synaptophysin in the cerebellar molecular layer. Testicular toxicity was assessed by quantification of seminiferous tubules with exfoliation of germ cells into the lumen and cell debris in the ducts of the epididymides. Testicular toxicity as well as neurotoxicity were evident in treated rats irrespective of which dietary fibre or supplement (sodium alginate, chlorophyllin) was added in the diet. Hence, there was no apparent influence of dietary fibre on the uptake of acrylamide from the gastrointestinal tract (Woo et al., 2007). Similar findings on the effects of dietary fibre and fat on the uptake of acrylamide in Wistar rats were reported by Sánchez et al. (2008). However, in that study, they used a different end-point—namely, the formation of acrylamide–valine (AA-Val) adducts in blood to assess acrylamide absorption.

In a study that investigated the influence of age and sex on the uptake of acrylamide in Wistar rats (six of each sex per group), Sánchez et al. (2008) reported that single doses of acrylamide (25 or 100 mg/kg bw) administered by gavage to females resulted in significantly ($P < 0.05$) increased AA-Val adduct levels relative to males at both doses (3.53- and 2.55-fold, respectively) 24 h after dosing. However, no differences between the sexes were observed in the levels of AA-Val adducts when acrylamide (25 mg/kg bw) was administered in the diet (via a fortified cookie) or after intravenous injection. Following single gavage administration of acrylamide (100 mg/kg bw) to female rats aged 1.5, 3 or 14 months, the authors observed an age-related reduction in mean AA-Val levels; the AA-Val concentrations in the 1.5-month-old rats were 30.1% higher than those in the 14-month-old rats.

In a study designed to investigate whether dosing male F344 rats (eight per group) with high oral doses of acrylamide (0, 5, 10 or 50 mg/kg bw per day) in the presence of either high (23.9%) or low (7%) corn oil in a semi-synthetic diet would modulate the incidence and severity of azoxymethane-induced aberrant crypt foci (precancerous lesions that can develop in the colons of both rodents and humans) after 8 weeks of treatment, there were no signs of toxicity, but rats given the highest dose of acrylamide (50 mg/kg bw per day) ate significantly less food in the high- or low-fat diets and had a correspondingly lower body weight relative to controls. Irrespective of dietary fat level, rats given the highest dose of acrylamide had significantly lower total aberrant crypt foci ($P < 0.05$) and lower large aberrant crypt foci (those with four or more crypts per focus; $P < 0.001$) compared with their respective controls. In addition, a significantly lower number of large aberrant crypt foci ($P = 0.046$) was noted in 10 mg/kg bw per day rats with high fat, relative to the high fat control (Raju & Mehta, 2009).

2.1.2 Biotransformation

Results from studies in rodents and human volunteers indicate that acrylamide is extensively converted to a range of metabolites that are excreted in urine (Sumner, MacNeela & Fennell, 1992; Sumner et al., 2003; Fennell et al., 2005, 2006; Boettcher et al., 2006; Fuhr et al., 2006; Doerge et al., 2007; Doroshyenko et al., 2009). Both rodents and humans are able to convert acrylamide, through cytochrome P450 2E1 (CYP2E1), to the nucleophilic reactive epoxide glycidamide

(Sumner, MacNeela & Fennell, 1992; Sumner et al., 1999; Settels et al., 2008; Doroshyenko et al., 2009). Orally ingested acrylamide in rodents and humans is extensively conjugated with glutathione to form the mercapturic acid, *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine (AAMA), and finally oxidized to its corresponding sulfoxide; the final oxidation step to the sulfoxide is not observed in mice or rats (Kopp & Dekant, 2009). The importance of glutathione conjugation in reducing acrylamide reactivity is suggested by an increased number of deoxyribonucleic acid (DNA) strand breaks when intracellular glutathione levels were depleted in rat hepatocytes and Chinese hamster lung fibroblasts (V79) in vitro (Puppel et al., 2005).

In mice and rats, about 9–29% of a single oral dose is excreted in urine as *N*-acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA), whereas in human clinical studies, only between 0.7% and 6% is excreted (Table 1). A comparison of the extent to which rodents preferentially metabolize acrylamide via glycidamide comes from a consideration of the cumulative ratio of racemic GAMA to AAMA at doses less than or equal to 3 mg/kg bw (Table 1). This ratio, GAMA/AAMA, is in the order of 40, 3 and 1 for mice, rats and humans, respectively, and is consistent with PBPK modelling, indicating only modest differences in acrylamide biotransformation between rats and humans (see section 2.1.3). As anticipated, the inhibition of CYP2E1 activity with disulfiram in humans resulted in an increase in acrylamide and AAMA excretion in the order of 1.34-fold and 1.15-fold, respectively, and a corresponding reduction in GAMA excretion of 0.44-fold (Doroshyenko et al., 2009).

Compared with the clinical studies, there are several other studies (Kellert et al., 2006; Urban et al., 2006; Bjellaas et al., 2007a; Hartmann et al., 2008; Kopp et al., 2008; Heudorf, Hartmann & Angerer, 2009) that have reported a greater range for the molar ratio of GAMA to AAMA in the urine of the general population. The reason for this difference is not entirely clear, but it may be related to the considerable interindividual variability in CYP2E1 activity, with various data sets generally supporting a 4-fold to 20-fold difference in enzyme level per milligram of microsomal protein (Neafsey et al., 2009). A lower level of CYP2E1 activity would be anticipated to influence the extent of first-pass metabolism, resulting in higher blood concentrations of acrylamide and AAMA, with correspondingly lower concentrations of glycidamide and GAMA.

In a group of 53 adults (20 males aged 45 ± 13 years; 33 females aged 41 ± 11 years), including 6 smokers (2 males, 4 females), the GAMA to AAMA ratio ranged from 0.01 to 0.2, with a median value of 0.07, in non-smokers and from 0.03 to 0.09, with a median value of 0.06, in smokers. The calculated exposures to acrylamide based on a 24 h dietary recall were 21 µg and 26 µg for non-smokers and smokers, respectively, irrespective of sex. The median dietary exposure to acrylamide was estimated to be 0.47 µg/kg bw per day (range 0.17–1.16 µg/kg bw per day). There was a poor correlation between the estimated dietary exposure and the amount of acrylamide and its metabolites excreted in urine (Bjellaas et al., 2007a). In a smaller-scale study that involved only five non-smoking adults (three females, two males) and one male smoker, the same investigators had earlier reported a higher median GAMA to AAMA ratio of 0.46 in non-smokers and a ratio

Table 1. Comparison of molar percentages of dose excreted in urine of rodents and humans after oral administration of acrylamide[a]

Species	Dose (mg/kg bw)	% of dose excreted in urine						GAMA/AAMA	Total as % of dose[b]
		AA	AAMA	AAMA-SO	GA	GAMA	Glyceramide		
Mouse	50[c]	NQ	21.0 ± 1.10	ND	8.6 ± 1.1	17 ± 0.60	2.70 ± 0.60	0.81	50.4
	0.1[d]	0.6–0.7	5–9	ND	16–18	9–22	ND	1.8–2.4	33–48
Rat	50[c]	NQ	34.0 ± 1.80	ND	2.8 ± 0.50	12 ± 0.60	1.20 ± 0.40	0.35	50.7
	50[e]	NQ	38	ND	3.9	10.5	0.6	0.28	53
	3[f]	NQ	29.0 ± 4.50	ND	ND	21 ± 2.42	ND	0.72	50.0 ± 8.60
	0.1[d]	2	31	ND	6	27–29	ND	0.93	64–66
	0.02[g]	ND	29.7 ± 5.13	ND	ND	25.4 ± 6.20	ND	0.86	55.1 ± 11.8
	0.1[g]	ND	34.9 ± 7.40	ND	ND	26.7 ± 4.64	ND	0.77	61.7 ± 10.5
Human	3[f]	NQ	22.0 ± 5.30	4.20 ± 1.10	0.79 ± 0.24	ND	3.30 ± 1.10	—[h]	34.0 ± 5.70
	0.013[i]	ND	45.1	ND	ND	2.8	ND	0.06	47.7
	0.5[j]	4.67 ± 1.34	31.2 ± 6.55	8.26 ± 2.39	0.43 ± 0.20	0.82 ± 0.16	ND	0.03	45.6 ± 8.50
	1[j]	5.02 ± 1.65	34.4 ± 5.21	8.68 ± 1.21	0.63 ± 0.33	0.82 ± 0.11	ND	0.03	49.9 ± 6.30
	3[j]	3.23 ± 0.49	27.8 ± 7.99	7.25 ± 2.40	0.65 ± 0.21	0.70 ± 0.22	ND	0.03	39.9 ± 9.90
	0.0005[k]	ND	41.4 ± 3.47	7.19 ± 1.40	ND	3.83 ± 0.78	ND	0.09	52.4 ± 3.59
	0.02[k]	ND	37.4 ± 2.92	6.33 ± 1.77	ND	3.23 ± 0.69	ND	0.09	46.9 ± 3.70
	0.0124[l]	4.4 ± 1.5	50.0 ± 9.4	ND	ND	5.9 ± 1.2	ND	0.12	60.3 ±11.2
	0.014[m]	2.9	58	ND	ND	1.4	ND	0.024	71[n]

Table 1 (contd)

AA, acrylamide; GA, glycidamide; ND, not determined; NQ, not quantified; SO, sulfoxide

[a] All information given is referenced to collection periods of 24 h after administration.

[b] Total amount excreted within 24 h after exposure calculated as percentage of dose.

[c] Sumner, MacNeela & Fennell (1992). Gavage male rats; gavage male mice.

[d] Doerge et al. (2007). Gavage male mice; gavage male rats.

[e] Sumner et al. (2003). Gavage male rats.

[f] Fennell et al. (2005). Gavage male rats; oral administration, 24 male volunteers.

[g] Kopp & Dekant (2009). Gavage male rats.

[h] GAMA not measured, so ratio not quantified.

[i] Boettcher et al. (2006). Oral administration, male volunteer ($n = 1$). Excretion within 22 h following exposure.

[j] Fennell et al. (2006). Oral administration, male volunteers; same samples, but more sensitive assay than for Fennell et al. (2005).

[k] Kopp & Dekant (2009). Oral administration, male and female volunteers (three of each sex). Excretion within 22 h following exposure.

[l] Fuhr et al. (2006). Oral administration (potato crisps; USA = chips), male and female volunteers (three of each sex). Excretion over 72 h.

[m] Doroshyenko et al. (2009). Oral administration (potato crisps), male and female volunteers (eight of each sex; mean body weight assumed to be 70 kg). Excretion over 72 h.

[n] After 72 h.

of 0.25 in the smoking individual (Bjellaas et al., 2005). The ratio range for non-smokers was 0–2.44. In another study, Kellert et al. (2006) reported the median molar ratio of GAMA to AAMA to be 0.12 (range not reported) in 13 adult (age not specified) non-smokers, 0.16 in 12 adult occasional smokers and 0.07 in 13 adult smokers (≥5 cigarettes per day). In a study involving only six non-smoking adults, Kopp et al. (2008) reported a median ratio of 0.09. Urban et al. (2006) reported higher ratios in a large-scale population study involving 60 smoking (49 females and 11 males) and 60 non-smoking (37 females and 23 males) adults. The median molar ratio of GAMA to AAMA in the urine was 0.18, with a range between 0.07 and 1.43, for non-smokers. In smokers, the median and molar ratio range were slightly less, at 0.13 and 0.06–0.67, respectively. There was also a very poor correlation between the reported dietary exposure over a 7-day period and the urinary excretion of AAMA ($r = 0.313$, $P = 0.015$) or GAMA ($r = 0.202$, $P = 0.121$) (Urban et al., 2006). Hartmann et al. (2008) also found a higher median ratio in a population of 91 individuals (45 males, 46 females), including children and adults, with ages ranging between 6 and 80 years. They reported a median GAMA to AAMA ratio of 0.3, with a range between 0.004 and 1.4. Interestingly, while the median ratios among children were little different from those of adults, they tended to have a smaller range, with the lower end of the range for 6- to 18-year-olds being approximately 10-fold higher than that observed for adults (i.e. 0.2 relative to 0.02 in adults, except for the group aged 31–39 years, which had a lower value of 0.004). In another study that focused on 110 children (63 boys and 47 girls) aged 5–6 years, the median GAMA to AAMA ratio was 0.42 ± 0.17 (Heudorf, Hartmann & Angerer, 2009).

2.1.3 Physiologically based pharmacokinetic (PBPK) modelling

(a) Description of different models

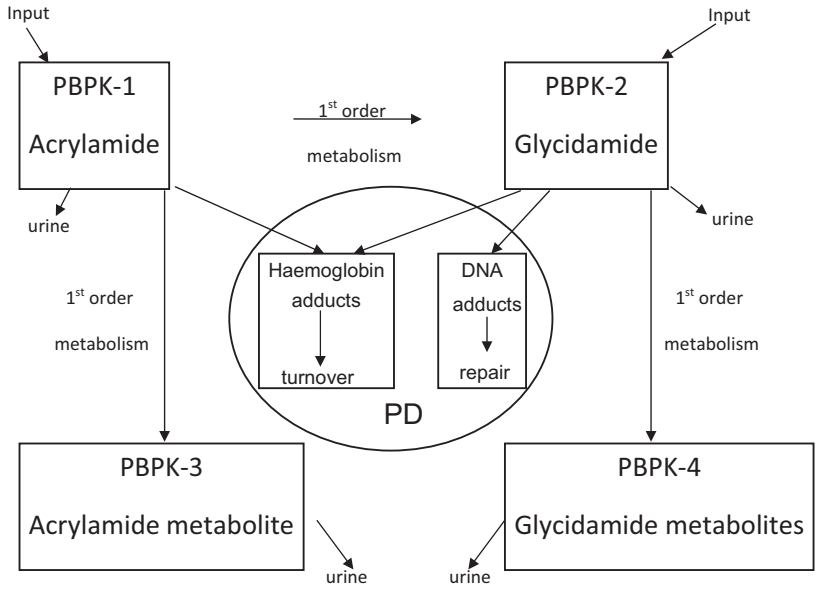
Several publications have reported various approaches to PBPK modelling of acrylamide absorption, metabolism and disposition, with the goal of predicting human internal exposures to acrylamide and glycidamide.

Kirman et al. (2003) used male F344 rat data to model the distribution of acrylamide to five compartments (arterial blood, venous blood, liver, lung and all other tissues lumped together) and linked the enzymatic metabolism by Michaelis-Menten kinetics of acrylamide to glycidamide in the liver by CYP2E1, epoxide hydrolase–catalysed hydrolysis of glycidamide and glutathione-*S*-transferase (GST)-catalysed conjugation of acrylamide and glycidamide, followed by elimination in urine of their mercapturate conjugates. Distribution of glycidamide was modelled similarly to acrylamide. The reaction of acrylamide and glycidamide with haemoglobin and other tissue macromolecules was also included. Physiological parameters for the rat (body weight, organ size, organ blood flow, etc.) were obtained from the published literature. Tissue/blood partition coefficients for acrylamide and glycidamide were estimated using chemical-specific properties. Input data were derived primarily from rodent measurements of total radioactivity from [^{14}C]acrylamide administration in blood and tissues (Miller, Carter & Sipes, 1982), acrylamide concentrations in blood and nerve tissue (Raymer et al., 1993) and urinary excretion data (Sumner et al., 1992). Despite a limited number of input

data, the model parameters provided an adequate description for most of the kinetic data available for acrylamide using a single set of input values. No kinetic data for glycidamide were available. Although no human modelling was attempted, the reported rat model was considered by the authors as "a first step in providing a tool to assist in developing (human) exposure limits" (Kirman et al., 2003).

Young, Luecke & Doerge (2007) used a general-purpose PBPK model to simulate a much more extensive number of literature data sets. The general model structure is shown in Figure 1. This study used four PBPK models under one shell, with multiple input and output options. Each PBPK unit was composed of 28 organ/tissue/fluid components that were maintained independently or connected through metabolic pathways. Acrylamide (AA), glycidamide (GA) and their glutathione (GS) conjugates (AA-GS and GA-GS) occupied the four PBPK units. Partition coefficients for acrylamide and glycidamide were derived from measured values obtained following gavage administration to F344 rats and B6C3F1 mice (Doerge et al., 2005a,b). Tissues other than those specifically analysed for acrylamide or glycidamide were assigned to be in the blood compartment. The specific organ/tissue weights and blood flows were based on literature values for the respective animal species, sex and total body weight. Optimization was based on minimizing the weighted sum of squares of the difference between each data point and its simulated value. The model was fit initially using a comprehensive plasma and tissue data set for acrylamide and glycidamide in blood and tissues from low-dose studies of acrylamide (100 µg/kg bw single exposure by intravenous, gavage and dietary routes; 1 mg/kg bw per day repeated drinking-water exposures) and equimolar glycidamide administered by intravenous and gavage routes (Doerge et al., 2005a,b). Urinary excretion of parent acrylamide, glycidamide and mercapturates for acrylamide and glycidamide was also measured. Subsequently, relevant rodent data from the literature were also modelled. In addition, a pharmacodynamic (PD) module was used to link circulating concentrations of acrylamide and glycidamide with the formation of haemoglobin adducts (AA-Val and GA-Val) and the tissue concentrations of glycidamide with the formation of DNA adducts (N7-glycidamide–guanine, or N7-GA-Gua). First-order kinetics were used in all cases because there was no advantage in imposing Michaelis-Menten kinetics, particularly on the acrylamide to glycidamide conversion, at any dose level (0.1–75 mg/kg bw). This finding was consistent with the very high Michaelis-Menten constant (K_m) values for oxidation of acrylamide to glycidamide (4–14 mmol/l) from rodent and human hepatic microsomes (Tareke et al., 2006). The PBPK/PD model of Young, Luecke & Doerge (2007) fit all of the data available for low and high doses of acrylamide and glycidamide in rodents and dietary doses of acrylamide in humans. Glycidamide data were fit first because it was the simplest simulation, then the glycidamide parameters were held constant to optimally fit the acrylamide dosing data. Finally, the PD adduct formation and decay data were simulated holding the PK parameters constant. Inclusion of generalized tissue macromolecular binding parameters for acrylamide and glycidamide was evaluated but found to have little impact on the data fits. Human simulations were based on model considerations similar to those described for rodents and focused on available exposure and elimination data from the literature that were specifically related to dietary administration of low acrylamide doses (~1 µg/kg bw per day). When

Figure 1. PBPK/PD model structure (Young, Luecke & Doerge, 2007)



possible, allometry was used to scale based upon body weights as an alternative means to validate parameters. No serum concentration data from human exposure studies were available in 2007, so the individual excretion kinetics for acrylamide, AA-GS and GA-GS from a low-dose acrylamide dietary administration (12.4 µg/kg bw) to three male and three female volunteers reported by Fuhr et al. (2006) were used as the foundation to estimate absorption, metabolism and excretion parameters for the human model. As glycidamide was not found above the limit of detection (LOD) in any urine sample, and glyceramide, the hydrolysis product of glycidamide, has never been detected in humans following dietary exposure, an estimate of the excretion of these metabolites was made based on the ratio of total GA to AA-GS excretion from a single oral acrylamide dose of 3 mg/kg bw reported in Fennell et al. (2005). These data were supplemented by the AA-Val and GA-Val adduct data from dietary exposures to acrylamide in the general human population reported by Boettcher et al. (2005), which were used to estimate a human exposure dose and in turn to estimate human internal dosimetry for acrylamide and glycidamide using published estimates of mean daily exposure (Doerge et al., 2008).

The Young, Luecke & Doerge (2007) approach to modelling produced statistically significant differences in the metabolic parameters when comparing sex, dose and route of administration in rats, although the range of values and their standard deviations were fairly small. The values of metabolic parameters for the mouse were within the same range as the rat values. Human parameters derived from dietary administration studies, when discrepant from the rodent parameters, appeared to scale appropriately based on allometry. A popular alternative modelling

approach is to fit all data to a single set of parameters that would represent all of the data across species. This approach is most often used when only the dose is being varied; the downside of this procedure is that the data fit can be compromised, in that no single set of data at any dose is fit optimally.

Internal dosimetry in humans consuming dietary acrylamide was simulated as steady-state concentrations in blood and specified tissues, using as input data the estimated mean dietary exposure (e.g. 0.4 µg/kg bw per day) in the USA and the Netherlands and measurements of urinary metabolites and haemoglobin adducts from acrylamide and glycidamide from non-smokers (Doerge et al., 2008). The predicted steady-state concentrations from daily consumption of this level of acrylamide in the diet were approximately 2.8 nmol/l for acrylamide and 0.27 nmol/l for glycidamide in blood, with comparable concentrations in a range in selected tissues. This exposure was predicted to produce DNA adduct levels in selected human tissues in the range of 0.3–0.4 N7-GA-Gua per 10^8 nucleotides. Simulations of adult male rats given the same 0.4 µg/kg bw dose of acrylamide produced steady-state blood concentrations of acrylamide and glycidamide of 0.40 and 0.19 nmol/l, respectively, with tissue N7-GA-Gua levels in the range of 0.04–0.1 adducts per 10^8 nucleotides.

Walker et al. (2007), using a recalibration of the original model parameters from Kirman et al. (2003), sought to improve upon several identified limitations of the original model: the uncertainty about the assumption for total urinary elimination of acrylamide-derived species based on 24 h urine collection; the lack of incorporation of haemoglobin adduct measurement data; and the use of a single default partition coefficient for glycidamide. Data for haemoglobin adducts were incorporated by adapting methodology for their use in calculating acrylamide and glycidamide circulating areas under the curve (AUCs) (Calleman, 1996). Partition coefficients for glycidamide were assumed to be equal to those for acrylamide and were used for both rodent and human simulations. These modifications led to recalibrated sets of model parameters that were used to fit rat and human data sets with the goal of simulating human AUCs from defined exposures to acrylamide. The human model was calibrated against human haemoglobin adduct and urinary metabolite data sets derived from human volunteers given a single oral dose of acrylamide (0.5–3 mg/kg bw; Fennell et al., 2005). This model was also used by the United States Environmental Protection Agency (USEPA) in a risk assessment of acrylamide (USEPA, 2010). Walker et al. (2007) also modelled the effect of perinatal development and interindividual variability based on the ontogeny of CYP2E1 activity and hepatic glutathione concentrations. These Monte Carlo simulations suggested modest differences in internal dosimetry for acrylamide and glycidamide between children and adults, with early-life differences predicted to be greater for acrylamide than for glycidamide.

Sweeney et al. (2010), including two of the authors of Kirman et al. (2003), reported an updated physiologically based toxicokinetic (PBTK) model for acrylamide in humans and rats that included all the relevant kinetic information available at that time. The resulting model parameters were expanded and refined from those in Kirman et al. (2003) and extended to humans. This modelling effort used all the male F344 rat data sets, including partition coefficients, blood and tissues, haemoglobin adducts and urinary metabolites, previously fit by Young, Luecke & Doerge (2007) and Walker et al. (2007). The human model was fit

using the haemoglobin adduct and urinary metabolite data from Fennell et al. (2005, 2006) derived from human volunteers given a single oral dose of acrylamide (0.5–3 mg/kg bw); time courses of urinary mercapturic acid metabolites derived from human volunteers given a single oral dose of acrylamide (20–100 µg/kg bw; Kopp & Dekant, 2009); urinary metabolites derived from human volunteers given a single oral dose of acrylamide (12.4 µg/kg bw; Fuhr et al., 2006); and urinary metabolite and haemoglobin adduct data derived from human volunteers given a single oral dose of acrylamide (15 µg/kg bw; Doroshyenko et al., 2009). Output data for internal dosimetry (i.e. steady-state concentrations or AUCs for acrylamide and glycidamide) were not reported except for an interspecies comparison between male rats and humans. Using simulated circulating AUCs for acrylamide and glycidamide as the output metrics, administration of a single acrylamide dose of 100 µg/kg bw to rats was equivalent to a human acrylamide dose of 23 µg/kg bw and a human glycidamide dose of 130 µg/kg bw. This rat-to-human equivalent dose relationship was reported to be linear up to doses of 2 mg/kg bw.

(b) Comparisons of PBPK model predictions for internal dosimetry

Although the format of model output data reported by Sweeney et al. (2010) was not directly comparable with those reported by Young, Luecke & Doerge (2007) and Walker et al. (2007), some comparisons of the three models' output for rat and human internal dosimetry for acrylamide and glycidamide are possible. As shown in Table 2, AUCs for acrylamide and glycidamide in male F344 rats predicted by Young, Luecke & Doerge (2007) and Walker et al. (2007) were similar to those measured in male F344 rats following gavage administration of acrylamide at 100 µg/kg bw (Doerge et al., 2005b). The predicted AUCs from Young, Luecke & Doerge (2007) overlap the mean ± standard deviation (SD) values for measured values for acrylamide and glycidamide AUCs, but the predictions from Walker et al. (2007) are consistently 2- to 3-fold higher than the measured values.

Comparing model-predicted human AUCs was possible across all three models by using the reported human equivalent doses of 0.023 mg/kg bw for acrylamide and 0.130 mg/kg bw for glycidamide when AUCs were compared with those resulting from an acrylamide dose of 0.1 mg/kg bw in male rats (Sweeney et al., 2010). The AUCs measured in male F344 rats at an acrylamide dose of 100 µg/kg bw (Doerge et al., 2005b) were divided by the human equivalent factor

Table 2. Comparison of PBPK model predictions for male F344 rat internal dosimetry with measured values from a single acrylamide dose of 100 µg/kg bw

Study	$AUC_{0-\infty}$ AA (µmol/l × h)	$AUC_{0-\infty}$ GA (µmol/l × h)
Doerge et al. (2005b) (gavage study)	2.4 ± 0.51	1.3 ± 0.20
Young, Luecke & Doerge (2007)	2.4	1.1
Walker et al. (2007)	6.7	5.0
Sweeney et al. (2010)	Not reported	Not reported

(0.23 for acrylamide or 1.3 for glycidamide) to yield the human AUCs (Table 3). The predicted AUC for acrylamide varied by a factor of 3 across models, and the AUC for glycidamide varied by a factor of 6.8. In all cases, the predictions by Walker et al. (2007) were highest, those by Sweeney et al. (2010) were lowest and those by Young, Luecke & Doerge (2007) were intermediate. Because dose linearity was explicit in the results of Young, Luecke & Doerge (2007) and Walker et al. (2007) and implied by Sweeney et al. (2010), it was also possible to predict human internal dosimetry from a mean daily acrylamide exposure of 1 µg/kg bw (Table 4).

(c) Use of PBPK modelling for human cancer and neuropathy risk assessments

Two publications have used internal dosimetry simulations from PBPK models for risk assessment of neurotoxicity and cancer to reduce uncertainty in extrapolating across dose and species from studies of humans exposed to dietary levels of acrylamide. The first publication interpreted results from rodent studies as being consistent with a genotoxic mechanism for acrylamide carcinogenesis by virtue of its metabolism to glycidamide, DNA adduct formation (N7-GA-Gua), somatic cell mutagenesis and, ultimately, tumour formation (Doerge et al., 2008). This group used the Young, Luecke & Doerge (2007) PBPK model to estimate the levels of N7-GA-Gua DNA adducts in rat target tissues using lower confidence limit on the benchmark dose for a 10% response ($BMDL_{10}$) values as the acrylamide dose from benchmark dose (BMD) analysis of the chronic male and female F344 rat bioassay tumour incidence data from Johnson et al. (1986) (see section 8.1.1 below). These adduct levels in tumour target tissues were then compared with N7-GA-Gua levels in the analogous human tissues predicted to result from daily consumption of acrylamide in the diet at a dose of 0.4 µg/kg bw. Lifetime excess

Table 3. Comparison of PBPK model predictions for human internal dosimetry from a single acrylamide dose of 100 µg/kg bw

Study	$AUC_{0-\infty}$ AA (µmol/l × h)	$AUC_{0-\infty}$GA (µmol/l × h)
Young, Luecke & Doerge (2007)	16.7	1.6
Walker et al. (2007)	25.0	6.7
Sweeney et al. (2010) (calculated using rat gavage AUC)	10.4	1.0

Table 4. Comparison of PBPK model predictions for human internal dosimetry from a single daily acrylamide dose of 1 µg/kg bw

Study	$AUC_{0-\infty}$ AA (µmol/l × h)	$AUC_{0-\infty}$ GA (µmol/l × h)
Young, Luecke & Doerge (2007)	0.17	0.016
Walker et al. (2007)	0.25	0.067
Sweeney et al. (2010)	0.10	0.010

cancer risks were then calculated and were in the range of 1–4 × 10^4 for thyroid, central nervous system, peritesticular mesothelium and mammary gland. The respective margins of exposure (MOEs) were in the range of 260–960. These predicted excess risks were of a similar magnitude to those in a previously published quantitative cancer risk assessment for dietary acrylamide (Dybing & Sanner, 2003), and the MOEs were consistent with those previously published by the Committee (Annex 1, reference *177*) for mean and high levels of acrylamide consumption of 1 and 4 µg/kg bw per day, respectively. Similarly, Doerge et al. (2008) used the Young, Luecke & Doerge (2007) PBPK model to estimate the brain/nervous tissue concentrations of acrylamide from several studies reporting neuropathy in rat bioassays (Burek et al., 1980; Johnson et al., 1986; Friedman, Dulak & Stedman, 1995). BMD analysis provided $BMDL_{10}$ values for neuropathy, and the PBPK model then used those doses to predict rat brain/nervous tissue concentrations of acrylamide. Those concentrations in rats were then compared with the predicted value of brain/nervous tissue acrylamide in humans from daily consumption of acrylamide in the diet at a dose of 0.4 µg/kg bw to calculate MOEs. Using male and female rat neuropathy data from lifetime (2 years) exposures to acrylamide, the MOEs were in the range of 130–320; for a 90-day exposure to acrylamide, the MOE was 54 using the $BMDL_{10}$ values (Doerge et al., 2008). These MOEs were also similar to those previously published by the Committee for mean and high levels of acrylamide consumption of 1 and 4 µg/kg bw per day, respectively (Annex 1, reference *177*).

The model output for internal dosimetry from Sweeney et al. (2010) was used to interpret results from chronic rodent carcinogenicity studies as being primarily consistent with hormonal dysregulation in the carcinogenic mechanism of acrylamide and/or glycidamide (Tardiff et al., 2010). This group used the Sweeney et al. (2010) PBPK model to calculate $BMDL_{10}$ values (individual tissues, including thyroid, testes and mammary gland, as well as a geometric mean value) based on predicted AUC for either acrylamide or glycidamide and to use these in MOE comparisons with human internal exposures predicted from daily exposure to 1 µg/kg bw (mean consumption) or 4 µg/kg bw (high consumption). Using the geometric mean $BMDL_{10}$ values for male and female F344 rat tumorigenesis, MOEs were calculated to be 200 (mean human consumption) or 50 (high consumption), assuming that acrylamide is the toxic species, and 1200 or 300, respectively, assuming glycidamide to be the toxic species (Table 5). Similarly, for rat neuropathy results from 2-year exposures (Johnson et al., 1986; Friedman, Dulak & Stedman, 1995), MOEs were calculated to be 300 (mean human consumption) or 80 (high consumption), assuming that acrylamide is the toxic species, and 500 or 130, assuming that glycidamide is the toxic species (Table 5).

A comparison of model-predicted MOEs for cancer and neuropathy for the two PBPK modelling/mechanism approaches with the MOEs previously calculated by the Committee was possible for a daily acrylamide exposure of 1 µg/kg bw (see Table 5). In general, predicted MOEs for acrylamide were similar to those previously reported by the Committee (Annex 1, reference *177*) for female rat mammary gland tumours and microscopically detected peripheral nerve degeneration for mean daily acrylamide exposure of 1 µg/kg bw (Table 5).

Table 5. Comparison of PBPK model-predicted MOEs for cancer and neuropathy, comparing internal dosimetry from a daily dose at $BMDL_{10}$ values in F344 rats or a 1 µg/kg bw dose of acrylamide in humans with previous evaluation by the Committee

	MOE		
	Annex 1, reference *177*	Doerge et al. (2008)	Tardiff et al. (2010)
Cancer	300[a]	100[a]	200 (AA), 1200 (GA)[b]
Neuropathy	200[c]	83[d]	300 (AA), 500 (GA)[d]

[a] Human average consumer (1 µg/kg bw dose of acrylamide) versus female rat mammary gland tumours.
[b] Human average consumer (1 µg/kg bw dose of acrylamide) versus geometric mean $BMDL_{10}$ for all male and female rat tumour types.
[c] Human average consumer (1 µg/kg bw dose of acrylamide) versus $BMDL_{10}$ for male rat 90-day study (Burek et al., 1980).
[d] Human average consumer (1 µg/kg bw dose of acrylamide) versus average $BMDL_{10}$ values for neuropathy from male and female rat chronic bioassay exposure data (Johnson et al., 1986; Friedman, Dulak & Stedman, 1995).

2.2 Toxicological studies

2.2.1 Acute toxicity

There were no new data on the acute toxicity of acrylamide, but, as reported in the monograph of the sixty-fourth meeting (Annex 1, reference *177*), previously reported median lethal doses were generally above 150 mg/kg bw (Dearfield et al., 1995).

2.2.2 Short-term studies of toxicity

In a study designed to investigate hormonal dysfunction as a possible cause of tumour induction in endocrine-responsive tissues, acrylamide was administered to male Fischer 344 rats (20 per group) in their drinking-water at a concentration of 25, 100 or 500 µg/ml for 14 days. These concentrations delivered approximate doses of 2.5, 10 and 50 mg/kg bw per day to the treatment groups. Doses were chosen on the basis that the lowest resulted in carcinogenicity over a lifetime of exposure and the high dose would cause neurotoxicity. The end-points measured included serum levels of thyroid and pituitary hormones; target tissue expression of genes involved in hormone synthesis, release and receptors; neurotransmitters in the central nervous system that affect hormone homeostasis; and histopathological evaluation of target tissues. There were no deaths in any group. No clinical signs were observed at 2.5 or 10 mg/kg bw per day, but at 50 mg/kg bw per day, lethargy and hindlimb paralysis were evident alongside a reduction in body weight gain (7–8% relative to controls). There were no significant changes in messenger ribonucleic acid (mRNA) levels in hypothalamus or pituitary for thyrotropin releasing hormone, thyroid stimulating hormone (TSH), thyroid hormone receptor α and β, as

well as 10 other hormones or releasing factors; mRNA levels in thyroid for thyroglobulin, thyroid peroxidase, sodium–iodide symporter or type I deiodinases; serum TSH or triiodothyronine (T_3) levels (thyroxine [T_4] was decreased at high dose only); and dopaminergic tone in the hypothalamus and pituitary or increased cell proliferation (Mki67 mRNA and Ki-67 protein levels not elevated) in thyroid or pituitary. Relative to controls, there were no induced changes in cell morphology (i.e. hypertrophy, hyperplasia, karyomegaly, degeneration), cell proliferation or apoptosis at the highest dose. The authors suggested that these results were not consistent with hormonal dysfunction being a mode of action for the carcinogenicity of acrylamide in rodents (Bowyer et al., 2008a,b).

In order to determine an appropriate range of acrylamide and glycidamide doses for carcinogenicity studies in mice and rats, the United States National Center for Toxicological Research (NCTR) and National Toxicology Program (NTP) conducted four separate 13-week studies in B6C3F1 mice and F344 rats. In all four studies, groups of eight male and eight female animals were treated with either acrylamide or glycidamide at a concentration of 0, 0.14, 0.35, 0.70, 1.41 or 3.52 mmol/l in the drinking-water. All animals treated with acrylamide survived to the end of the 13-week study. With the exception of one female mouse treated with glycidamide at 1.41 mmol/l, all animals survived until the end of the 13-week study. The weights of male and female mice treated with acrylamide at 3.52 mmol/l were 86% and 94% of their respective control body weights. At an acrylamide concentration of 1.41 mmol/l, the weights of male mice were 91% of the weights of the control male mice. The weights of male and female mice treated with glycidamide at 3.52 mmol/l were approximately 90% of their respective control body weights. Hindlimb paralysis was observed in all mice treated with acrylamide at 3.52 mmol/l. Two of eight male mice at a glycidamide concentration of 3.52 mmol/l displayed hindlimb paralysis, and they also showed a low incidence (one of eight) of spinal cord degeneration and urinary bladder dilatation.

The weights of male rats exposed to acrylamide at 3.52 mmol/l were 73% of the weights of the control male rats, whereas the weights of similarly exposed females were 71% of the respective control weights. For all other groups, the body weights were unaffected by treatment after 13 weeks of exposure to acrylamide. Hindlimb paralysis was observed in all rats treated with acrylamide at 3.52 mmol/l. The weights of male and female rats treated with glycidamide at 3.52 mmol/l were 78% of their respective control weights. At a glycidamide concentration of 1.41 mmol/l, the weights of male and female rats were 87% of their respective control weights. In other groups, the body weights were not depressed by more than 10%. All of the rats treated with glycidamide at 3.52 mmol/l displayed hindlimb paralysis, and two of eight male rats also showed spinal cord degeneration and urinary bladder dilatation. The hindlimb paralysis observed in rats treated with acrylamide at 1.41 mmol/l precluded the use of this dose in the 2-year bioassay. Because of this, a high dose of acrylamide of 0.70 mmol/l was selected for the chronic 2-year drinking-water study in the rats, with the remaining acrylamide doses being 0.0875, 0.175 and 0.35 mmol/l. In order to facilitate comparisons between species and compounds, the same doses were used with the mice and with glycidamide (Beland, 2010) (see section 2.2.3).

In a study to investigate alterations in mRNA expression and histological signs of neurotoxicity in the rat forebrain following exposure to acrylamide in drinking-water for 14 days, male Fischer 344 rats (n = 7 for substantia nigra, striatum; n = 8 for parietal cortex) were treated with an acrylamide dose of 44 mg/kg bw per day. Changes in mRNA levels in the striatum, substantia nigra and parietal cortex were measured by complementary DNA (cDNA) array and/or reverse transcriptase polymerase chain reaction (RT-PCR) analysis. Treatment resulted in significantly decreased body weight and reduced locomotor activity. These physiological effects were not accompanied by prominent changes in gene expression in the forebrain. All the expression changes seen in the 1200 genes that were evaluated in the three brain regions were 1.5-fold or less, and most were not significant. Very few, if any, statistically significant changes were seen in mRNA levels of the more than 50 genes directly related to the cholinergic, noradrenergic, γ-aminobutyric acid–releasing (GABAergic) or glutamatergic neurotransmitter systems in the striatum, substantia nigra or parietal cortex. All the expression changes observed in genes related to dopaminergic function were less than 1.5-fold and not statistically significant, and the 5HT1b receptor was the only serotonin-related gene affected. No histological evidence of axonal, dendritic or neuronal cell body damage was found in the forebrain. Similarly, no microglial activation was observed. The authors concluded that acrylamide, even at maximally tolerable levels, induced neither marked changes in gene expression nor neurotoxicity in the motor and somatosensory areas of the central nervous system (Bowyer et al., 2009).

2.2.3 Long-term studies of toxicity and carcinogenicity

(a) Mouse

In a study that complied with the United States Food and Drug Administration's (USFDA) Good Laboratory Practice Regulations, groups of 48 male and female B6C3F1 mice received either acrylamide or glycidamide at a concentration of 0, 0.0875, 0.175, 0.35 or 0.70 mmol/l in their drinking-water for 2 years (Beland, 2010). Based on water consumption over 2 years, the mean acrylamide dose in males was 1.05, 2.23, 4.16 and 9.11 mg/kg bw per day for the 0.0875, 0.175, 0.35 and 0.70 mmol/l dose groups, respectively. In females, the corresponding acrylamide doses were 1.11, 2.25, 4.71 and 9.97 mg/kg bw per day. For glycidamide, the mean dose in males was 1.21, 2.68, 5.18 and 9.68 mg/kg bw per day for the 0.0875, 0.175, 0.35 and 0.70 mmol/l dose groups, respectively. In females, the corresponding doses for glycidamide were 1.39, 2.93, 5.72 and 13.13 mg/kg bw per day. Mice were monitored daily for clinical signs, whereas body weight, feed consumption and water consumption were measured weekly. At the conclusion of the study, surviving mice were euthanized, and a necropsy was performed. Necropsies were also performed for mice that either had died naturally or were sacrificed in extremis. Tissues examined included brain (cerebrum, cerebellum and brain stem), Harderian glands, heart, liver, lungs, pancreas, peripheral nerve (sciatic), ovaries, thyroid gland, parathyroid gland, skin, mammary glands, spinal cord (thoracic, lumbar and cervical), forestomach, glandular stomach and testes.

No clinical signs were observed among the mice given acrylamide. Male mice (28 at terminal sacrifice) treated with acrylamide at 0.70 mmol/l and female mice (15 and 25 at terminal sacrifice) treated with acrylamide at 0.35 and 0.70 mmol/l had reduced survival relative to controls (39 males and females at terminal sacrifice). Sporadic, although statistically significant, changes in body weight that did not exceed 6% of control weights occurred throughout the study. Water consumption was unaffected by the presence of acrylamide in male mice, but for females, there was a dose-related increase observed beginning at week 80. The incidences of neoplasia in various organs in mice given acrylamide are shown in Table 6.

There were also no clinical signs observed among the mice given glycidamide. Male mice (34, 26 and 25 survivors) treated with glycidamide at 0.175, 0.35 and 0.70 mmol/l and female mice (31 and 8 survivors, respectively) treated at 0.35 and 0.70 mmol/l had reduced survival relative to controls (males, 45; females, 41). Sporadic, although statistically significant, changes in body weight occurred throughout the study. Water consumption in females showed a dose-related increase beginning at week 80. The incidences of neoplasia in various organs in mice given glycidamide are shown in Table 7.

Several non-neoplastic lesions were considered to be treatment related. These included alveolar epithelial hyperplasia (lung) with a prevalence of 5 in high-dose males, whereas the control group displayed a prevalence of 0. Epithelial hyperplasia (forestomach) had a prevalence of 12 in high-dose males and 7 in high-dose females, whereas the control group males had 5 and females had 4. In addition, the following non-neoplastic changes had a higher incidence in treated animals, probably due to increased tumour formation: 1) cataracts and 2) myeloid hyperplasia in the bone marrow along with splenic haematopoietic cell hyperplasia.

(b) Rat

In an identical experimental protocol as described for B6C3F1 mice in the previous section, F344 rats (48 of each sex per group) received either acrylamide or glycidamide at a concentration of 0, 0.0875, 0.175, 0.35 or 0.70 mmol/l in their drinking-water for 2 years (Beland, 2010). Based on water consumption over 2 years, the mean acrylamide dose in males was 0.34, 0.67, 1.36 and 2.78 mg/kg bw per day for the 0.0875, 0.175, 0.35 and 0.70 mmol/l dose groups, respectively. In females, the corresponding doses were 0.45, 0.90, 1.88 and 4.09 mg/kg bw per day. For glycidamide, the mean dose in males was 0.39, 0.80, 1.59 and 3.40 mg/kg bw per day for the 0.0875, 0.175, 0.35 and 0.70 mmol/l groups, respectively. In females, the corresponding glycidamide doses were 0.55, 1.10, 2.27 and 4.72 mg/kg bw per day.

There were no clinical signs observed among the rats administered acrylamide. Survival among male rats was unaffected by the presence of acrylamide in the drinking-water, but females at 0.175, 0.35 and 0.70 mmol/l had a reduced survival relative to controls. A reduction in body weight gain among male and female rats at 0.70 mmol/l that commenced at week 8 resulted in a significant reduction in body weight at the conclusion of the study. The incidences of neoplasia in various organs and peripheral axonal degeneration in rats given acrylamide are shown in Table 8.

Table 6. Incidence of neoplasms in acrylamide-treated male and female B6C3F1 mice

Sex	Neoplastic or non-neoplastic finding	Poly-3 survival-adjusted incidence (%)				
		0 mmol/l[a]	0.0875 mmol/l[a]	0.175 mmol/l[a]	0.35 mmol/l[a]	0.70 mmol/l[a]
Male	Harderian gland adenoma	4.8*	29.1**	59.7**	78.8**	87.5**
	Harderian gland adenoma or carcinoma	4.8*	29.1**	59.7**	81.0**	87.5**
	Lung alveolar/bronchiolar adenoma	11.9*	13.8	29.8**	23.5	47.0**
	Lung alveolar/bronchiolar adenoma or carcinoma	14.3*	13.8	32.1**	23.5	49.5**
	Forestomach squamous cell papilloma	0.0*	4.5	4.6	13.5	15.3**
Female	Forestomach squamous cell papilloma or carcinoma	0.0*	4.5	4.6	15.7**	20.4**
	Harderian gland adenoma	0.0*	17.8**	44.7**	73.5**	74.9**
	Lung alveolar/bronchiolar adenoma	2.2*	8.9	13.7	29.2**	52.1**
	Lung alveolar/bronchiolar adenoma or carcinoma	4.5*	8.9	13.7	29.2**	54.8**
	Mammary gland adenocarcinoma	0.0*	8.9	13.8**	5.2	33.4**
	Mammary gland adenoacanthoma	0.0*	2.3	2.3	5.3	10.8**
	Mammary gland adenocarcinoma or adenoacanthoma	0.0*	8.9	13.8**	5.2	35.4**
	Ovarian benign granulosa cell tumour	0.0*	2.4	0.0	2.7	15.2**

* Significant ($P < 0.05$) trend; ** significantly different ($P < 0.05$) from the control group (0 mmol/l).

[a] Equivalent to 0, 1.05, 2.23, 4.16 and 9.11 mg/kg bw per day in males and 0, 1.11, 2.25, 4.71 and 9.97 mg/kg bw per day in females.

Table 7. Incidence of neoplasms in glycidamide-treated male and female B6C3F1 mice

Sex	Neoplastic or non-neoplastic finding	Poly-3 survival-adjusted incidence (%)				
		0 mmol/l[a]	0.0875 mmol/l[a]	0.175 mmol/l[a]	0.35 mmol/l[a]	0.70 mmol/l[a]
Male	Harderian gland adenoma	6.4*	37.6**	54.2**	76.5**	93.2**
	Harderian gland adenoma or carcinoma	29.8*	24.0	30.7	46.6	49.2**
	Lung alveolar/bronchiolar adenoma	0.0*	16.0**	16.5**	32.5**	42.4**
	Lung alveolar/bronchiolar adenoma or carcinoma	0.0*	16.0**	18.9**	32.5**	47.2**
	Skin squamous cell papilloma	0.0*	2.2	4.8	2.6	20.1**
	Forestomach squamous cell papilloma	0.0*	4.4	7.1	5.1	20.1**
	Forestomach squamous cell papilloma or carcinoma	0.0*	4.4	7.1	5.1	25.2**
Female	Harderian gland adenoma	4.4*	40.9	43.5	56.3	93.1**
	Lung alveolar/bronchiolar adenoma	6.7*	11.0	11.5	16.4	27.8**
	Lung alveolar/bronchiolar adenoma or carcinoma	8.9*	13.1	11.5	18.7	37.4**
	Mammary gland adenocarcinoma	2.2*	2.2	4.5	21.3**	30.0**
	Mammary gland adenoacanthoma	0.0*	0.0	0.0	2.4	32.6**
	Skin fibrosarcoma	0.0*	2.2	2.3	9.4	32.7**
	Forestomach squamous cell papilloma	2.2*	2.2	2.3	9.4	23.1**

* Significant ($P < 0.05$) trend; ** significantly different ($P < 0.05$) from the control group (0 mmol/l).

[a] Equivalent to 0, 1.21, 2.68, 5.18 and 9.68 mg/kg bw per day in males and 0, 1.39, 2.93, 5.72 and 13.13 mg/kg bw per day in females.

Table 8. Incidence of neoplasms and axon degeneration in acrylamide-treated male and female F344 rats

Sex	Neoplastic and non-neoplastic findings	Incidence of neoplasms and axon degeneration				
		0 mmol/l[a]	0.0875 mmol/l[a]	0.175 mmol/l[a]	0.35 mmol/l[a]	0.70 mmol/l[a]
Male	**Neoplastic**	**Poly-3 survival-adjusted incidence (%)**				
	Testicular mesothelioma	5.5*	5.7	2.7	13.1	22.9**
	Heart malignant schwannoma	2.8*	5.9	7.8	10.3	18.2**
	Pancreas islet adenoma	2.8*	5.8	10.4	2.7	18.0**
	Pancreas islet adenoma or carcinoma	2.8*	5.8	10.4	5.3	18.0**
	Thyroid gland follicular cell carcinoma	2.8*	5.8	7.9	15.8	17.6**
	Thyroid gland follicular cell adenoma or carcinoma	2.8*	8.6	10.5	15.8	25.9**
	Non-neoplastic	**Incidence (%)**				
	Peripheral nerve (sciatic) axon degeneration	5/48 (10.4)	7/48 (14.6)	7/48 (14.6)	11/48 (22.9)	23/48 (47.9)
Female	**Neoplastic**	**Poly-3 survival-adjusted incidence (%)**				
	Clitoral gland carcinoma	2.3*	14.4**	30.3**	8.1	24.3**
	Mammary gland fibroadenoma	36.4*	42.1	56.6**	57.8**	84.0**
	Mammary gland fibroadenoma or adenocarcinoma	38.5*	42.1	58.5**	57.8	84.0**
	Non-neoplastic	**Incidence (%)**				
	Peripheral nerve (sciatic) axon degeneration	4/48 (8.3)	3/48 (6.3)	1/48 (2.1)	4/48 (8.3)	19/48 (39.6)

* Significant ($P < 0.05$) trend; ** significantly different ($P < 0.05$) from the control group (0 mmol/l).
[a] Equivalent to 0, 9.34, 0.67, 1.36 and 2.78 mg/kg bw per day in males and 0, 0.45, 0.90, 1.88 and 4.09 mg/kg bw per day in females.

There were no clinical signs observed among the rats given glycidamide. Male and female rats treated at 0.35 and 0.70 mmol/l and males treated at 0.175 mmol/l had reduced survival relative to controls. Statistically significant reductions in body weight were observed in both sexes at 0.70 mmol/l. The incidences of neoplasia in various organs in rats given glycidamide are shown in Table 9.

For acrylamide-treated rats, the no-observed-adverse-effect level (NOAEL) for peripheral nerve (sciatic) axonal degeneration was 0.67 mg/kg bw per day in males and 1.88 mg/kg bw per day in females.

2.2.4 Genotoxicity

The results of recent genotoxicity studies with acrylamide and glycidamide that have been reported following the Committee's last review in 2005 (Annex 1, reference *177*) are summarized in Table 10. In accord with the previously reported findings, the new in vitro genotoxicity studies appear to confirm that acrylamide in the absence of activation is a poor mutagen but an effective clastogen. In contrast, glycidamide is a mutagen and clastogen.

In a study designed to investigate the mutagenicity of acrylamide and its epoxide glycidamide in tissues that had tumours following treatment for 2 years in carcinogenicity studies, Big Blue transgenic rats (eight of each sex per group) were treated with either acrylamide or glycidamide in drinking-water for 60 days. The average acrylamide and glycidamide doses achieved were anticipated to be approximately the same as and twice the doses used in a 2-year glycidamide carcinogenicity study in rats—namely, 5 and 10 mg/kg bw per day. Blood was collected for a micronucleus assay and the spleens for a lymphocyte *Hprt* mutant assay. Liver, thyroid, bone marrow and testis tissues from males and mammary gland tissue from females were collected for the *cII* mutant assay. Neither acrylamide nor glycidamide increased the frequency of micronucleated reticulocytes. In contrast, both acrylamide and glycidamide caused a small (2- to 3-fold), but significant ($P < 0.05$), increase in lymphocyte *Hprt* mutant frequencies, with the increases having a dose-related linear trend ($P = 0.045$ to $P < 0.001$). The frequencies of *cII* mutations in mammary gland, testis (target tissues) and liver (a non-target tissue) were unaffected by treatment. Both acrylamide and glycidamide produced weak positive increases in bone marrow (non-target) and thyroid (target) tissues. The results from this study suggest that under exposure conditions that are comparable with those known to produce tumours in 2-year bioassays, acrylamide and glycidamide are weak gene mutagens (*cII* rat bone marrow assay and *Hprt* lymphocyte assay) in the rat (Mei et al., 2010).

To investigate the mutation potential of acrylamide and glycidamide in lung tissue, Big Blue mice were treated with acrylamide at 0, 1.4 or 7.1 mol/l in drinking-water for up to 28 days. At approximate doses of 20 and 100 mg/kg bw per day, there was 3- to 5-fold increase in *cII* mutation frequency ($P \leq 0.01$) (Guo et al., 2009).

Table 9. Incidence of neoplasms in glycidamide-treated male and female F344 rats

Sex	Neoplastic or non-neoplastic finding	Poly-3 survival-adjusted incidence (%)				
		0 mmol/l[a]	0.0875 mmol/l[a]	0.175 mmol/l[a]	0.35 mmol/l[a]	0.70 mmol/l[a]
Male	Testicular mesothelioma	0.0*	2.8	11.0	28.1**	51.2**
	Heart malignant schwannoma	5.3*	8.2	8.3	17.1	26.3**
	Oral cavity papilloma squamous or papilloma	2.6*	5.4	0.0	5.9	23.7**
	Oral cavity squamous cell carcinoma, papilloma squamous or papilloma	5.3*	5.4	2.7	5.9	23.7**
	Thyroid gland follicular cell adenoma	5.4*	3.0	8.1	8.9	31.3**
	Thyroid gland follicular cell carcinoma	0.0*	5.8	8.2	2.9	18.2**
	Thyroid gland follicular cell adenoma or carcinoma	5.4*	8.8	16.0	11.6	46.2**
	Mononuclear cell leukaemia	49.4*	60.2	65.4	65.2	76.0**
Female	Clitoral gland carcinoma	9.3*	14.5	17.0	29.8**	45.1**
	Clitoral gland adenoma or carcinoma	20.8*	19.3	29.1	35.1	52.2**
	Mammary gland fibroadenoma	35.9*	59.3	81.3	85.1	90.5**
	Mammary gland fibroadenoma or adenocarcinoma	37.7*	59.3	82.6	86.3	91.6**
	Oral cavity squamous cell carcinoma, papilloma squamous or papilloma	2.3*	4.9	5.0	5.5	24.7**
	Thyroid gland follicular cell adenoma	0.0*	7.3	7.6	5.7	19.1**
	Thyroid gland follicular cell adenoma or carcinoma	0.0*	7.3	12.4	11.4	29.5**
	Mononuclear cell leukaemia	26.2*	23.1	47.3	47.7	69.6**

* Significant ($P < 0.05$) trend; ** significantly different ($P < 0.05$) from the control group (0 mmol/l).

[a] Equivalent to 0, 0.39, 0.80, 1.59 and 3.40 mg/kg bw per day in males and 0, 0.55, 1.10, 2.27 and 4.72 mg/kg bw per day in females.

Table 10. Summary of recent genotoxicity studies with acrylamide and glycidamide

Assay	Test system	Dose/concentration	Lowest effective dose	Result	Reference
Chromosomal alterations in mammalian cells in vivo					
Micronucleus	Rat (Sprague-Dawley) bone marrow cells (male)	0, 125, 150 or 175 mg/kg bw (AA), gavage	125	Positive	Yener & Dikmenli (2009)
Micronucleus	Mouse (B6C3F1/Tk) peripheral blood	0, 0.14 or 0.70 mmol/kg bw (AA and GA), IP in pups on PND 1, 8 and 15	No effective dose with mortality observed at 0.7 mmol/kg bw GA	Negative	Von Tungeln et al. (2009)
HPRT and *tk* loci	Mouse (B6C3F1/Tk1/1) spleen lymphocytes	0, 0.14 or 0.70 mmol/kg bw (AA and GA), IP in pups on PND 1 and 8	0.7 mmol/kg bw	Positive	Von Tungeln et al. (2009)
Mammalian gene mutation assays in vitro					
Thymidine kinase (*TK*) gene mutation	TK6 human lymphoblastoid cell line; no activation	2.5–14 mmol/l (AA)	14 mmol/l (AA)	Positive (with cytotoxicity)	Koyama et al. (2006)
		0.6–2.4 mmol/l (GA)	0.6 mmol/l (GA)	Positive	Koyama et al. (2006)
Thymidine kinase (*TK*) gene mutation	L5178Y/Tk+/− mouse lymphoma cells; no activation	2–18 mmol/l (AA)	12 mmol/l (AA)	Positive	Mei et al. (2008)
		0.125–4 mmol/l (GA)	2 mmol/l (GA)	Positive	Mei et al. (2008)
Chromosomal aberration	Chinese hamster V79; no activation	0–2 mmol/l (AA)	No effective dose (AA)	Negative	Martins et al. (2007)

Table 10 (contd)

Assay	Test system	Dose/concentration	Lowest effective dose	Result	Reference
Chromatid exchange	Chinese hamster V79; no activation	0–2 mmol/l (AA)	0.5 mmol/l (AA)	Positive	Martins et al. (2007)
		0–1 mmol/l (GA)	0.025 mmol/l (GA)	Positive	Martins et al. (2007)
Micronucleus	Human lymphoblastoid TK6 cells; no activation	2.5–14 mmol/l (AA)	14 mmol/l (AA)	Positive	Koyama et al. (2006)
		0.6–2.4 mmol/l (GA)	0.6 mmol/l (GA)	Positive	Koyama et al. (2006)
Comet	No activation	2.5–14 mmol/l (AA)	14 mmol/l (AA)	Negative	Koyama et al. (2006)
	No activation	0.6–2.4 mmol/l (GA)	0.6 mmol/l (GA)	Positive	Koyama et al. (2006)

AA, acrylamide; GA, glycidamide; IP, intraperitoneally; PND, postnatal day

2.2.5 Reproductive and developmental toxicity

(a) Reproductive toxicity

No reproductive toxicity studies were identified.

(b) Developmental toxicity

In a study designed to investigate the developmental effects of acrylamide on the nervous and male reproductive systems, pregnant CD(SD)IGS Sprague-Dawley rats (four per group) were treated with acrylamide at 0, 25, 50 or 100 mg/l in the drinking-water from gestational day 6 to postnatal day (PND) 21. On PND 4, litters were culled randomly to preserve eight pups, mostly four of each sex per litter. Daily observation for clinical signs, including gait abnormalities and mortality of dams and pups, was conducted throughout the experimental period. The extent of acrylamide exposure in the pups was estimated by measuring haemoglobin–acrylamide adduct and acrylamide concentrations on PND 14 and comparing them with maternal levels on PND 21. A separate group of pups from two untreated dams received acrylamide at 50 mg/kg bw by intraperitoneal injections 3 times a week from PND 2 to PND 21. The acrylamide dose received by the dams was estimated from water intake.

Dams treated with acrylamide at 100 mg/l displayed gait abnormalities from PND 2, which then progressed to become severe by PND 21. Body weights of the dams and pups were also reduced in parallel with the progression of neurotoxicity. The reduction achieved statistical significance among the pups, but not in the dams. At 50 mg/l, a slightly abnormal gait appeared from PND 18. Feed and water consumption were also reduced at 100 mg/l during the lactation period. Based on water intake, the daily dose of acrylamide during gestation and lactation was 3.7 ± 0.3, 7.9 ± 1.7 and 14.6 ± 2.5 mg/kg bw per day at 25, 50 and 100 mg/l, respectively. There were no treatment-related deaths or clinical signs among the pups from treated dams, but pups treated with acrylamide at 50 mg/kg bw per day by intraperitoneal injection had gait abnormalities from PND 15.

Histopathological analysis revealed central chromatolysis of ganglion cells in the trigeminal nerves of dams at 50 mg/l and 100 mg/l and in pups treated by intraperitoneal injection with acrylamide. The severity of the lesion in dams was reported to be three mild and one moderate at 50 mg/l and three moderate and one severe at 100 mg/l. In nine pups treated by intraperitoneal injection, the severity was reported to be mild in seven and moderate in the other two. All five male pups from dams treated at 100 mg/l also showed evidence of delayed spermatogenesis, with three of five having mild symptoms and the other two moderate. The four male pups treated by intraperitoneal injection at 50 mg/kg bw per day had similar effects, with two of four having mild symptoms, whereas the other two had moderate effects.

Morphometric data on the sciatic nerve of dams at 100 mg/l showed a significant increase in the number of degenerated axons and myelinated nerves less than 3 μm in diameter. In the cerebellar molecular layer, a significant increase of dot-like synaptophysin-immunoreactive structures was also detected at 100 mg/l. While no differences in these parameters were observed in the pups of dams at

100 mg/l, similar degradation was observed in pups dosed intraperitoneally with 50 mg/kg bw per day. No acrylamide was detected in serum or milk of dams or pups, but evidence of acrylamide exposure came from the presence of AA-Val adducts. The level of AA-Val in pups ranged between 15- and 17-fold less than observed in dams at 25, 50 and 100 mg/l. It seems likely that this low level of exposure to acrylamide could account for the absence of any neurotoxicity in the pups (Takahashi et al., 2009).

The effect of daily, low-level acrylamide exposure on food-motivated behaviour in rats was investigated with dosing commencing prenatally on gestation day 6 and continuing to PND 85. Acrylamide was administered to presumed pregnant Fischer 344 rats (9–10 per group) by gavage at doses of 0, 0.1, 0.3, 1 or 5 mg/kg bw per day. On PND 1, litters were culled to either four of each sex or three of one sex and four or five of the other, as the original litter size and sex ratio permitted. Fostering between litters of the same treatment group to achieve the appropriate numbers and sex ratio was also conducted. On PNDs 1–22, pups were gavaged with the same dose their dams had received. On PND 22, pups were weaned and pair housed with a same-sex littermate, and acrylamide exposure continued at 0, 1, 3, 10 and 50 mg/l in drinking-water. The reason for changing the mode of acrylamide administration was not reported. In order to motivate the rats to perform for food reinforcers during testing, they were placed on a restricted diet. This resulted in body weights that were around 90% of those of rats fed ad libitum, based on historical control data. One male and one female pup per litter were tested under a progressive ratio schedule of food reinforcement from approximately 6 to 12 weeks of age. There were 14 progressive ratio sessions completed over approximately 6 weeks. The behavioural testing was achieved through food rewards after lever pressing, whereas the progressive ratio task, which required multiple presses of a lever for a food reward, was introduced at 3–6 weeks of age.

Results over 6 weeks of testing indicated a significant treatment effect of acrylamide on number of reinforcers earned, with Tukey's Honestly Significant Difference (HSD) post hoc tests revealing significantly fewer reinforcers earned in the 5 mg/kg bw per day dose group than in controls. A significant effect of acrylamide on response rate was also observed, with Tukey's HSD post hoc tests revealing a significantly lower response rate in the 5 mg/kg bw per day group than in controls. There is also a relatively linear dose–response for acrylamide at other doses, although no significant interactive effects of treatment, day, sex or post-reinforcement pause were observed. These data suggest that acrylamide exposure at 5 mg/kg bw per day can produce measurable decrements on aspects of food-motivated behaviour (Garey & Paule, 2007).

2.2.6 Special studies

(a) Covalent binding to nucleic acids and proteins

In a study designed to correlate acrylamide dose with the extent of DNA alkylation, male B6C3F1 mice (10 per group) were treated by oral gavage at doses of 0, 0.125, 0.25, 1, 2, 4, 6, 8, 12, 16 or 24 mg/kg bw per day for 28 days. The presence of micronuclei in peripheral blood reticulocytes and erythrocytes was

estimated by flow cytometry. Glycidamide and acrylamide haemoglobin adducts (GA-Val, AA-Val) in plasma and N7-GA-Gua adducts in liver were also monitored. No animal died or had clinical signs of toxicity. Statistically significant increases in body weight gain were observed in some groups, but there was no correlation with dose. There was a gradual linear increase in the number of micronuclei in erythrocytes, which achieved statistical significance at doses of 6 mg/kg bw per day and above, whereas in reticulocytes, significance occurred at doses greater than or equal to 4 mg/kg bw per day. Using an internal marker of acrylamide exposure, such as haemoglobin (GA-Val, AA-Val) or DNA (N7-GA-Gua) adduct concentrations, there was a much better fit for a model with a threshold of 1–2 mg/kg bw per day relative to a linear model (Zeiger et al., 2009).

Johansson et al. (2005) investigated the nature of glycidamide-induced mutagenesis in mammalian cells using normal and DNA repair–defective Chinese hamster cell lines. They used three separate cell lines that were deficient in base excision repair, nucleotide excision repair or homologous recombination, respectively. The results obtained on the rate of incisions in base excision repair and nucleotide excision repair suggested that lesions induced by glycidamide are repaired by short patch base excision repair rather than long patch base excision repair or nucleotide excision repair. Furthermore, a large proportion of the glycidamide-induced lesions at doses up to 8 mmol/l per hour gave rise to strand breaks that are repaired by a mechanism not involving poly-adenosine diphosphate (ADP) ribose polymerase. The authors speculated that these strand breaks, which may be the result of alkylation of the backbone phosphate, are misrepaired by homologous recombination during replication, thereby leading to a clastogenic rather than a mutagenic pathway.

(b) Inflammatory markers

In a preliminary study, Jin et al. (2009) investigated a possible relationship between dietary acrylamide and inflammatory markers in rats in the presence of low and high concentrations of dietary fat. Male F344 rats (eight per group) were fed a semi-synthetic diet containing corn oil at either 70 or 174 g/kg together with acrylamide at 0, 5, 10 or 50 mg/kg for 8 weeks. A number of measured parameters were altered, such as C-reactive protein, intercellular adhesion molecule-1, paraoxonase-1 levels in serum and 8-hydroxydeoxyguanosine levels in urine. In the rats fed the low-fat diet, the administered acrylamide dose was significantly and positively correlated with urinary 8-hydroxydeoxyguanosine and serum paraoxonase-1 activity and negatively with homocysteine. In the rats fed the high-fat diet, acrylamide was significantly and negatively correlated with serum paraoxonase-1 activity, C-reactive protein and intercellular adhesion molecule-1 levels. The biological significance of these changes will need to be further investigated.

(c) Kinesin

To test the hypothesis that kinesin was a common site of action of acrylamide in producing a range of toxic effects, in particular those affecting cell division, Sickles et al. (2007) isolated genes of kinesin-related proteins from rat testes using

recombinant DNA techniques. Using appropriate protein systems, the effects of acrylamide, glycidamide and propionamide (a non-neurotoxic metabolite) on the function of two of the identified kinesin motors (i.e. KIFC5A and KRP2) were investigated. KIFC5A microtubule bundling activity, required for mitotic spindle formation, was measured in a microtubule binding assay. Both acrylamide and glycidamide caused a similar concentration-dependent reduction in the binding of microtubule. Acrylamide or glycidamide concentrations of 100 µmol/l reduced the binding activity by 60%. KRP2 microtubule disassembling activity was assayed using the quantity of tubulin disassembled from taxol-stabilized microtubule. Both acrylamide and glycidamide inhibited KRP2-induced microtubule disassembly. Glycidamide was substantially more potent, with significant reductions of 60% being achieved at 500 µmol/l, whereas a comparable inhibition by acrylamide required a concentration of 5 mmol/l. Propionamide had no significant effect on either kinesin, except KRP2 at 10 mmol/l. The investigators concluded that acrylamide may act on multiple kinesin family members and produce toxicities in organs highly dependent on microtubule-based functions.

2.3 Observations in humans

2.3.1 Enzyme polymorphism

In order to determine whether there was an association between the concentration of AA-Val and GA-Val adducts and genetic polymorphisms in a range of enzymes known to be involved in the biotransformation of acrylamide, such as CYP2E1, epoxide hydrolase (EPHX1) and glutathione-*S*-transferases (GSTM1, GSTT1 and GSTP1), Duale et al. (2009) genotyped 49 volunteers—18 males with a mean age of 45 (range 26–65) and 31 females with a mean age of 41 (range 24–60). Two of the males and four of the females were smokers. Duale et al. (2009) then matched concentrations of AA-Val and GA-Val to the polymorphisms found in order to identify any associations. The mean concentrations of AA-Val and GA-Val in non-smokers were 40.00 ± 2.25 pmol/g globin and 20.41 ± 1.34 pmol/g globin, respectively. In smokers, the mean adduct levels were 154.00 ± 19.00 pmol/g globin and 76.50 ± 10.74 pmol/g globin for AA-Val and GA-Val, respectively. There were no significant differences between males and females in the ratio of GA-Val to AA-Val adduct levels. Testing the molar ratio of GA-Val to AA-Val against various polymorphisms revealed no association with CYP2E1 genotypes. However, for individuals with the EPHX1 139Arg allele in exon 4, which corresponds to increased enzyme activity, there was a highly significant correlation, with higher ratios of GA-Val to AA-Val relative to individuals with the wild-type allele 139His ($P = 0.007$). Paradoxically, the 139Arg allele was also linked to reduced AA-Val concentrations, which was an unexpected association. For the GST genotypes, statistically significant associations were reported for increased GA-Val to AA-Val ratio for the null alleles for the GSTM1 and GSTT1 genotypes ($P = 0.039$ and $P = 0.006$, respectively). In addition, the absolute concentrations of GA-Val were significantly increased for the null genotypes for both enzymes. An analysis of the effects of various combinations of genotypes suggested that individuals with some specific genotype combinations had a significantly higher ratio of GA-Val to AA-Val in the blood. These combinations were 1) GSTM1 null and GSTT1 null; 2) CYP2E1 179Val

and GSTM1 null; 3) CYP2E1 179Val, GSTM1 null and GSTT1 null; and 4) CYP2E1 179Val, GSTT1 null, EPHX1 113Tyr and EPHX1 139Arg. Other combinations of alleles did not reveal significant associations.

2.3.2 Biomarkers of exposure

Electrophilic compounds like acrylamide and glycidamide are able to interact with reactive carboxyl, amino and sulfhydryl groups on amino acids in proteins. As albumin and haemoglobin are the most predominant proteins in blood, acrylamide and glycidamide readily form covalent adducts with the reactive amino acids of these proteins. The acrylamide and glycidamide protein adducts are typically long-lived in the body relative to the biological half-life of any unbound acrylamide or glycidamide. For example, in humans, the mean lifespan of erythrocytes is in the order of 120 days, whereas in the rat, it is only around 60 days. The long lifespan of erythrocytes means that measured levels of haemoglobin adduct reflect a time-weighted average over the lifetime of the erythrocyte. Hence, the same level of haemoglobin adducts can be produced by a single exposure or a repeated exposure over an extended period of time, as long as the cumulative exposure is the same. This characteristic limits the usefulness of these biomarkers for dose–response modelling under circumstances where there is variability in the magnitude and frequency of exposure.

Other measures of exposure of acrylamide are mercapturic acid metabolites of acrylamide and glycidamide in urine and free acrylamide in plasma and urine, reflecting the exposure during the preceding few days. As many studies looking at the association between acrylamide and cancer risk are based upon estimates of dietary exposure, studies comparing exposure with AA-Val adduct levels are of primary interest; these can be considered as validation studies for the use of AA-Val as a biomarker of dietary exposure. An aspect to be considered in validation studies is the time frame of each instrument measuring exposure. As mentioned, haemoglobin adducts reflect the acrylamide exposure of the last 3 or 4 months, whereas food frequency questionnaires (FFQs) aim to assess the usual diet over a period of time prior to its application, in most cases 1 year. On the other hand, as urinary excretion of acrylamide metabolites reflects short-term exposure on the day (or a few days) before urine collection, the suitable instruments for diet assessment are 24 h diet recall or a food diary collected over the previous days. A major advantage of comparing FFQ-based acrylamide exposure with AA-Val is that measurement errors in the FFQ are likely to be independent of errors in adduct levels; thus, they could be used together when measured simultaneously in the same study to get a more accurate estimate of the (unknown) true exposure. However, the two measures are not directly comparable, as the FFQ measures dietary exposure, whereas adduct levels are also influenced by intersubject differences in absorption and metabolism. Given this difference, the correlations between the FFQ and adduct measures can be seen as a lower bound of the true validity of the questionnaire assessment of acrylamide exposure. In contrast, as adducts can be formed by acrylamide independent of the origin of the exposure, it is important to know the background levels of AA-Val among individuals who are non-smokers and who are not exposed occupationally, as they may actually reflect acrylamide from dietary exposure.

Thus, before dealing with validation studies, some publications reporting AA-Val levels in the population were considered. In addition to publications on adducts, the Committee analysed publications dealing with urinary levels of acrylamide metabolites and short-term dietary exposure, as well as some studies with potentially relevant information on the metabolism and possible effects of acrylamide in humans. Although studies dealing with biomarkers include different approaches, the purpose of most epidemiological studies is to assess the health effects of long-term dietary exposure to acrylamide; thus, our primary interest focuses on validation studies dealing with haemoglobin adducts and FFQs.

(a) Studies reporting background levels of haemoglobin–acrylamide adducts in the population

Chevolleau et al. (2007) reported AA-Val and GA-Val concentrations in a random sample of the French population (29 adult males and 39 adult females; 8 males and 8 females were smokers) aged 18–77 years. The limits of quantification (LOQs) for the AA-Val and GA-Val adducts were 0.2 and 0.4 pmol/g globin, respectively. The total mean and median concentrations of AA-Val were 33 and 26 pmol/g globin, respectively, with a range of 9–163 pmol/g globin; for GA-Val, they were 23 and 21 pmol/g globin, respectively, with a range of 12–62 pmol/g globin. For smokers, the mean and median levels were higher, at 61 and 56 pmol/g globin, respectively, in males (range 24–119 pmol/g globin) and 46 and 27 pmol/g globin, respectively, in females (range 16–163 pmol/g globin). For smokers and non-smokers combined, the ratio of GA-Val to AA-Val adducts had a median value of 0.76 and a mean value of 0.84 (range 0.44–2.17). As the range of ratios of GA-Val to AA-Val adducts for smokers and non-smokers combined was rather large, the investigators suggested that it may reflect a large difference in CYP2E1 activities within the population.

The variability of acrylamide exposure was analysed in the European Prospective Investigation into Cancer and Nutrition study (Vesper et al., 2008). The study population included 510 subjects from Denmark, France, Germany, Greece, Italy, the Netherlands, Spain, Sweden and the United Kingdom. Within each country, 60 subjects (30 in France, only women) were selected, equally distributed by sex and smoking status. AA-Val and GA-Val were analysed by high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS). Overall, the mean concentration of AA-Val was 92.5 pmol/g globin, with a range of 14.5–623 pmol/g globin, a median concentration of 62.6 pmol/g globin and 5th- and 95th-percentile concentrations of 28.1 and 244 pmol/g globin, respectively; for GA-Val, the mean concentration was 72.2 pmol/g globin, with a range of 7.8–377 pmol/g globin, a median concentration of 51.9 pmol/g globin and 5th- and 95th-percentile concentrations of 22 and 179 pmol/g globin. The GA-Val to AA-Val ratio had a mean of 0.84 (range 0.18–1.67) and tended to decrease with the concentration of AA-Val. Regarding AA-Val, the mean concentration in non-smokers was 48.4 pmol/g globin; within countries, the concentrations of AA-Val varied between 38.9 pmol/g globin (Denmark) and 74.3 pmol/g globin (United Kingdom). Among smokers, the mean concentration was 137 pmol/g globin, ranging from 70.3 pmol/g globin (France) to 167 pmol/g globin (Denmark). For GA-Val, in

non-smokers, the mean concentration was 43.3 pmol/g globin, varying between 30.8 pmol/g globin in Denmark and 67 pmol/g globin in the United Kingdom; among smokers, the mean was 101 pmol/g globin, ranging between 59.4 pmol/g globin in France and 119 pmol/g globin in the United Kingdom. A linear regression model with the log-transformed concentration of adducts as the dependent variable was used to assess its association with potential determinants, introduced as dependent variables in the model. In the non-smoker group, AA-Val was inversely related with alcohol consumption and body mass index (BMI) (both $P < 0.05$); for GA-Val, the inverse association was significant only for alcohol. Among smokers, AA-Val was inversely associated with BMI and education level and positively with the number of cigarettes smoked, whereas for GA-Val, only the association with education level and number of cigarettes smoked remained significant.

In Bavaria (Germany), 1008 volunteers aged 3–84 years were recruited in 2003–2004 (Kütting et al., 2009). Overall, the mean AA-Val concentration was 28 pmol/g globin in non-smokers and 83 pmol/g globin in smokers. Restricting the analysis to adults ($n = 898$), the mean (range) AA-Val levels were 83.2 pmol/g globin (8.2–331 pmol/g globin) and 27.1 pmol/g globin (3–68.1 pmol/g globin) for smokers and non-smokers, respectively. In a subsample of 91 non-smokers from this population (aged 6–80 years), in addition to AA-Val, GA-Val and urinary excretion of acrylamide metabolites were measured (Hartmann et al., 2008). The median (range) AA-Val and GA-Val concentrations were 30 pmol/g globin (15–71 pmol/g globin) and 34 pmol/g globin (14–66 pmol/g globin), respectively, with a median GA-Val/AA-Val ratio of 1.1 (0.4–2.7). For urinary metabolites, the median concentrations were 29 µg/l for AAMA and 7 µg/l for GAMA.

(b) Validation studies: relationship between AA-Val adducts and dietary acrylamide exposure

The Malmö Diet and Cancer cohort included subjects aged 45–73 years recruited between 1991 and 1996 in Sweden (Hagmar et al., 2005). A random sample of 142 subjects was selected, stratified by sex and smoking status within three groups: two of them were formed based on whether consumption of the main sources of acrylamide according to the FFQ was high ($n = 82$) or low ($n = 20$); the third was selected at random, without considering acrylamide exposure. AA-Val analysis was performed with gas chromatography (GC) coupled with MS/MS. Among the randomly selected subjects, the median level of AA-Val was 31 pmol/g globin in non-smokers, compared with 152 pmol/g globin in smokers. Among non-smokers, men with estimated high dietary acrylamide exposure had significantly higher AA-Val than did men with lower acrylamide exposure, but no such difference was seen for women. The opposite pattern was seen among smokers. In non-smokers, the AA-Val concentration varied by a factor of 5, whereas among smokers, it varied by a factor of 10. There was considerable overlap in AA-Val levels between different dietary groups.

Within the same population, Wirfält et al. (2008) estimated acrylamide dietary exposure using a method combining a 7-day menu book and a 168-item diet history questionnaire, applying the database of the Malmö Diet and Cancer cohort, based upon the Swedish National Food Administration's database for acrylamide

content in foods. In the random sample, the median exposure was 25 µg/day (range 10–62 µg/day). Pearson's correlations between AA-Val and acrylamide dietary exposure were $r = 0.36$ ($P = 0.002$) in smokers and $r = 0.43$ ($P < 0.001$) in non-smokers. In a linear regression model using the log-transformed adduct concentration as the dependent variable, the AA-Val variance (R^2) explained by all foods containing acrylamide was 0.13 among non-smokers and 0.25 among smokers; the latter increased to 0.32 when the amount of tobacco smoked was included in the model as a co-variable. This positive association among smokers was also consistent by sex, and there was a significant association in men among non-smokers, but there was no significant association among non-smoking women. Even when using dietary assessment methods with high relative validity, most of the variation in acrylamide remains unexplained.

Sixty randomly selected non-smokers and 60 smokers selected to ensure that a wide range of cigarette consumption levels was included in the study were recruited in Munich, Germany, in 2002 (Urban et al., 2006). Acrylamide dietary exposure was estimated using a 7-day diary and concentration data from the German Federal Agency for Consumer Protection and Food Safety. Urinary AAMA and GAMA concentrations were measured by liquid chromatography (LC) coupled with MS/MS, and AA-Val concentrations were measured by GC-MS. Among non-smokers, the mean concentrations were 73.1 and 15.9 ng/ml for AAMA and GAMA, respectively, whereas for smokers, they were 185.7 and 27.6 ng/ml. The concentrations of AA-Val were 27.6 and 81.8 pmol/g globin for non-smokers and smokers, respectively. There was a weak correlation between urinary excretion of acrylamide metabolites and acrylamide exposure at day 7, with $r = 0.313$ ($P = 0.015$) for AAMA and $r = 0.202$ ($P = 0.121$) for GAMA. No significant correlation was found between AA-Val concentrations and acrylamide exposure.

A group of 1033 volunteers was recruited from the Bavarian population in Germany (Kütting, Uter & Drexler, 2008). A total of 898 adults provided a blood sample and completed an FFQ including 19 acrylamide-related food items. Acrylamide exposure was estimated using data from the German Federal Institute of Risk Assessment; AA-Val concentrations were determined by GC-MS. Smokers had 3-fold higher mean AA-Val levels than did non-smokers. Among non-smokers, the Spearman rank correlations were $r_S = 0.178$ (0.089–0.268) in women and $r_S = 0.168$ (0.063–0.273) in men. In linear regression, AA-Val concentration was poorly associated with acrylamide exposure; together with sex and BMI, acrylamide exposure explained only 8% of the log-transformed AA-Val concentration.

The concentrations of AA-Val and GA-Val haemoglobin adducts were measured in a study involving 20 male and 33 female volunteers (mean ages of males and females were 45 ± 13 years and 41 ± 11 years, respectively) in Norway (Bjellaas et al., 2007b). Of these 53 volunteers, only 6 (2 males, 4 females) were smokers, who smoked between 7 and 21 cigarettes per day (median 14). The haemoglobin adduct concentrations were compared with dietary acrylamide exposure estimates derived from FFQs and a database listing the acrylamide content in various foods. The LOQs for the AA-Val and GA-Val adducts were 2 and 6 pmol/g globin, respectively. The median estimated dietary exposure of acrylamide was 13.5 µg/day (range 4.1–72.6 µg/day) in non-smokers and 18.3 µg/day

(range 7.8–32.0 µg/day) in the six smokers. Male non-smokers were calculated to have a higher median acrylamide exposure relative to non-smoking females: 16.6 µg/day (range 18.6–72.6 µg/day) and 12.8 µg/day (range 4.1–30.2 µg/day), respectively. Non-smokers had median AA-Val and GA-Val adduct concentrations of 36.8 pmol/g globin (range 17.9–65.5 pmol/g globin) and 18.2 pmol/g globin (range 6.7–45.6 pmol/g globin), respectively. In smokers, the values were 165.8 pmol/g globin (range 98.8–211 pmol/g globin) and 83.2 pmol/g globin (range 29.1–99.0 pmol/g globin), respectively. Using linear regression analysis, a statistically significant positive correlation was found between the AA-Val adduct concentration and the intake of chips/snacks and crispbread. However, GA-Val adduct levels did not correlate with consumption of any of the main food groups, nor did the AA-Val adduct or GA-Val adduct concentrations correlate with estimated total dietary exposure to acrylamide. There was also no correlation between adduct concentrations and 24 h urinary excretion of mercapturic acid metabolites of acrylamide and glycidamide in the same subjects reported in an earlier study (Bjellaas et al., 2007a). The molar ratio between GA-Val and AA-Val adducts varied considerably (from 0.12 to 1.08) between individuals, and this variability could not be explained by differences in acrylamide exposure alone.

In a similar study in the USA with the same outcome, Tran et al. (2010) compared the AA-Val and GA-Val levels reported in the 2008 National Health and Nutrition Examination Survey (NHANES) biomonitoring data with estimates of long-term dietary acrylamide exposure. The NHANES survey data reported on the concentrations of AA-Val and GA-Val haemoglobin adducts in 5306 participants aged 3 and older (1019 children aged 3–12; 561 males and 640 females aged 13–19; and 1408 males and 1678 females 20+ years of age). Owing to the absence of individual AA-Val and GA-Val measurements, the total number of subjects with FFQ, cotinine (a nicotine metabolite) and AA-Val was 4799; the total number with FFQ, cotinine and GA-Val was 4892. Long-term dietary exposure estimates were derived from FFQ responses and 24 h dietary recall data. The FFQ collected information on food consumed during the previous 12 months, including seasonal food consumption. A database listing the acrylamide content in various foods was generated from a number of food sample analyses that the USFDA had undertaken during 2002–2006 and then again in 2009. The mean estimate of dietary acrylamide exposure for the population aged 3 years and over was 0.44 µg/kg bw per day, whereas the 95th-percentile exposure was 1.15 µg/kg bw per day. For children aged between 3 and 12 years, the mean dietary acrylamide exposure was 0.86 µg/kg bw per day, and the 95th-percentile exposure was 2.39 µg/kg bw per day. The mean AA-Val and GA-Val concentrations in all subjects were 72.4 pmol/g globin and 72 pmol/g globin, respectively. There was a strong correlation between AA-Val and GA-Val levels (r^2 = 67%). However, when dietary acrylamide consumption was compared with AA-Val and GA-Val adduct levels using linear regression, the magnitude of the correlation was small ($r^2 \leq 3.3\%$; $P \leq 0.05$).

Finally, a validation study was carried out in women in the USA from the second Nurses' Health Study (NHS-II) (Wilson et al., 2009c). The study report is based on the analysis of blood samples and questionnaires for 342 women (36 smokers, 296 non-smokers; 10 of the original study population were excluded for

various reasons) from 1999. Dietary exposure was estimated from the 130-item FFQ and the USFDA's Exploratory Analysis of Acrylamide in Foods; AA-Val and GA-Val concentrations were measured by HPLC-MS/MS. The median time between blood sampling and completion of the FFQ was 8 months; a second blood sample was measured in 45 women to assess reproducibility, with a median time between samples of 23 months. Among smokers, median adduct levels were 97.3 pmol/g globin for AA-Val and 137.5 pmol/g globin for GA-Val; these values were 43.9 and 49.4 pmol/g globin, respectively, among never smokers (GA-Val to AA-Val ratio of 1.10). All validation studies were carried out among non-smokers, for whom mean dietary acrylamide exposure was 19.3 µg/day (0.27 µg/kg bw per day). The adduct levels of acrylamide and glycidamide correlated strongly with each other ($r = 0.69$). Reproducibility of adduct measurements was also high, with intra-class correlation coefficients of $r_I = 0.78$ for AA-Val and $r_I = 0.80$ for GA-Val. Correlations of dietary acrylamide exposure with adduct levels were $r = 0.29$ (0.17–0.40) for AA-Val, $r = 0.35$ (0.24–0.46) for GA-Val and $r = 0.34$ (0.23–0.45) for the sum of AA-Val and GA-Val. These correlations were estimated with log-transformed adduct levels as the dependent variable, adjusted by laboratory batch, age, BMI, energy intake and alcohol consumption. They were further corrected by within-person measurement error using the intra-class correlation coefficients obtained in the reproducibility study (de-attenuation). The latter had actually a small impact given the high reproducibility; however, adjustment by energy intake substantially improved validity owing to the reduction of within-person measurement error of intake. Adduct levels and acrylamide exposure are not expected to correlate perfectly, as adducts account for exposure, absorption and metabolism of acrylamide over the previous 4 months and are not specifically related to dietary acrylamide exposure. In a cross-classification by acrylamide exposure and adducts (AA-Val + GA-Val), 31% of subjects were classified in the same quartile.

(c) Validation studies: relationship between AA-Val adducts and occupational exposure to acrylamide

Although occupational exposure has been monitored mainly by measurement of urinary excretion of acrylamide metabolites, one publication has also used haemoglobin adduct measurement for such purposes (Jones et al., 2006). A total of 60 workers (23 smokers, 37 non-smokers) in the United Kingdom, including manufacturing, maintenance and laboratory workers, provided two blood samples 3 months apart. Environmental acrylamide exposure was measured during this period by a total of 285 airborne samples, analysed by HPLC with ultraviolet (UV) detection. The mean environmental exposure was 30 µg/m^3, about 10 times lower than the United Kingdom's maximum exposure level (MEL) of 300 µg/m^3; the maximum observed concentration of 282 µg/m^3 was slightly below the MEL. The mean AA-Val level over the 3-month period was 178.3 pmol/g globin. Airborne acrylamide concentrations and AA-Val concentrations correlated well ($r = 0.61$). Based upon the regression equation, long-term exposure at the MEL was predicted to give rise to a mean AA-Val concentration of 1550 pmol/g globin. Although there were no explicit controls (unexposed), 13 workers were exposed to acrylamide concentrations in air below 10 µg/m^3; these workers had mean AA-Val

concentrations of 32 pmol/g globin (8 non-smokers) and 51 pmol/g globin (5 smokers). The figure for non-smokers is in good agreement with reported background levels of AA-Val. The results of this study confirm that exposure to acrylamide at the workplace is associated with a high level of adducts; this points out the importance of excluding any occupational exposure from studies on dietary exposure.

(d) Validation studies: relationship between urinary excretion of acrylamide metabolites and short-term dietary acrylamide exposure

A German study described above (Urban et al., 2006) measured urinary acrylamide metabolites in addition to haemoglobin adducts and acrylamide exposure in 120 subjects. Among non-smokers, mean urinary concentrations were 73.1 and 15.9 µg/l for AAMA and GAMA, respectively, whereas for smokers, they were 185.7 and 27.6 ng/ml. There was a weak correlation between urinary excretion of acrylamide metabolites and acrylamide exposure at day 7, with $r = 0.313$ ($P = 0.015$) for AAMA and $r = 0.202$ ($P = 0.121$) for GAMA.

A validation study in Norway, the results of which for AA-Val and GA-Val have been described above (Bjellaas et al., 2007b), also reported results on the relationship between exposure collected in a 24 h dietary recall and acrylamide metabolites in a 24 h urine sample (Bjellaas et al., 2007a). The study included 6 smokers and 44 non-smokers. The time periods for urine collection were before 18:00, after 18:00 and morning urine. Urinary metabolites were analysed by LC-MS/MS. The median dietary exposures were 26 µg/day in smokers and 21 µg/day in non-smokers (see above). The median urinary concentrations were 32 µg/l and 3 µg/l for AAMA and GAMA, respectively, for non-smokers and 184 µg/l and 10 µg/l, respectively, for smokers. There was no correlation between total exposure to acrylamide and the excretion of acrylamide metabolites during 24 h. However, statistically significant correlations between dietary exposure before 12:00 and urinary excretion before 18:00 ($r = 0.36$, $P < 0.05$) and between estimated exposure after 18:00 and excretion of acrylamide in morning urine ($r = 0.32$, $P < 0.05$) were found. In a linear regression model, adjusted for weight and sex, intake of coffee, aspartic acid and starch were identified as independent predictors of urinary acrylamide excretion, with $R^2 = 0.49$ ($P < 0.001$).

Another Norwegian study aimed to explore three different methods to assess acrylamide dietary exposure in the Norwegian Mother and Child Cohort study (Brantsaeter et al., 2008). Participants were 119 healthy pregnant women who answered an FFQ, completed a 4-day weighted food diary and provided 24 h urine collection at the end of the food diary period. Acrylamide dietary exposure was estimated using data from the Norwegian Food Safety Authority and the database of the European Commission's Institute for Reference Materials and Measurements (IRMM). In addition to the FFQ and food diary, the mean exposure was also estimated by a probabilistic model using 2 days of the food diary. Estimated acrylamide exposures (median) were 33.7 µg/day for the FFQ and 28.5 µg/day for the food diary; expressed per unit body weight, the median exposures were 0.48 µg/kg bw per day for the FFQ, 0.41 µg/kg bw per day for the food diary and 0.42 µg/kg bw per day for the probabilistic model. The median excretion based on

urinary acrylamide metabolites (AAMA and GAMA) was 11.2 µg/day in non-smokers (n = 116) and 50.1 µg/day in smokers (n = 3). There was a positive relationship between dietary acrylamide exposure and urinary excretion data; using exposure expressed per unit body weight, the correlation coefficients were r = 0.26 (P = 0.005) for the FFQ and r = 0.34 (P < 0.001) for the food diary. Classification into quintiles by acrylamide exposure and excretion showed that 65% of participants were classified into the same or adjacent quintiles.

The relationship between dietary acrylamide exposure and urinary excretion has also been assessed in children in a study in Germany (Heudorf, Hartmann & Angerer, 2009). A random sample of 108 children, aged 5–6 years, was recruited during medical examinations. A spot urine sample was collected, and parents provided information on dietary habits and exposure to environmental tobacco smoke. Urinary acrylamide metabolites were analysed by LC-MS/MS. Mean concentrations were 57.8 µg/l for AAMA and 18.3 µg/l for GAMA. Children who regularly consumed french fries, chips and other fried potato products, as well as other fried foods and biscuits, had higher levels of acrylamide metabolites in their urine; the difference was significant only for french fries. Children who were exposed to environmental tobacco smoke at home did not exhibit higher levels of acrylamide metabolites in urine. This was further confirmed by the lack of correlation between acrylamide metabolites and cotinine levels in urine.

2.3.3 Epidemiological studies: cancer

(a) Occupational exposure

At the previous meeting of the Committee, the available epidemiological evidence was based on two prospective studies that examined the mortality patterns of workers exposed to acrylamide (Sobel et al., 1986; Collins et al., 1989). Sobel et al. (1986) reported the findings of a cohort of 351 male workers who had potential exposure to acrylamide in an industrial facility in Michigan, USA, among which 29 deaths from all causes were observed (38 expected). Another prospective study by Collins et al. (1989) included 8854 workers in three factories in the USA and one in the Netherlands, among which 2293 were considered as exposed to acrylamide (cumulative exposure >0.001 mg/m^3-year); there was a significant decrease in mortality from all causes. No significant associations were seen for overall cancer death or specific tumour sites. In 1999, an updated analysis of the latter study with extended follow-up and additional assessment of exposure (Marsh et al., 1999; Schulz et al., 2001) reported a significant excess mortality for pancreatic cancer (standardized mortality ratio [SMR] = 2.26, 95% confidence interval [CI] 1.03–4.29) for subjects with cumulative exposure above 0.3 mg/m^3-year. No consistent exposure–response relationship was observed, but further reanalysis combining two intermediate categories of exposure (0.001–0.029 and 0.03–0.29 mg/m^3-year) to avoid the small number of cases obtained a monotonically increasing risk with increasing exposure. One interesting feature of this study is the magnitude of exposure. As it has been calculated in a recent review (Exon, 2006), the average cumulative exposure at the workplace was 0.25 mg/m^3-year; assuming a daily inhalation of 10 m^3 of air and 100% absorption, the cumulative exposure in 1 year would be equal to 912.4 mg. This exposure is close to the estimated lifetime

exposure of 844 mg for a subject with average daily exposure to 0.033 mg over a 70-year lifespan.

More recently, Marsh and colleagues reported results of an 8-year extended update of the USA cohorts and a 21-year extended update of the cohort from the Netherlands (Marsh et al., 2007). This update includes 8508 workers from the USA cohorts and 344 (out of 346) from the Dutch cohort; the latter had not been considered in the previous update in 1999. There were 275 new deaths in the USA cohorts, yielding a total of 4650, and 71 deaths in the Dutch cohort. Historical reconstruction of acrylamide exposure allowed definition of the average intensity of exposure (mg/m^3) as the ratio of cumulative exposure (in mg/m^3-year) to duration of exposure (years). Compared with the general population, in the USA cohorts, there was a statistically significant 7% decrease in total mortality and a non-significant 4% increase in mortality by all malignant neoplasms. No association was observed for pancreatic cancer (SMR 0.94; 95% CI 0.70–1.22). The only significant association was for lung cancer mortality, with an SMR of 1.18 (95% CI 1.08–1.30). The increase in risk for lung cancer was confined to only one of the three plants and had been reported previously and attributed to muriatic acid exposure (Marsh et al., 1999). In the Dutch plant, there were significant decreases in both mortality by all causes and death by all neoplasms; a non-statistically significant excess mortality was reported only for tumours of the liver and thyroid, based upon two cases and one case, respectively. The risk for pancreatic cancer was further analysed in the USA cohorts by comparing the risk of death across groups with different levels of exposure within the same cohort (internal comparisons). Once adjusted for smoking and time since first acrylamide exposure, the relative risks (RR) were 1.59 (95% CI 0.46–5.51) and 1.78 (95% CI 0.50–6.37) for the highest categories of cumulative and average intensity of exposure, respectively, compared with the group with the lowest exposure; there was no dose–response relationship for any of the quantitative acrylamide exposure variables.

The earlier study in a chemical plant from Michigan, USA (Sobel et al., 1986), has been updated with additional vital status follow-up, extra workers and better acrylamide exposure assessment (Swaen et al., 2007). All but 2 of the 371 individuals from the original study were included in the update; in addition, 327 employees were identified whose job history indicated that they had worked in the acrylamide facility during the study period. Before the end of follow-up, 141 out of the total 696 workers were deceased, compared with an expected number of 172.1, with an SMR of 81.9 (95% CI 69.0–96.6); there was also a 5% (non-significant) decrease in mortality from all neoplasms. Five deaths by pancreatic cancer were reported, with an SMR of 222 (95% CI 72–519). Several tumour sites showed some excess mortality, but none of them reached statistical significance. The only cause of death with significant excess risk was diabetes, with an SMR of 289 (95% CI 138–531). To exclude different coding practices, an analysis was conducted using all the employees of the same company (unexposed to acrylamide) as the reference group, and the positive association persisted. However, given the rather prevalent risk factors of diabetes, it is possible that small differences in their distribution between the cohort and the reference group could explain this unexpected result. Furthermore, there was no exposure–response relationship. Thus, it was concluded that the increase in diabetes mortality is most likely not related to acrylamide.

Taken together, the extended analyses of these two occupational cohorts do not provide support for a relationship between acrylamide exposure at the workplace and cancer mortality. The updated results revealed considerably lower relative risks of mortality from pancreatic cancer (controlled for smoking) than in previous analyses of the same cohorts; furthermore, the lower 95% confidence limits of the updated estimates were below 1, so that association with death by pancreatic cancer was not statistically significant.

(b) Dietary exposure

In the previous monograph (Annex 1, reference *177*), the only information available that considered the risk associated with dietary exposure to acrylamide came from case–control studies originally designed to assess the potential cancer risk of dietary factors other than acrylamide. In a series of hospital-based case–control studies in Italy and Switzerland (Pelucchi et al., 2004) and two Swedish population-based studies (Mucci et al., 2003, 2004), no association was found between indicators of acrylamide exposure and tumours of the oral cavity, pharynx, larynx, oesophagus, colon and rectum, breast, ovary, urinary bladder and kidney. An update of the Italian-Swiss study with better assessment of acrylamide dietary exposure did not modify the pattern of negative results (Pelucchi et al., 2006, 2007). Although the Swedish studies are of relatively high quality from the methodological point of view, some limitations preclude making definitive conclusions. The need for a better design, mainly using a prospective approach, complete inclusion of potential confounders and better assessment of dietary acrylamide exposure, probably using biomarkers, was acknowledged.

(i) Prospective studies based on estimated dietary exposure to acrylamide

Several prospective studies have reported results on the relationship between estimated dietary exposure to acrylamide and tumours at a number of different sites. Although papers are usually site specific, all publications arising from the same prospective study share common features. Thus, in this section, the results are grouped by study of origin; first, the main characteristics of each cohort are described, and then all the papers using data from this cohort are reported in detail.

The Netherlands Cohort Study (NLCS)

The Netherlands Cohort Study on diet and cancer (NLCS) began in 1986 with the enrolment of 58 279 men and 62 573 women aged 55–69 years. Follow-up for cancer detection was by means of record linkage with regional cancer registries and the Netherlands Pathology Registry. At baseline, all participants completed an FFQ with 150 food items, including all relevant sources of acrylamide. Estimates of acrylamide exposure were obtained by applying the acrylamide content in foods from the analyses made by the Dutch Food and Consumer Product Safety Authority and the IRMM database to the reported food consumption data. Analyses of the NLCS are based upon a case–cohort approach: a subcohort of 5000 men and women was randomly sampled from the entire cohort at baseline, taken

as a "control" group for each tumour site. In each analysis, all incident cases of the tumour of interest and the subcohort are used; however, as specific exclusions can be applied in each analysis, the number of subjects from the subcohort may vary across different papers.

Hogervorst et al. (2007) reported results in relation to some hormone-related cancers in women. After 11.3 years of follow-up, and taking into account exclusions for several reasons, the analyses were based upon 1796 women from the subcohort and 221 cases of endometrial cancer, 195 cases of ovarian cancer and 1350 cases of breast cancer. On average, the subcohort had a daily exposure to 21 µg of acrylamide (0.32 µg/kg bw per day); the median exposure was 17.9 µg/day, and medians of the first and fifth quintiles were 9.5 and 36.8 µg/day, respectively. After adjustment for relevant confounders, the hazard ratio (HR) and 95% confidence intervals for an increase of 10 µg of acrylamide per day were 1.04 (0.91–1.19) for endometrial cancer, 1.11 (0.99–1.25) for ovarian cancer and 0.99 (0.92–1.06) for breast cancer. For ovarian cancer, the risk was higher among never smokers (HR 1.17; 95% CI 1.01–1.36). The HR of the highest versus lowest quintile for never smokers was also significant for endometrial cancer (HR 1.99; 95% CI 1.12–3.52).

After a slightly longer follow-up (13.3 years), another paper reported results based upon 339 cases of renal cell carcinoma, 1210 cases of urinary bladder cancer and 2246 cases of prostatic cancer (Hogervorst et al., 2008a). The subcohort for this analysis included 4232 subjects, 2011 of whom were men. On average, the subcohort had a daily exposure to 21.8 µg of acrylamide (0.30 µg/kg bw); the corresponding values for men and women were 22.5 µg (0.29 µg/kg bw) and 21.0 µg (0.32 µg/kg bw), respectively. After adjustment for relevant confounders, there was a marginally significant increase in risk for renal cell cancer, with an HR of 1.10 (95% CI 1.01–1.21) for an increase of 10 µg of acrylamide exposure per day. This association was no longer significant when the analysis was restricted to never smokers. No association was observed for bladder cancer or prostatic cancer, with HRs close to unity.

The relationship of acrylamide exposure with gastrointestinal cancer was assessed after 13.3 years of follow-up (Hogervorst et al., 2008b). This work included a subcohort of 4045 subjects, 2190 cases of colorectal cancer, 563 cases of gastric cancer, 349 cases of pancreatic cancer and 216 cases of oesophageal cancer. The average daily exposure to acrylamide in the subcohort was 21.8 µg (0.30 µg/kg bw). There was no association with any of these tumour sites in analyses controlling for relevant confounders. Analyses by sub-localization were also carried out (colon and rectum separately, and cardia or non-cardia gastric cancers); all the analyses were also performed among never or former smokers, but without revealing significant associations.

After 13.3 years of follow-up, 2231 cases of lung cancer were identified in the same cohort (Hogervorst et al., 2009a). In the subcohort of 4438 subjects, the average daily exposure to acrylamide was 22.6 µg (0.29 µg/kg bw) for men and 21.0 µg (0.32 µg/kg bw) for women. Subgroup analyses were carried out according to smoking status and histological type of tumour. There was no association between estimated dietary exposure to acrylamide and lung cancer in men. In

women, there was a statistically significant inverse association, with an HR of 0.82 (95% CI 0.69–0.96) for 10 µg/day of acrylamide exposure, stronger among never smokers (HR 0.78; 95% CI 0.61–1.00). The decrease in risk was restricted to subjects with adenocarcinoma, with an HR of 0.61 (95% CI 0.45–0.81), whereas no association was observed for squamous, small cell or large cell tumours.

In total, 259 cases of primary brain cancer were ascertained during a follow-up of 16.3 years (Hogervorst et al., 2009b). After some exclusions, 238 cases were compared with a subcohort of 4438 subjects. Within the cases, 191 were microscopically verified; 168 were astrocytic glioma, including 148 of high grade. The average daily exposure to acrylamide in the subcohort was 21.8 µg (0.30 µg/kg bw). After adjustment for relevant confounders, no association was found for any type of brain cancer; all the estimates were slightly below 1, none of them being statistically significant.

Finally, dietary acrylamide exposure was analysed in relation to head–neck and thyroid cancers after 16.3 years of follow-up (Schouten et al., 2009). After some exclusions, 357 cases of head–neck cancer and 36 cases of thyroid cancer were compared with a subcohort of 4232 subjects. On average, the subcohort had a daily exposure to 21.8 µg acrylamide (0.30 µg/kg bw); the corresponding estimates by sex were 22.5 µg (0.29 µg/kg bw) for men and 21.1 µg (0.32 µg/kg bw) for women. Subgroup analyses were carried out by sex and smoking status and for specific site within head–neck tumours: oral cavity, pharynx (oro- and hypopharynx) and larynx. For most comparisons, no association was found with dietary acrylamide exposure. The only statistically significant association observed was an excess risk of oral cavity cancer for non-smoking women (21 cases), with an adjusted HR of 1.28 (95% CI 1.01–1.62) for an increase of 10 µg/day of acrylamide exposure.

The Swedish Mammography Cohort (SMC)

The Swedish Mammography Cohort (SMC) was established in 1987–1989 in Västmanland County and in 1988–1990 in Uppsala County in central Sweden. At baseline, a complete questionnaire was obtained for 66 651 women born between 1914 and 1948 (74% of the source population), 39 226 of whom completed a second questionnaire in 1997. After excluding those with missing or implausible information and prevalent cancer cases, the cohort included about 61 000 women, approximately 36 000 of whom completed a second questionnaire. Ascertainment of cancer cases was achieved by linkage of the cohort with the national and regional Swedish cancer registries. The dietary assessment tool was an FFQ with 67 food items (96 in the second questionnaire). Acrylamide dietary exposure was estimated using information from the Swedish National Food Administration and other published data on the acrylamide content of foods in Sweden.

In the follow-up of the SMC to the middle of 2003 (13.4 years on average), 504 cases of colon cancer and 237 cases of cancers of the rectum were identified (Mucci, Adami & Wolk, 2006). The average daily exposure to acrylamide in this cohort of women with a mean age of 54 years was 24.6 µg (0.38 µg/kg bw); the median exposure was 24.1 µg/day, and the means of the first and fifth quintiles were 12.8 and 37.9 µg/day, respectively. After adjustment for relevant confounders, no

association was seen for colorectal cancer. Point estimates for the HRs of the highest compared with the lowest quintiles of acrylamide exposure were 0.9 and 1.0 for colon and rectal cancers, respectively; none were statistically significant.

Larsson and colleagues (Larsson, Akesson & Wolk, 2009a,b,c) examined the risk of three hormone-related cancers in the SMC, followed up until the end of 2007 (17.5 years on average). The cohort for the three studies was almost the same: women had a mean age of 56.4 years. The average daily exposure to acrylamide was 24.6 µg (0.38 µg/kg bw). All the analyses included relevant confounding factors for the tumour site of interest. A total of 2952 incident cases of invasive breast cancer were diagnosed, among which 2062 had information available on estrogen and progesterone receptors (Larsson, Akesson & Wolk, 2009a). Subgroup analyses were carried out according to estrogen/progesterone receptor status, and a complementary analysis was done using information from the second questionnaire. No statistically significant associations were found. For the whole group, the HR for the highest compared with the lowest quartile was 0.91 (95% CI 0.80–1.02). The analysis of ovarian cancer included 368 incident cases of invasive epithelial ovarian tumours (Larsson, Akesson & Wolk, 2009b). There was no association between acrylamide exposure and the risk of ovarian cancer. The HR for the highest compared with the lowest quartile was 1.17 (95% CI 0.72–1.89); the effect was more prominent among smokers, but still non-significant. Finally, 687 cases of endometrial carcinoma were diagnosed during follow-up (Larsson, Akesson & Wolk, 2009c). No association with acrylamide exposure was found; the HR for the highest compared with the lowest quartile was 1.12 (95% CI 0.79–1.59); the effect was higher among never smokers, but again non-significant.

The Cohort of Swedish Men (CSM)

Parallel to the second phase of the SMC, the Cohort of Swedish Men (CSM) was established in 1997 in Västmanland and Orebro counties, including men aged 45–79 years. After some exclusions, the cohort was composed of 45 306 men. The methods for case ascertainment and estimation of dietary acrylamide exposure were the same as for the SMC, using the second questionnaire with 96 food items. The potential risk of dietary acrylamide exposure in relation to prostate cancer was assessed in this cohort (Larsson et al., 2009d). In total, 2696 cases of prostate cancer were identified up to the end of 2007 (mean follow-up of 9.1 years), among which 1088 were localized cases and 951 advanced cases (for the remaining, the stage at diagnosis was unknown). The average daily acrylamide exposure in this cohort was 36.1 µg, with a median daily exposure of 35.4 µg; the corresponding medians for the first and fifth quintiles were 23.7 and 49.8 µg/day, respectively. Subgroup analyses were performed according to the stage and separately for never smokers, taking into account relevant confounders. No evidence was found that acrylamide exposure was associated with prostate cancer. The HR of the highest compared with the lowest quintile was 0.88 (95% CI 0.70–1.09); the estimate was 1.07 for localized cases, 0.98 for advanced cases and 0.91 for never smokers, none of them being statistically significant.

Swedish Women's Lifestyle and Health Cohort (SWLHC)

The Swedish Women's Lifestyle and Health Cohort (SWLHC) is actually the Swedish part of the Norwegian-Swedish Women's Lifestyle and Health Cohort. In Sweden, a sample of women born between 1943 and 1962 (aged 30–49 years) was randomly selected from the Swedish Central Population Registry and Statistics in 1991. In total, 49 259 women (51.3% of the selected group) were recruited. Case ascertainment was by means of linkage with population-based cancer registries. Mucci, Sandin & Magnusson (2005) estimated the dietary acrylamide exposure and its potential relationship with breast cancer in 43 404 women followed until 2002. During this period (mean follow-up of 11.3 years), 667 incident breast cancers were identified. Dietary acrylamide exposure was estimated using a semiquantitative FFQ, including most relevant sources of acrylamide, combined with data from the Swedish National Food Administration. The mean daily acrylamide exposure in the cohort was 25.9 µg; the average exposures for the first and fifth quintiles were, respectively, 12 µg/day and 44 µg/day. Less than 1.5% of participants consumed more than 1 µg/kg bw per day. Overall, there was no association with breast cancer risk. Compared with the lowest quintile, the highest had an adjusted HR of 1.19 (95% CI 0.91–1.55); there was no significant trend in risk across quintiles of acrylamide exposure.

Nurses' Health Study II (NHS-II)

The NHS-II is a prospective cohort of female registered nurses aged 25–42 years at the start of the study in 1989. Follow-up questionnaires were sent biennially to update information on lifestyle and health. The usual diet was assessed by means of an FFQ with over 130 food items, and acrylamide exposure was estimated using data from the USFDA and additional data from the Swedish National Food Administration. The paper of interest (Wilson et al., 2009a) focused on women who completed the first questionnaire in 1991 and was restricted to premenopausal women; after some exclusions, the cohort for analysis included 90 628 women with a mean age of 36 years. The average daily acrylamide exposure was 20.2 µg (0.32 µg/kg bw); the corresponding means for the first and fifth quintiles were 10.8 µg/day and 37.8 µg/day, respectively. During a follow-up of 14 years (until the middle of 2005), 1179 cases of breast cancer were identified in total. The exposure to acrylamide was not associated with risk of premenopausal breast cancer, after accounting for potential confounders. The HR comparing the highest with the lowest quintile was 0.92 (95% CI 0.76–1.11); there was no significant trend in risk across quintiles of acrylamide exposure. The same pattern was observed in subgroups analysed according to smoking habits or extrogen/progesterone receptor status.

(ii) Epidemiological studies based on acrylamide–haemoglobin adducts

Only two studies have been published recently using acrylamide–haemoglobin adducts (AA-Val) as indicators of acrylamide exposure. One is a case–control study, and the other is nested in a prospective cohort.

The Cancer of the Prostate in Sweden (CAPS) is a population-based case–control study carried out in four of the six areas covered by regional cancer registries

(Wilson et al., 2009b). Cases were subjects aged 35–79 years diagnosed with cancer of the prostate in the four participating registries during 2001 and 2002. Controls were randomly selected from the Swedish Population Registry, frequency matched to cases by region of residence and age. The participation rates were 74% for the cases and 67% for the controls. After some exclusions because of missing or implausible information, 1499 cases and 1118 controls were included, with mean ages of 67 years and 68 years, respectively. The usual diet was assessed by means of a 261-item FFQ, and acrylamide exposure was estimated using information from the Swedish National Food Administration database. As a biomarker of exposure, AA-Val concentrations in blood were measured from a random sample of 170 cases and 161 controls, all of them non-smokers. Among controls, the mean daily exposure to acrylamide was 44.5 µg (0.56 µg/kg bw), with a range of 8–125 µg (0.08–1.59 µg/kg bw). The mean adduct level was 53.7 pmol/g globin, with medians for the first and fourth quartiles of 32 pmol/g globin and 56 pmol/g globin, respectively. The correlation between acrylamide exposure and AA-Val concentration was 0.19 (0.08–0.29), but increased to 0.35 after adjusting for calories; this improvement in the correlation is due to the reduction of the within-person measurement error. There was no association between acrylamide exposure and the risk of prostate cancer, adjusted for relevant confounders: the RRs for a 10-unit increase in level were 1.00 (95% CI 0.86–1.16) for AA-Val and 0.99 (95% CI 0.92–1.06) for acrylamide exposure. The data on the AA-Val levels suggest that they have a skewed distribution, with many high values driving up the mean. Furthermore, the RRs for a constant increase in the exposure (i.e. 10 units) assume a constant increase in risk along the exposure range, which is not the case; however, the RR of the highest compared with the lowest quartile was also close to 1. Quartiles are based upon the ranking of subjects according to the exposure, and they are not influenced by the skewness of the distribution. The pattern did not change according to the stage of tumour at diagnosis (advanced or localized, high/low prostate-specific antigen level, high/low-grade disease according to Gleason index).

The only prospective study using adducts as biomarkers of acrylamide exposure published to date is a nested case–control analysis within the Danish Diet, Cancer and Health (DDCH) study (Olesen et al., 2008). Women resident in Copenhagen and Aarhus with ages between 50 and 64 years were invited to participate in the study between 1993 and 1997. In total, 29 875 women were enrolled (37% of the target). Identification of cases was obtained by linkage with the Danish Cancer Registry. The analysis presented focused on postmenopausal women. Until the end of 2000 (average follow-up of 4 years), 434 cases of post-menopausal breast cancer were detected; one control was selected for each case, alive and free of the disease at the time of diagnosis, matched by age, menopausal status and hormone replacement therapy use. After exclusion of subjects with missing information or without a blood sample, 372 case–control pairs with median age of 57 years remained for analysis. Haemoglobin adducts of acrylamide (AA-Val) and glycidamide (GA-Val) were determined by LC-MS. Among controls, the median concentrations (with 5th and 95th percentiles) were 47 (18–205) pmol/g globin for AA-Val and 28 (9–99) pmol/g globin for GA-Val. The median concentrations were significantly higher in smokers than in non-smokers: 122 versus

35 pmol/g for AA-Val and 60 versus 21 pmol/g for GA-Val. Overall, there was no significant association with postmenopausal breast cancer: the HRs for a 1-unit increase in the adduct concentration (in $\log_{10}$ scale) were 1.05 (95% CI 0.66–1.69) and 0.88 (95% CI 0.51–1.52) for AA-Val and GA-Val, respectively, once adjusted for potential confounders. There was a significant increase in risk for AA-Val among smokers, only after adjusting for the amount and duration of tobacco smoked at baseline, with an HR of 3.1 (95% CI 1.0–9.7); this effect was even stronger when the analysis was restricted to estrogen receptor positive (ER+) cases, with an HR of 4.9 (95% CI 1.2–2.0). However, the association between breast cancer and smoking is not completely understood, and therefore this result is not easily interpreted. It must be noted that the adduct concentration is expressed in $\log_{10}$ scale, so the HRs reported correspond to a 10-fold increase in the dose; this means that these HRs actually reflect the increase in breast cancer risk between the 5th and the 95th percentiles of the AA-Val concentration.

3. ANALYTICAL METHODS

3.1 Chemistry

Acrylamide (CH_2=CH-CO-NH_2; 2-propenamide), a colourless and odourless crystalline powder with a melting point of 84.5 °C and a high boiling point of 136 °C (at 3.3 kPa), is soluble in water, acetone and ethanol (Smith, Prues & Oehme, 1996).

3.2 Description of analytical methods

3.2.1 Common and established methods

It can be concluded from recent reviews and proficiency test reports that isotope dilution LC-MS/MS and GC-MS(/MS) are most widely used for the quantification of acrylamide in heat-treated foods (Wenzl, de la Calle & Anklam, 2003; Zhang, Zhang & Zhang, 2005; Wenzl et al., 2006, 2009; Wenzl, Lachenmeier & Gökmen, 2007; Karasek, Sziláguy & Wenzl, 2008; Zhang, Ren & Zhang, 2009). LC-based methods enable determination of acrylamide as such, whereas GC-based methods generally include derivatization of acrylamide prior to further workup and analysis. Isotope dilution, using isotope-labelled acrylamide as an internal standard, is generally needed to adjust for ion suppression in LC-MS/MS and for variable derivatization yields in GC-MS(/MS) methods, as well as for general workup losses.

3.2.2 Screening tests

To achieve rapid screening, high throughput and low cost, some biological methodologies have been considered, including genetic techniques and enzyme-linked immunosorbent assay (ELISA). The small size of acrylamide (71 daltons) has defeated attempts to raise useful antibodies for screening immunoassays. Recently, 3-mercaptobenzoic acid and *N*-acryloxysuccinimide derivatives of acrylamide were used to obtain effective immunogen compounds that were used as a basis for the development of immunoassays for the determination of acrylamide in food extracts

after derivatization (Preston, Fodey & Elliott, 2008; Zhou et al., 2008). Hasegawa et al. (2007) developed a biosensor (MJCU017) for screening analysis of acrylamide in common foods, such as powdered green tea, coffee, tomato juice and sports drinks. The accuracy and sensitivity of those test methods still need to be optimized, and their analytical results should be confirmed by other robust methods.

3.2.3 Validated methods

A European interlaboratory study was conducted to validate one GC-MS and one LC-MS/MS analytical procedure for the determination of acrylamide in bakery products (crispbreads, biscuits) and potato products (chips), within a concentration range from about 20 µg/kg to about 9000 µg/kg. The LC-MS/MS method showed superior performance compared with the GC-MS method and was deemed fit for purpose (Wenzl et al., 2006). Wenzl et al. (2009) further subjected a slightly modified method for the determination of acrylamide in roasted coffee to method validation by collaborative trial. Method performance parameters satisfied internationally accepted criteria.

In China, national standard GB/T 5009.204-2005, a GC-MS method for the determination of acrylamide in food, partially based on previous methods from the USFDA, was published in 2005 (Ministry of Health, People's Republic of China, 2005). It was later developed (GB 5009.204-2010) to include an isotope dilution LC-MS/MS analytical procedure (Zhao et al., 2005), and then a collaborative trial validation of this new updated standard GB 5009.204 was performed by seven Chinese laboratories (Zhao et al., 2005; Ministry of Health, People's Republic of China, 2010).

3.2.4 Analytical quality control

The need for a certified reference material of acrylamide in a food matrix is emphasized by competent authorities as a tool to improve comparability and ensure the accuracy and traceability of analytical results. Such materials are available today from at least three sources: IRMM (http://irmm.jrc.ec.europa.eu), German Federal Institute for Materials Research and Testing (http://www.bam.de) and the United Kingdom's Food Analysis Performance Assessment Scheme (FAPAS) (http://www.fapas.com) (Dabrio et al., 2008; Koch et al., 2009). Also, proficiency tests have been and are still being offered (e.g. by FAPAS) in order to assess the capability of analytical laboratories and methods.

3.2.5 Developments in pretreatment

(a) Extraction

Water is most commonly used to extract acrylamide from foods, but polar solvents are sometimes used (Karasek, Sziláguy & Wenzl, 2008). Incomplete extraction is a possible cause of erroneous results in the analysis of acrylamide. This might occur when the food is not sufficiently macerated or when a short extraction time or low extraction temperature is used, especially when these conditions are combined (Petersson et al., 2006). Formation of acrylamide during

the extraction procedure is another possible error source, which is an easily neglected factor (Hoenicke et al., 2004). Other possible pitfalls during the extraction procedure include contamination of acrylamide from labware such as syringe filters and ultrafilters (Fohgelberg et al., 2005) and thermal degradation of acrylamide. Recently, some studies found that acrylamide can significantly co-evaporate with water (Rufián-Henares & Morales, 2006; Chu & Metcalfe, 2007). One study suggested that high-pH extraction would release "hidden" acrylamide from the food matrix (Eriksson & Karlsson, 2006). Later work showed that this high-pH effect was probably due to the formation of acrylamide from Maillard reaction intermediates and should thus be regarded as an extraction artefact (Goldmann et al., 2006; Perez Locas & Yaylayan, 2008).

(b) Cleanup

The use of multiple-cartridge solid-phase extraction (SPE) is widespread—for example, the combination of ENV+ (a crosslinked polystyrene-based polymer) and Strata-X-C (a cation exchange polymer) (Bermudo et al., 2008). Layered SPE cartridges have also been used to simplify the cleanup procedures. Soares, Cunha & Fernandes (2006) found that adding a layer of C_{18} sorbent to the Isolute Multimode sorbent with a ratio of 1:3 was ideal to eliminate the most relevant contaminants in some complex food matrices, such as coffee. Single SPE cartridges, such as Isolute Multimode (a hydrophobic interaction sorbent) (Mizukami et al., 2006; Rufián-Henares, Delgado-Andrade & Morales, 2006) and Oasis HLB (Zhang et al., 2005), have also been used, as have other techniques, including solid-phase microextraction (El-Ghorab, Fujioka & Shibamoto, 2006) and matrix solid-phase dispersion (Fernandes & Soares, 2007).

Mastovska & Lehotay (2006) developed a fast and easy combined solvent extraction and cleanup procedure. Homogenized food samples were extracted with a mixture of hexane, water, acetonitrile, magnesium sulfate and sodium chloride. Water facilitated the extraction of acrylamide, hexane defatted the sample and the salt combination induced separation of the water and acetonitrile layers and forced the majority of acrylamide into the acetonitrile layer. The upper hexane layer was discarded, and an aliquot of the acetonitrile extract was cleaned up by dispersive SPE. The final extracts were analysed either by LC-MS/MS or by GC-MS.

3.2.6 Developments in instrumental analysis

(a) GC-MS

GC-based methods usually include derivatization of acrylamide, which is performed with hydrobromic acid and saturated bromine (Br_2) solution in many laboratories. GC-MS methods with or without derivatization of acrylamide were systematically reviewed by Castle & Eriksson (2005). Recently, the derivatization method was improved by using potassium bromate and potassium bromide in an acidic medium (Zhang et al., 2006). The use of these reactants is more convenient and safe, and the reaction is performed in about 30 min at cold storage temperature with good reproducibility.

Reliable analysis of underivatized acrylamide is also possible by GC-MS(/MS), but great care must then be taken to remove asparagine and sugars from the extract in order to avoid acrylamide formation in the heated injection port of the GC (Dunovská et al., 2006). Furthermore, the compound 3-hydroxypropionitrile may be coeluted with acrylamide, causing falsely high acrylamide values (Biedermann & Grob, 2008). The problem could be solved by using a more polar column (Carbowax 1000). Alternatively, it was possible to get 3-hydroxypropionitrile eluted after acrylamide using a high molecular weight Carbowax combined with adequate tuning of the separation conditions.

(b) LC-MS/MS

The LC-MS/MS methods are, in principle, based on the method published by Rosén & Hellenäs (2002), further modified in various reports (Zhang, Zhang & Zhang, 2005; Wenzl, Lachenmeier & Gökmen, 2007). For the chromatographic part, Rosén, Nyman & Hellenäs (2007) comparatively investigated the effect of different solid phases on the chromatographic retention of acrylamide. The best retention was achieved with a phase comprising porous graphitic carbon (Hypercarb) using water as the mobile phase.

A majority of established methods employ electrospray ionization (ESI). Marín et al. (2006) instead recommended the Ion Sabre atmospheric pressure chemical ionization as the interface and obtained improved sensitivity for acrylamide (LOD 0.03 µg/l) and less matrix effects compared with ESI.

For improvement of the chromatographic step, ultraperformance liquid chromatography (UPLC) coupled to MS/MS was exploited (Zhang et al., 2007). Compared with routine LC-MS/MS, the UPLC-MS/MS method supplies a rapid procedure for the quantification of acrylamide with a run time of only 3 min. Furthermore, the hybrid particles used in UPLC columns often showed unique selectivity compared with conventional HPLC packings (Churchwell et al., 2005). The advantage of the UPLC method is also related to an increase in the run efficiency and resolution, because the particles with 1.7 µm size in UPLC columns allow the chromatographic analysis under much higher pressure and faster flow rate.

The formation of acrylamide in foods has been shown to correlate with pre-processing levels of asparagine, fructose and glucose. Previous studies used HPLC and an amino acid analysis kit to quantify the contents of sugars and asparagine, respectively (Knol et al., 2005). Nielsen et al. (2006) developed an LC-MS/MS method for simultaneous analysis of acrylamide (LOD 0.013 mg/kg), asparagine (LOD 1.8 mg/kg), glucose (LOD 96 mg/kg), fructose (LOD 552 mg/kg) and sucrose (LOD 23 mg/kg) in bread.

(c) Other techniques

Microemulsion electrokinetic chromatography, a capillary electrophoresis (CE) technique, has been applied for the determination of acrylamide without derivatization (Bermudo et al., 2004; Zhou et al., 2007). A lower LOD was obtained for CE after derivatization of acrylamide with 2-mercaptobenzoic acid to obtain an

ionic compound (LOD 0.07 µg/ml) (Bermudo et al., 2006a). To further improve LODs and to spread the applicability of the method over a wide range of samples, field amplified sample injection (FASI) was proposed (Bermudo et al., 2006b). Based on the FASI-CE technique, Bermudo et al. (2007) demonstrated the applicability of CE coupled to MS/MS for the analysis of acrylamide in foodstuffs and obtained good linearity and precision. Besides the FASI-CE method, a non-aqueous CE method (Bakan & Erim, 2007) and a relative field amplified sample stacking technique (Tezcan & Erim, 2008) were also developed and reported as simple, rapid and inexpensive choices.

Chromatographic methods with conventional detection techniques, generally cheaper and easier to operate than MS, have been developed for the determination of acrylamide in some foods. For LC analysis, UV (Paleologos & Kontominas, 2005; Wang et al., 2008) or diode array detection (Geng, Jiang & Chen, 2008; Gökmen et al., 2005) at wavelengths of 210 and 225 nm was applied. Pulsed electrochemical detection was also used (Casella, Pierri & Contursi, 2006). For GC analysis, electron capture detection has been used as an alternative to MS (Zhang et al., 2006; Zhu et al., 2008). Besides LC and GC techniques, a thin-layer chromatography method with fluorescence detection after derivatization with dansulfinic acid was also reported (Alpmann & Morlock, 2008).

4. EFFECTS OF PROCESSING

4.1 Heat-induced formation of acrylamide in foods

4.1.1 Formation from asparagine and sugar by Maillard reaction

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 have a positive influence on the reactions.

The main route of acrylamide formation in heated food is the Maillard reaction. Upon heating, free asparagine reacts with reducing sugars or other carbonyl compounds to form acrylamide. Alternative routes of formation have been demonstrated, but the relative importance of these routes under different conditions in food matrices has not been fully elucidated. In model systems, it was shown that, depending on pH, temperature and moisture level, the Schiff base, which is the first interaction product between reducing sugars and amino acids, can undergo Amadori rearrangement, the Strecker reaction or cyclizations to generate nitrogen-containing heterocyclic compounds. However, before these transformations, the Schiff base may undergo isomerization reactions, which further increase the Maillard reaction products. One of these isomerization reactions can form a non-stabilized azomethine ylide, through oxazolidin-5-one formation (Chu & Yaylayan, 2009).

In a recent study, experiments were done with two precursors of acrylamide: *N*-(D-glucos-1-yl)-3′-aminopropionamide or *N*-(1-deoxy-D-fructos-1-yl)-3′-aminopropionamide, which can form acrylamide either directly or through the

formation of 3-aminopropionamide (3-APA). In both dry and wet conditions, *N*-(D-glucos-1-yl)-3′-aminopropionamide gave the highest acrylamide yield (Perez Locas & Yaylayan, 2008). These outcomes have to be corroborated in food, as matrix and moisture content play important roles in the processes mentioned above.

4.1.2 Formation from oil degradation products

Although no correlation between fatty acid composition and acrylamide formation could be found (Mestdagh et al., 2005), it was suggested that additional pathways from lipids may exist (Gertz & Klostermann, 2002; Becalski et al., 2003; Gertz, Klostermann & Kochhar, 2003; Yasuhara et al., 2003; Rüdiger, 2004; Ehling, Hengel & Shibamoto, 2005). Earlier studies show that acrylamide can be formed from acrylic acid upon heating in the presence of ammonia (Yasuhara et al., 2003), but this could not be verified by Mestdagh et al. (2005). This was probably due to differences in the time of heating, 7 min at 170 °C instead of 30 min. Although several oil degradation products were tested by Mestdagh et al. (2005), only the heated model system containing acrolein together with asparagine showed a significant increase in acrylamide formation. The formation mechanism was then likely to be the Maillard reaction, with acrolein, instead of sugar, being the carbonyl reaction partner to asparagine. However, the contribution of acrolein to the overall formation of acrylamide appeared to be negligible in the presence of a reducing sugar, indicating that in foodstuffs, the importance of acrolein and other oil degradation products is probably small (Mestdagh et al., 2008).

4.1.3 Formation from 3-aminopropionamide (3-APA)

Zyzak et al. (2002) reported 3-APA as being an intermediate in acrylamide formation from the reaction between asparagine and reducing sugar. The presence, as a transient intermediate, of relatively high amounts of 3-APA was later detected in several heated foods, such as roasted coffee and cocoa, and in popcorn (Granvogl et al., 2007).

However, 3-APA can also be formed biochemically in non-heated raw potato through enzymatic decarboxylation of asparagine (Granvogl et al., 2004). The efficacy with which 3-APA is transformed into acrylamide during heat treatment is more than 12-fold higher than that associated with the generation of acrylamide from asparagine (Granvogl & Schieberle, 2006). In addition to raw potato, 3-APA has also been found in small amounts in olives (Amrein et al., 2007), cheese (Granvogl & Schieberle, 2006) and cocoa (Granvogl & Schieberle, 2007). No correlation was found between 3-APA and acrylamide in potato crisps (USA = chips) (Amrein et al., 2007), but further studies are needed before its possible importance as an alternative acrylamide precursor can be evaluated.

4.1.4 Formation from wheat gluten

The formation of acrylamide through pyrolysis of alanine-containing protein is suggested as an electrocyclic domino reaction in which cinnamic acid is also formed. This pathway requires higher temperatures for acrylamide formation compared with the formation from asparagine and reducing sugars. A 20% increase

in acrylamide formation was reported by Claus et al. (2006) when sugar and asparagine-free gluten were added to dough samples. This finding is, however, in contrast with those of an earlier study in which the addition of gluten to crackers resulted in a decrease in acrylamide content (Levine & Smith, 2005). Since these findings on acrylamide formation from wheat gluten in 2006, no additional research has been published on this alternative pathway. More studies would be of interest, as this pathway could set an upper limit for how efficient mitigation can be achieved by asparagine removal (e.g. by using asparaginase in bakery products).

4.1.5 Formation in olives

Olives can form very high amounts of acrylamide during processing, although the temperature does not exceed 120 °C. Levels of asparagine and 3-APA in olives were not high enough to explain formation by known mechanisms. Other formation mechanisms have been suggested (e.g. involving acrylic acid or dehydroalanine), but no experimental evidence has been presented so far (Casado & Montano, 2008).

4.2 Acrylamide intermediates and reaction products in food

A model study by Perez Locas & Yaylayan (2008) suggested that some acrylamide precursors (e.g. decarboxylated Amadori product) can undergo incomplete reaction and accumulate in food products. During storage, such precursors can react with glucose and subsequently undergo a base-catalysed Hofmann-type elimination to finally form acrylamide. This mechanism might explain why highly alkaline (pH 12) extraction resulted in a much higher acrylamide yield compared with extraction at lower pH for some foods (Eriksson & Karlsson, 2006; Goldmann et al., 2006). It has been demonstrated in an animal study that the “extra” acrylamide measured after alkaline extraction does not correspond to bioavailable acrylamide (Vikstrom et al., 2008).

Rydberg et al. (2003) proposed an elimination reaction of acrylamide where it reacts with the amino acid side-chains. A very recent model study in which pure acrylamide was mixed with amine compounds showed that acrylamide reacts with small amino compounds to form its Michael adduct during storage at or above 35 °C. Interestingly, subsequent heating for 20 min at 180 °C reversed the reaction to release some of the acrylamide (Zamora, Delgado & Hidalgo, 2010). It is known that the levels of acrylamide in roasted coffee and cocoa powder significantly decrease during storage. Baum et al. (2008) showed in a study with [^{14}C]acrylamide-spiked coffee powder that acrylamide was covalently bound to the insoluble matrix to a large extent and thus remained in the filter cake upon subsequent brewing. Acrylamide was the only labelled low molecular weight compound that could be detected in the brew by radio-HPLC, although up to 50% of the radioactivity in the brew could not be accounted for.

5. PREVENTION AND CONTROL

The risk management of acrylamide in fried food, in terms of reducing consumer exposure, has so far relied mainly on voluntary actions from the food industry to reduce the acrylamide levels in their products. Additionally, many national authorities provide some information to consumers, usually through their web sites, on how acrylamide can be reduced in home cooking. To some extent, dietary advice is also given.

The Codex Alimentarius Commission has issued an international Code of Practice for the Reduction of Acrylamide in Foods. The scope is to provide national and local authorities, manufacturers and other relevant bodies with guidance to prevent and reduce the formation of acrylamide in potato and cereal products. The Code of Practice was adopted at step 8 (final draft stage) by the Thirty-second Session of the Codex Alimentarius Commission, held in 2009 (FAO/WHO, 2009). The Commission concluded that the Code could be updated when new technology and data for the mitigation of acrylamide formation in other products (e.g. coffee) become available.

A comprehensive collection of different mitigation methods has been compiled and critically reviewed by the Confederation of the Food and Drink Industries of the European Union (CIAA) in the format of an acrylamide “toolbox”. The first toolbox, which was issued in 2005, has since been updated with 12 newer versions. The 2009 version (CIAA, 2009a) was extended to include information from food and beverage manufacturers in the USA, provided by the Grocery Manufacturers Association. The extension was suggested to mark progression towards a “global” acrylamide toolbox.

Based on the acrylamide toolbox, a series of acrylamide mitigation brochures or pamphlets was developed jointly by the European Commission (Directorate-General for Health and Consumers) and the CIAA. The pamphlets, first published in 2007 and revised in 2009, include two-page summaries for each of five relevant food sectors: biscuits, crackers and crispbread, bread products, breakfast cereals and fried potato products. The pamphlets, which are primarily aimed at assisting small and medium-sized enterprises, have been translated into more than 20 languages.

5.1 Mitigation methods

The CIAA toolbox for acrylamide mitigation is currently based on 14 different listed parameters that can be controlled and optimized for reduced acrylamide levels in industrial food production, namely:

- Agronomical
  - Sugars
  - Asparagine
- Recipe
  - Raising agents

  - Other minor ingredients (e.g. glycine and divalent cations)
  - pH
  - Dilution
  - Rework

- Processing
  - Fermentation
  - Thermal input and moisture control
  - Pretreatment (e.g. washing, blanching, divalent cations)
  - Asparaginase

- Final preparation
  - Colour end-point
  - Texture/flavour
  - Consumer guidance

The parameters list is of a generic nature, providing a range of tools from which each manufacturer is expected to select and try out what is most suited for the food product in question. This is supported by a main section in which various measures that can be taken to control the parameters are discussed for each of the main food groups—potato, cereal, and coffee and coffee mixtures. The section also gives specific information on the stage at which the supporting studies have been conducted—laboratory scale, pilot scale or industrial scale.

Among all possible mitigation measures, a limited number have so far been reported by the toolbox to have been successfully applied to mitigate acrylamide at the industrial level. These include, for example:

- choosing potato varieties with low levels of reducing sugars, storing the potatoes above 6 °C and controlling the sugar levels by analysis or a fry test;
- avoiding wheat grains grown in sulfur-deprived soils;
- cutting thicker strips and hot water blanching (french fries);
- including calcium salts and/or acids in the recipe for formulated potato snacks and bread;
- avoiding the addition of reducing sugars (bread, bakery wares, breakfast cereals);
- replacing ammonium bicarbonate with other raising agents (biscuits);
- using asparaginase for enzymatic removal of asparagine in doughs (crispbreads and biscuits);
- controlling thermal input;
- controlling final moisture content and colour.

No efficient mitigation methods for acrylamide in roasted coffee or coffee surrogates have so far been presented.

Several recent reviews have critically reviewed and discussed the mitigation methods and future options (Amrein et al., 2007; Foot et al., 2007; Grob, 2007; Konings et al., 2007; Claus, Carle & Schieber, 2008; Friedman & Levin, 2008; Muttucumaru et al., 2008; Anese et al., 2009; Zhang, Ren & Zhang, 2009). The use of the enzyme asparaginase is still identified as one of the most promising methods. Asparaginase for food industry use is now available from different commercial suppliers. Selective removal of the key precursor asparagine can potentially almost inhibit acrylamide formation with limited effect on the overall Maillard reaction cascade that gives fried foods their characteristic flavour and colour. The efficiency is, however, limited by technological difficulties, such as those related to penetration and mobility in the food matrix and effects on the enzyme activity. Reductions of acrylamide by 34–92% in dough-based applications, by 60–85% in french fries and up to 60% in potato chips were achieved in food model and pilot-scale testing reported by one producer of commercial asparaginase (Hendriksen et al., 2009). Further testing and process development are needed before its full applicability in real food production can be evaluated.

Other ways of acrylamide reduction via lowered asparagine are through agronomical factors and plant breeding. In addition to important recent findings of high asparagine concentrations in wheat from sulfur-deprived soils, more research is needed on factors behind the large seasonal variations in grain cereals. Also, the industry (CIAA, 2009b) has requested research on providing cereal varieties with low asparagine content.

Owing to the high content of asparagine in potatoes, reducing sugars are normally the rate-limiting acrylamide precursor. Nevertheless, substantial reduction of asparagine levels will efficiently mitigate formation. Potato chips (USA = french fries) and potato crisps (USA = chips) from a new intragenic potato with very low levels of asparagine, developed by silencing asparagine synthetase genes through DNA transformation, accumulated as little as 5% of the acrylamide present in wild-type controls (Rommens et al., 2008).

Another interesting area is the use of low molecular weight additives, such as acids, amino acids, divalent cations and antioxidants. Recent studies in food models have indicated a high mitigation potential of antioxidant-containing extracts from various fruits and plants (Ciesarova, Suhaj & Horvathova, 2008; Hedegaard et al., 2008; Zhang, Ren & Zhang, 2009).

Although measures to reduce sugar levels in potatoes and control frying conditions were implemented early, there still seems to be a potential for further improvements from improved control of these factors. It has been suggested from restaurant trials and monitoring of sugar levels in potatoes in Switzerland that an average acrylamide concentration of about 50 µg/kg should be achievable for french fries (Grob, 2007). For comparison, an average concentration of 350 µg/kg was obtained in an all-European monitoring exercise in 2007 (EFSA, 2009).

5.2 Mitigation achievements

Successful mitigation results have been reported by the food industry for potato products. The major achievements seem to have been made in potato

crisps and french fries during the first years after the discovery of acrylamide in foods (Matissek & Raters, 2005; Foot et al., 2007; Wenzl & Anklam, 2007) (CIAA, 2009b). The average weekly acrylamide levels in German potato crisps produced from stored potatoes were about 800–1000 µg/kg in the years 2002–2003 and about 400–600 µg/kg in the years 2004–2009 (Association of the German Confectionery Industry: http://www.bdsi.de/de/positionen_themen/acrylamid/verbraucherinformationen/). An almost identical trend in acrylamide levels in french fries ready cooked according to on-pack instructions over the same time period was reported by the European Potato Processors' Association (CIAA, 2009b). Less reduction in acrylamide levels in crisps produced from fresh potatoes might indicate that mitigation was largely achieved by controlling sugar levels in stored potatoes. Acrylamide levels in potato crisps sampled in the year 2008 in Spain were nearly 50% lower than those found in an investigation carried out 4 years earlier (Arribas-Lorenzo & Morales, 2009). A comparison of two different European databases of acrylamide levels obtained in 2003–2006 and in 2007 showed geometric mean levels in potato crisps of 514 µg/kg and 366 µg/kg, respectively (EFSA, 2009). By contrast, the mean concentration in french fries was lower in the years 2003–2006 than in 2007: 178 µg/kg and 227 µg/kg, respectively.

Mitigation seems to have been less successful in general terms for bread and other cereal products (Konings et al., 2007), although significant reductions have been reported more recently for some specific products. For example, the levels in Dutch spice cake were reduced from approximately 1000 µg/kg to 350 µg/kg from 2002 to 2006, presumably by removing ammonium carbonate from the recipe. Also, more than a 50% reduction was reported for non-fermented crispbread by changing the oven profile (Konings et al., 2007) and, more recently, by the addition of asparaginase enzyme (CIAA, 2009b).

Mitigation after 2003 has been reported mainly for food types with comparably high acrylamide levels or single products that are in the high end with respect to acrylamide levels within their food type. Although this might significantly reduce the exposure for some individuals or population subgroups, it will have little effect on the general population exposure in most countries. This conclusion is supported by repeated national exposure studies in the USA and in Sweden. No significant differences were seen when comparing three different exposure assessments carried out in 2003–2006 in the USA (Friedman & Levin, 2008). Similar observations can be made from an ongoing Swedish trend study in which products from all food groups of major importance for acrylamide exposure were sampled twice every year starting from 2005 (Swedish National Food Administration, 2009).

It should be pointed out that reliable evaluation of mitigation results is very difficult as a result of the high variability in acrylamide levels. For example, annual variations in the composition of raw materials (e.g. due to agricultural conditions) can result in significant differences in acrylamide levels in food products. It might therefore take several years before acrylamide mitigations achieved, for example, by changes in production methods will be detected.

Long-term monitoring also presents analytical challenges, requiring strict laboratory quality control programmes. For example, a comparison made by the

European Food Safety Authority (EFSA) suggested that an apparent reduction in exposure from coffee could have been caused by analytical difficulties with this specific food matrix in early studies (EFSA, 2009). Another source of error could be comparison of data sets with non-identical product composition (e.g. roasted and instant coffee or different types of bread).

6. LEVELS AND PATTERNS OF CONTAMINATION IN FOOD COMMODITIES

6.1 Surveillance data

At the current meeting, the Committee reviewed data from 31 countries (Table 11) on the occurrence of acrylamide in different foods analysed between 2004 and 2009. The total number of analytical results (single or composite samples) was 12 582, with 61% coming from Europe, 28% from Asia, 9% from North America, 1% from the Pacific and 1% from Latin America. No data were received from Africa. The Committee noted that the occurrence data evaluated at its present meeting were more comprehensive than the data submitted at the sixty-fourth meeting. Most countries used validated analytical methods and employed quality control programmes to ensure the reliability of the data.

The choices of food items used in the acrylamide monitoring were based on what has become known since 2002–2003 on the formation of acrylamide in foodstuffs and on the recommendations made by the Committee at its sixty-fourth meeting. As acrylamide is formed during heat treatment and concentrations in cooked products depend on methods of cooking, several commodities have been analysed in processed/cooked foods using different cooking methods.

Table 11. Summary of acrylamide occurrence data from various countries for the 2004–2009 period

Region	Country	Number of analytical results	% of values below LOR
Asia	China	1 316	30
	Democratic People's Republic of Korea	149	20
	Japan	1 631	10
	Turkey	431	16
Subtotal		*3 527*	*19*
Europe	Austria	51	31
	Belgium	188	28
	Central and Eastern Europe	300	0
	Czech Republic	132	14

Table 11 (contd)

Region	Country	Number of analytical results	% of values below LOR
	Denmark	3	0
	Estonia	50	3
	Finland	83	1
	France	201	27
	Germany	4 796	2
	Greece	41	29
	Ireland	103	25
	Italy	26	0
	Latvia	38	39
	Lithuania	41	46
	Netherlands	584	5
	Norway	233	11
	Poland	119	3
	Slovakia	52	38
	Spain	107	7
	Sweden	249	3
	Switzerland	11	9
	United Kingdom	233	3
Subtotal		*7 641*	*5*
Latin America	Brazil	114	8
North America	Canada	644	8
	USA	483	28
Subtotal		*1 241*	*16*
Pacific region	Australia/New Zealand	163	39
Total		*12 582*	*20*

LOR, limit of reporting (LOD and LOQ)

6.2 National occurrence

National occurrence data on acrylamide were reported by 31 countries. Most samples were analysed by either GC-MS(/MS) or LC-MS(/MS) methods, where the LOD and LOQ ranged from 1 to 60 µg/kg and from 2 to 100 µg/kg, respectively, for different food commodities. To harmonize the national occurrence data, all data

below the limit of reporting (LOR) (below the LOD or LOQ where the percentage of non-quantified values was less than 60% for major contributing foods) have been assigned as follows: data below LOD = ½ LOD and data below LOQ = ½ LOQ, following the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) guidelines (GEMS/Food-EURO, 1995).

6.2.1 Australia

Australia submitted acrylamide data, published in 2006 by the Government of South Australia (GSA, 2006), in non-carbohydrate-based foods. Acrylamide concentrations were obtained from 77 food samples purchased from commercial shops in June 2005. Foods including coffee, cereal, beverages, chocolate, prune juice, nuts, commercial soups, olives, canned beans, meat pies, fried rice, frozen pizza, hash browns, fish, chicken and beef were analysed, as recommended from a list provided by Food Standards Australia New Zealand (FSANZ). Samples were analysed using the LC-MS/MS technique. The LOD and LOQ ranged from 3 to 25 µg/kg and from 10 to 50 µg/kg, respectively, for different foods. Eighty per cent of values were reported to be below the LOQ. The mean concentrations in food where acrylamide was quantified were black olives (345 µg/kg, 1 sample), hash browns (320 µg/kg, 3 samples), prune juice (93 µg/l, 1 sample) and coffee ready to drink (2.7 µg/l, 41 samples).

6.2.2 Brazil

Brazil submitted data from a recent acrylamide survey on 114 individual food samples purchased from supermarkets, fast food restaurants and restaurants in the region of Campinas from 2004 to 2006; the main results have been published by Arisseto et al. (2007). Analyses were performed using LC-MS/MS. The LOD and LOQ were 10 µg/g and 20 µg/kg, respectively. Eight per cent of the values were reported to be below the reporting limits. The highest contributing food groups and mean acrylamide concentrations in those food groups were french fries (331 µg/kg), potato chips (612 µg/kg), potato “palha” (549 µg/kg), crackers (179 µg/kg), toast (100 µg/kg), bread (41 µg/kg), breakfast cereals (32 µg/kg), cassava starch biscuit and cassava flour (22 µg/kg) and coffee instant or roasted powder (350 µg/kg).

6.2.3 Canada

Canada submitted the results of a survey conducted in 2009 that was undertaken to establish the prevalence of acrylamide in the Canadian diet (Health Canada, 2009). Acrylamide concentrations were obtained from 644 ready-to-eat samples purchased at local Ottawa grocery stores and also collected from local fast food outlets. Analyses were performed using LC-ESI-MS/MS. The LOD and LOQ were, respectively, 3 µg/g and 10 µg/kg. Eight per cent of the values were reported to be below the LOR. The main analysed food groups and associated mean acrylamide concentrations were instant coffee (666 µg/kg), crackers crispbread (586 µg/kg), dark chocolate (570 µg/kg), potato chips (537 µg/kg), french fries (426 µg/kg), cocoa powder (348 µg/kg), mixed nuts (315 µg/kg), corn chips and popcorn (270 µg/kg), fruit juices (nectars and prunes, 200 µg/kg), cookies

(197 µg/kg), pretzels (148 µg/kg), baby food prunes (140 µg/kg), crackers others (137 µg/kg), cereals wheat, oat, corn or rice (120 µg/kg), peanut butter (103 µg/kg), baby food sweet potatoes (70 µg/kg), bread (54 µg/kg), milk chocolate (23 µg/kg), pizza (17 µg/kg) and coffee ready to drink (9 µg/kg).

6.2.4 China

China submitted levels of acrylamide in foods using the GEMS/Food format (Chinese CDC, 2009). Acrylamide concentrations were obtained for 1316 individual food samples purchased in 2005–2007 from a range of food outlets in China. Samples were analysed using the LC-MS/MS technique. The LOD and LOQ were 1–40 µg/kg and 3–133 µg/kg, respectively, for different foods. Thirty per cent of the values were reported to be below the LOR. The highest contributing food groups together with the mean acrylamide concentrations in those groups were potato chips (740 µg/kg), pastry and biscuits (279 µg/kg), popcorn (262 µg/kg), coffee beans, roasted (259 µg/kg), dried grapes (currants, raisins and sultanas, 155 µg/kg), peppers chili (123 µg/kg), peanuts and hazelnuts (94 µg/kg), wheat germ (91 µg/kg), white bread (72 µg/kg), poultry meat products (58 µg/kg), soya beans dry (56 µg/kg), rice-based cereal (51 µg/kg), cocoa mass (48 µg/kg), tea green and black (fermented and dried) (45 µg/kg), spices (42 µg/kg) and oat products (25 µg/kg).

6.2.5 Democratic People's Republic of Korea

The Democratic People's Republic of Korea submitted results on acrylamide occurrence (Academy of Health and Food Science of the Democratic People's Republic of Korea, 2009). Concentrations of acrylamide in 149 traditional and national food samples were obtained. No information was provided on the method of analysis or on the LOR. The food groups and their mean concentrations of acrylamide were as follows: potato chips (963 µg/kg), biscuits (169 µg/kg), crackers (130 µg/kg), potato snacks (74 µg/kg), popcorn (88 µg/kg), tea (ready to drink, 85 µg/kg), confectionery (76 µg/kg), meat and fish products (fried) (52 µg/kg) and chocolates (44 µg/kg).

6.2.6 European Union (including Norway and Switzerland)

The European Union provided two sets of data. The first set, for 3241 acrylamide levels in food, was collected from March 2004 until June 2006; these data came from the published database of the European Commission's IRMM (EC, 2006), which was established between 2003 and 2006 (Wenzl & Anklam, 2007). The second set, for 3381 analytical results, was reported from member states and Norway for the period 2007–2008 according to an agreed-upon sampling procedure recommended by the European Commission (EC, 2007). A scientific report on "Monitoring of acrylamide levels in food" has recently been published by EFSA (2009). Overall, 6622 analytical results for acrylamide content in foods sampled since the sixty-fourth meeting were evaluated. Seventy-two per cent were from Germany, with the remaining 28% from other European countries. Most analyses were performed using LC-MS/MS and GC-MS(/MS). The LOD and LOQ ranged from 0.5 to 60 µg/kg and from 1.7 to 100 µg/kg, respectively, for the different

food commodities. Six per cent of the values were reported to be below the LOR. The analysed food groups and their mean acrylamide concentrations were as follows: specific foods for people with diabetes (bakery wares, dietetic foods, sweets) (1256 µg/kg), coffee substitute, extracts (1151 µg/kg), potato crisps (524 µg/kg), gingerbread (551 µg/kg), coffee roasted (249 µg/kg), potato chips (361 µg/kg), precooked potato chips (308 µg/kg), biscuits (including infant biscuits) (302 µg/kg), bread (197 µg/kg), breakfast cereals (120 µg/kg), processed cereal-based baby food (39 µg/kg) and jarred baby food (21 µg/kg).

6.2.7 France

France submitted levels of acrylamide in foods as consumed from its second Total Diet Study (TDS), in the GEMS/Food format (AFSSA, 2009). Acrylamide concentrations were obtained from 197 composite food samples purchased from a range of commercial shops during 2008–2009 in eight regions and 33 cities. These composites were reflective of market shares for the brands most commonly consumed, as reported in the last national individual food consumption survey. The food sampling strategy has been described in Sirot et al. (2009). Samples were analysed using LC-ESI-MS/MS. The LOD and LOQ were 4 µg/kg and 10 µg/kg, respectively. Twenty-seven per cent of the values were reported to be below the LOR. The main analysed food groups and their mean acrylamide concentrations were potato crisps (954 µg/kg), potato chips (724 µg/kg), salted biscuits (248 µg/kg), biscuits (203 µg/kg), coffee ready to drink (68 µg/kg), chocolate (41 µg/kg), bread/pastry/rolls (26 µg/kg), breakfast cereals (16 µg/kg) and meat and fish products (12 µg/kg).

6.2.8 Japan

Japan submitted acrylamide levels in foods in the GEMS/Food format (Japan Ministry of Health, Labour and Welfare, 2009). Acrylamide concentrations were obtained for 1631 individual food samples collected during the 2005–2008 period in seven randomly selected supermarkets in six major cities in Japan. Analyses were performed using GC-MS/MS. The LOD and LOQ of the method were 5 µg/kg and 20 µg/kg, respectively. Ten per cent of the values were reported to be below the LOR. The main analysed food groups and their mean acrylamide concentrations were potato crisps (1202 µg/kg), potato chips (410 µg/kg), black sugar (463 µg/kg), pan-fried vegetables (snow peas, 393 µg/kg; bean sprouts and asparagus, 100 µg/kg; broccoli, cabbage, pumpkin, eggplant, haricot beans, onion, 30 µg/kg), biscuits (197 µg/kg), curry (71 µg/kg), rice crackers (54 µg/kg), fried potato snacks (50 µg/kg) and coffee ready to drink (9 µg/kg).

6.2.9 Netherlands

The Netherlands submitted acrylamide data obtained during 2005–2007 (Dutch Food and Consumer Product Safety Authority, 2009). Acrylamide concentrations were obtained for 359 individual food samples. No information was available on the method of analysis or reporting limits. The main analysed food groups and their mean acrylamide concentrations were salty biscuits (517 µg/kg), cookies (339 µg/kg), toast (297 µg/kg), coffee, roasted (246 µg/kg), bread (white,

brown, raisin and wholemeal; 189 µg/kg), peanuts (185 µg/kg), potato chips (159 µg/kg), table olives (147 µg/kg), baby food (89 µg/kg), cocoa products (87 µg/kg), others biscuits (69 µg/kg) and rusks and rye bread (19 µg/kg).

6.2.10 New Zealand

New Zealand submitted acrylamide data on coffee from a survey conducted by the New Zealand Food Safety Authority in 2008 (New Zealand Food Safety Authority, 2009). Acrylamide concentrations in 86 composite samples of coffee, ready to drink, were provided: cappuccino, flat white, ground, latte, instant black and white, short and long black, and mocha. No information was provided on the analytical method or reporting limits. The mean concentration found from all coffee samples ready to drink was 6.26 µg/kg.

6.2.11 Norway

Norway has published results on levels of acrylamide in foods (Brantsaeter et al., 2008). Acrylamide concentrations were obtained from 233 Norwegian foods (Norwegian Food Safety Authority, 2006). No information was provided on the method of analysis or reporting limits. The food groups analysed and their mean acrylamide concentrations were potato crisps (780 µg/kg), biscuits (518 µg/kg), crispbread (459 µg/kg), potato chips (279 µg/kg), breakfast cereals (120 µg/kg), bread (17 µg/kg) and coffee ready to drink (17 µg/kg).

6.2.12 Poland

Poland submitted levels of acrylamide in foods (Poland National Food and Nutrition Institute, 2009). Acrylamide concentrations were obtained for 119 traditional and national food samples collected in restaurants and shops and homemade. Samples were analysed by a GC-MS/MS method after derivatization. The LOD and LOQ were 20 µg/g and 40 µg/kg, respectively. Three per cent of the values were reported to be below the LOR. The main analysed food groups and their mean acrylamide concentrations were potato chips (792 µg/kg), potato crisps (399 µg/kg), ground coffee (392 µg/kg), biscuits (339 µg/kg), wheat and rice gruel (153 µg/kg), breakfast cereals (149 µg/kg), homemade dishes with meat and vegetables (77 µg/kg) and white bread (59 µg/kg).

6.2.13 Spain

Results on levels of acrylamide from a survey of biscuits and bread derivatives in Spain have been published (Rufian-Henares, Aribas-Lorenzo & Morales, 2007). Acrylamide concentrations were measured in a series of 107 samples of commercial products randomly purchased in different supermarkets from the autonomous community of Madrid: commercial biscuits (62 samples), bread crust (24 samples), bread sticks (10 samples) and crackers (11 samples). Samples were analysed using the LC-MS technique. The LOD and LOQ were 10 µg/kg and 30 µg/kg, respectively. The foods analysed and their mean acrylamide concentrations were biscuits (423 µg/kg), bread sticks (157 µg/kg), crackers (140 µg/kg) and crispbread (87 µg/kg).

6.2.14 Sweden

Sweden submitted occurrence data from an ongoing trend study on acrylamide levels in food products sold in the country (Swedish National Food Administration, 2009). Acrylamide concentrations were reported for 168 composite samples collected in food stores and restaurants from November 2005 to April 2009. Samples were chosen to represent the major contributors to acrylamide exposure in Sweden (i.e. potato products, cereal products and coffee). Analyses were performed using LC-MS/MS. No information was provided on the reporting limits. The analysed food groups and reported mean acrylamide concentrations were potato crisps (773 µg/kg), potato chips (326 µg/kg), biscuits (273 µg/kg), crispbread (269 µg/kg), breakfast cereals (117 µg/kg), soft bread (45 µg/kg) and coffee, ready to drink (12 µg/kg).

6.2.15 Turkey

Turkey published a survey on acrylamide levels in foods from the Turkish market (Senyuva & Gökmen, 2005; Ölmez et al., 2008). In total, 431 samples of processed foods and traditional Turkish foods, especially desserts, were analysed for acrylamide content using a GC-MS method after bromine derivatization (LOD and LOQ were 10 µg/kg and 30 µg/kg, respectively) or an LC-MS method (LOD 6–10 ng/g and LOQ 15–20 ng/g for different food matrices). More than 16% of the samples were reported to be below the LOR. The food commodities and their mean acrylamide concentrations were as follows: breakfast cereals (130 µg/kg), biscuits and crackers (346 µg/kg), potato crisps (622 µg/kg), corn crisps (287 µg/kg), nuts and seeds (roasted, 98 µg/kg), instant and Turkish coffee (262 µg/kg), cakes (206 µg/kg), cookies (126 µg/kg), grilled vegetables (127 µg/kg) and traditional Turkish foods (300 µg/kg).

6.2.16 United States of America

The USA published acrylamide data on selected food items in their TDS conducted in 2005 and 2006 (USFDA, 2006). The TDS in the year 2005 involved samples collected from four regions (West, North-central, South and North-east, market baskets 1–4), and the 2006 study involved one region (North-central, market basket 2). For each market basket, samples of each TDS food were collected from grocery stores and fast food restaurants in three cities within the region, prepared table-ready and composited for analysis. In total, 483 composite samples were analysed for acrylamide by the LC-MS method (LOD 10 µg/kg). Twenty-eight per cent of the samples were found to be below the LOR. The major foods and their mean concentrations of acrylamide were as follows: baby food (cookies, teething biscuits, etc.) (251 µg/kg), baby food (vegetable/meat based) (22 µg/kg), breakfast cereals (94 µg/kg), potato chips (398 µg/kg), french fries (425 µg/kg), casseroles and stews (24 µg/kg) and milk products (7 µg/kg).

6.2.17 Summary of national occurrence data

National mean concentrations of acrylamide in major foods were found to range from 399 to 1202 µg/kg for potato crisps; from 159 to 963 µg/kg for potato

chips; from 169 to 518 µg/kg for biscuits (USA = cookies); from 87 to 459 µg/kg for crispbread and crackers; and from 3 to 68 µg/l for coffee (ready to drink). The Committee noted that the mean concentration ranges of acrylamide in the above foods are similar to those reviewed in its previous evaluation at the sixty-fourth meeting.

6.3 International occurrence

Acrylamide levels obtained from individual food items have been organized according to the GEMS/Food consumption cluster diet categorization (WHO, 2006). In total, 11 036 analytical results were compiled from 27 countries, with 66% from Europe, 6% from North America, 27% from Asia, 1% from the Pacific region and 1% from Latin America. Ten per cent (1276 analytical results) of the data were not included, as aggregate data only were provided (individual data not available), some mixed foods could not be classified, data on spices and condiments were excluded and food names were given in a foreign language.

In order to take into account the censored data in the calculations of dietary exposure, international recommendations described in the GEMS/Food-EURO (1995) consultation report have been applied. As the percentage of non-quantifiable values was less than 60%, the following treatment was used: data below LOD = ½ LOD, and data below LOQ = ½ LOQ. This accounts for 5% of the samples from Europe, 17% of those from North America, 32% of those from Latin America, 32% of those from the Pacific region and 39% of those from Asia.

A summary of the concentration data for acrylamide found in several food commodities from 2004 to 2009 is presented in Table 12. Food groups have been divided into subgroups according to the cooking process, as defined at the sixty-fourth meeting of the Committee. A differentiation has been made between raw, boiled and canned products and processed food (dried, fried, baked, grilled). The highest average levels of contamination were found for the following food commodities: chicory roots (2470 µg/kg), potato crisps (USA = chips) (956 µg/kg), coffee extracts/substitute (705 µg/kg), gingerbread (572 µg/kg), sugar unrefined (445 µg/kg), potato chips (USA = french fries) (410 µg/kg), peas dry (349 µg/kg), coffee decaffeinated, not brewed (331 µg/kg), coffee (ground, instant or roasted, not brewed) (314 µg/kg), pastry and biscuits (288 µg/kg), potato chip, croquettes (frozen, not ready to serve) (245 µg/kg), baby food (dry powder) (237 µg/kg), fruits fried, processed (214 µg/kg), breads and rolls (207 µg/kg), cocoa mass, powder (194 µg/kg), breakfast cereals (149 µg/kg), potato baked (including cassava) (147 µg/kg), oilseed (131 µg/kg), cereals and pasta processed (toasted, fried, grilled) (127 µg/kg), baby food (biscuits, rusks, etc.) (121 µg/kg) and tree nuts (104 µg/kg). Other food commodities generally had mean levels well below 100 µg/kg.

In comparing global mean acrylamide levels for commodity groups with the levels obtained at the sixty-fourth meeting, the Committee noted that the acrylamide level in rye products had decreased significantly ($P < 0.001$). No significant differences were observed for products made from potato, barley, rice, wheat, maize or oats.

Table 12. Summary of the distribution of acrylamide concentrations in several commodities from 2004 to 2009

Commodities	Number of samples	*N* < LOR (%)	Mean concentration (µg/kg)[a]	CV (%)[b]	90th-percentile concentration (µg/kg)	97.5th-percentile concentration (µg/kg)	Reported maximum concentration (µg/kg)
Cereals and cereal-based products	5183	10	273	177	667	1539	8066
Cereals and pasta, raw and boiled	177	28	30	93	57	99	218
Cereals and pasta processed (toasted, fried, grilled)	149	29	127	255	273	388	3817
Cereal-based processed products, all	4857	9	286	173	696	1569	8066
Breads and rolls	1481	15	207	213	497	936	81
Pastry and biscuits (USA = cookies)	2311	8	288	153	730	1458	6798
Gingerbread	621	0.6	572	133	1490	2578	6891
Breakfast cereals	414	9	149	144	327	690	1649
Pizza	20	45	20	90	36	62	81
Fish and seafood (including breaded, fried, baked)	44	19	64	106	156	179	349
Eggs	13	85	18	23	20	28	31
Meats and offals (including coated, cooked, fried)	137	17	42	174	91	217	671
Milk and milk products	13	85	6	123	15	21	23
Nuts and oilseeds	201	31	111	207	311	704	1658
Oilseed	53	19	131	196	341	747	1548
Tree nuts	148	36	104	211	206	685	1658

Table 12 (contd)

Commodities	Number of samples	*N* < LOR (%)	Mean concentration (µg/kg)[a]	CV (%)[b]	90th-percentile concentration (µg/kg)	97.5th-percentile concentration (µg/kg)	Reported maximum concentration (µg/kg)
Pulses	196	66	50	214	160	393	620
Beans	20	40	40	141	148	179	187
Peas	14	0	349	53	595	617	620
Soya bean	54	33	63	112	160	215	382
Soya sauce	108	96	7	68	10	17	17
Roots and tubers (potato and potato products)	3451	29	532	116	1263	2281	5500
Potato puree/mashed/boiled (including cassava, taro)	44	64	23	69	51	69	71
Potato baked (including cassava)	92	3	147	135	421	696	1,027
Potato crisps (USA = chips)	878	0.5	956	77	2	3100	5500
Potato chips (USA = french fries)	2332	2	410	124	898	1799	5269
Potato chip, croquettes (frozen, not ready to serve)	105	10	245	82	486	763	931
Stimulants and analogue (decaffeinated/coffee substitute)	1014	1	427	142	966	2091	4700
Coffee (brewed), ready to drink	254	15	17	173	40	79	245
Coffee (ground, instant or roasted)	324	3	314	83	558	856	3025
Coffee extracts, substitute	227	1	705	82	1	2370	3779
Coffee decaffeinated	8	0	331	45	474	561	590

Table 12 (contd)

Commodities	Number of samples	*N* < LOR (%)	Mean concentration (µg/kg)[a]	CV (%)[b]	90th-percentile concentration (µg/kg)	97.5th-percentile concentration (µg/kg)	Reported maximum concentration (µg/kg)
Cocoa mass, powder	56	25	194	124	494	627	1260
Cocoa products	74	22	78	165	190	366	826
Green tea (roasted)	59	42	47	150	96	294	368
Chicory roots	12	0	2470	38	3	4343	4700
Sugars	76	3	332	153	865	2038	2300
Sugar refined	24	0	86	104	162	293	438
Sugar unrefined	52	4	445	130	1	2218	2300
Vegetables	239	25	52	265	100	311	1767
Raw, boiled and canned	170	23	67	241	152	152	1767
Processed (toasted, fried, grilled)	69	30	16	89	37	54	68
Fruits	124	7	110	183	251	558	1630
Fresh	17	29	54	187	178	316	332
Dried	61	2	47	108	112	154	258
Fried, processed	46	7	214	137	398	911	1630
Alcoholic beverages (beer, cider, gin, wine)	59	69	17	82	20	65	84

Table 12 (contd)

Commodities	Number of samples	*N* < LOR (%)	Mean concentration (µg/kg)[a]	CV (%)[b]	90th-percentile concentration (µg/kg)	97.5th-percentile concentration (µg/kg)	Reported maximum concentration (µg/kg)
Baby food (cereals and pasta, raw and boiled)	20	55	13	89	30	33	35
Baby food (canned, jarred)	83	29	58	131	108	261	399
Baby food (dry powder)	6	0	237	61	412	455	470
Baby food (biscuits, rusks, etc.)	187	11	121	109	253	400	1100

[a] Data below the reporting limits (LOD or LOQ) have been assumed to be half of those limits.
[b] Coefficient of variation (standard deviation divided by mean, %).

7. FOOD CONSUMPTION AND DIETARY EXPOSURE ESTIMATES

7.1 National estimates of dietary exposure

Since the previous evaluation of acrylamide at the sixty-fourth meeting of the Committee, data on dietary exposure to acrylamide for eight countries (Brazil, China, France, Ireland, New Zealand, Norway, Spain, United Kingdom) have become available and were evaluated at this meeting. All regions were represented, except for Africa, for which no dietary exposure data were available. National dietary exposures were calculated mainly using a deterministic assessment. The modelling combined national individual consumption data with mean occurrence data obtained from national monitoring surveys and with the consumer body weights reported in consumption surveys.

7.1.1 Brazil

A recent publication from Arisseto et al. (2009) gives an overview of the acrylamide dietary exposure for a population of Brazilian adolescents. Acrylamide concentrations used in calculations came from 73 individual food samples purchased at supermarkets, fast food restaurants and restaurants in the region of Campinas from 2004 to 2006. Dietary exposures to acrylamide were generated deterministically using the mean concentrations and food consumption data collected from a 24 h dietary recall survey on a representative sample of 578 adolescents aged from 11 to 17 years from the administrative region of Campinas. Concentration values below the reporting limits (8% of the total samples) were assigned a concentration equal to ½ LOR. The dietary exposure estimates were approximately 0.12 µg/kg bw per day on average and 0.8 µg/kg bw per day for the 97.5th percentile. The main foods contributing to total exposure for this adolescent population were potato chips (60%), bread (13%), salt biscuit (11%) and coffee (9%).

7.1.2 China

China submitted estimates of dietary exposure to acrylamide based on the results of the third (2000) and fourth (2007) Chinese TDSs (Chinese CDC, 2009). Acrylamide concentrations of 144 food composites from 665 food samples prepared as consumed were used in the calculations. Dietary exposure calculations were performed using a deterministic method, combining mean acrylamide concentrations from the food group composites with their associated food consumptions. Concentration values reported below the reporting limits (50% of values below the LOR) were assigned a concentration equal to ½ LOR. In 2000, the third Chinese TDS included 4320 persons 15 years of age and older (Chinese CDC, 2009). It covered four baskets from 12 provinces, municipalities and autonomous regions in mainland China. The average exposure estimates for the whole population increased from 0.19 µg/kg bw per day in 2000 to 0.29 µg/kg bw per day in 2007. Main food contributors to total exposure in the 2000 Chinese TDS were cooked vegetables, including fried and grilled potato (53.7%), cereals and pasta, processed (26.4%), potato, boiled and baked only (10.9%) and pulses (5.9%), whereas those in the 2007 Chinese TDS were cooked vegetables, including

fried and grilled potato (48.4%), cereals and pasta, processed (27.1%), potato and potato products, baked (8.0%) and pulses (5.8%).

A recent publication from Chen et al. (2008) gives an overview of the dietary exposure to acrylamide in a Chinese population. Acrylamide concentrations used in calculations were from 349 individual food samples purchased at local supermarkets and stores in Beijing in 2005 and 2006. Analyses were performed using LC-MS/MS. The LOD and LOQ of the method ranged from 0.8 to 10 µg/kg and from 4 to 25 µg/kg, respectively. Food consumption data for Chinese people were collected in 2002 from the National Nutrition and Health Survey results for 55 768 persons 15 years of age and older (MHPRC, 2004). Concentrations reported to be below the reporting limits (23% of total samples) were assigned a concentration equal to ½ LOR. Dietary exposures to acrylamide were generated deterministically using the mean concentration in food commodities and associated food consumption data at the food group commodity level. The mean dietary exposure for adults in the Chinese population was estimated to be 0.4 µg/kg bw per day, and the 97.5th-percentile exposure was 1.5 µg/kg bw per day. The main food contributors to total exposure were flour and flour products (55%), potato crisps and potato chips (18%) and spices (13%).

7.1.3 France

France submitted data on dietary exposures to acrylamide from an updated evaluation based on the results of its second TDS (AFSSA, 2009). Acrylamide concentrations used in calculations were from 197 individual food composite samples purchased from a range of commercial shops from 2008 to 2009 and prepared as consumed (Sirot et al., 2009). Analyses were performed using LC-ESI-MS/MS. The LOD and LOQ of the method were 4 µg/kg and 10 µg/kg, respectively. Dietary exposure calculations were performed deterministically using the mean acrylamide concentrations of foods and the food consumption by each individual reported in the national individual food consumption survey completed in 2006 with 4079 participants aged 3–79 years. Concentrations below the reporting limits (27% of total samples) were assigned a concentration equal to ½ LOR. The dietary exposure estimates for the whole population aged 17 years and older were 0.4 µg/kg bw per day on average and 1.0 µg/kg bw per day for the 95th percentile. Children aged from 3 to 17 years had exposures ranging from 0.7 to 1.8 µg/kg bw per day. The main food contributors to total exposure were potato chips (45–60%), coffee ready to drink (29.5%), biscuits (4–11%), salted biscuits (5–8%) and bread (5%).

7.1.4 Ireland

A publication from Mills et al. (2008) provides estimates of dietary exposure to acrylamide for the population of Ireland (Mills et al., 2008). Acrylamide concentrations (n = 7000) used in calculations were taken from the European Union's acrylamide monitoring database (EC, 2006). Dietary exposures for acrylamide were generated in a probabilistic way using CREMe 2.0 food model software from O'Reilly Institute, Trinity College, Dublin. Distributions of food concentrations and individual food consumption data obtained from the North/South

Ireland Food Consumption Survey (954 adults aged 18–64 years) were used. The dietary exposure estimates for the adult population were 0.6 µg/kg bw per day on average and 1.8 µg/kg bw per day for the 97.5th percentile. The main food group contributors to total exposure were potato and potato products (48%), bread (34%) and biscuits (10%).

7.1.5 New Zealand

New Zealand submitted results from an updated acrylamide dietary exposure analysis for the New Zealand population (Love & Grounds, 2006). Concentration data from a New Zealand database for main foods and from European and USA databases for other foods were combined with New Zealand food consumption data derived from national nutrition surveys performed for adults in 1999, children in 2003 and infants in 2005. The average dietary exposure estimates ranged from 0.7 to 0.9 µg/kg bw per day for the adult population 19 years of age and older; from 1.4 to 1.6 µg/kg bw per day for adolescents aged 11–14 years; and from 1.7 to 2.3 µg/kg bw per day, respectively, for infants 6–12 months of age and preschool children 1–6 years of age. No data on high-percentile (95th–97.5th percentile) exposures were provided. The main foods contributing to total exposure were potato chips (9–23%), potato crisps (10–22% for adolescents, preschool children and infants), roasted potatoes (17–20% for adolescents and preschool children) and wheat biscuits (13% for preschool children 1–3 years of age only).

7.1.6 Norway

A publication from Brantsaeter et al. (2008) gives estimates of dietary exposure to acrylamide for pregnant women. Acrylamide concentrations (n = 466) used in calculations were mainly for Norwegian foods reported by the Norwegian Food Safety Authority. Dietary exposures to acrylamide were generated deterministically using the mean concentrations and food consumption data collected from a validated FFQ given to 119 pregnant women aged from 22 to 44 years surveyed at Baerum hospital in 2003–2004. The dietary exposure estimates were 0.5 µg/kg bw per day on average and 0.9 µg/kg bw per day for the 95th percentile. The main food contributors to total exposure were potato crisps (16%), crispbread (22%), snacks, peanuts, popcorn (12%) and bread (11%).

7.1.7 Spain

The publication from Rufián-Henares, Arribas-Lorenzo & Morales (2007) gives an overview of dietary exposure to acrylamide in the Spanish population from consumption of biscuits, bread derivatives, breakfast cereals and potato chips. Acrylamide concentrations used in calculations came from more than 160 samples of commercial products: potato chips, breakfast cereals, biscuits, bread crust, bread sticks and crackers randomly purchased in different supermarkets from the autonomous community of Madrid. Dietary exposure calculations were performed deterministically using mean acrylamide concentrations in food and food consumption data from a national household survey of Spanish consumers (MAPA, 2005). Dietary exposure for the whole population was estimated to be 0.2 µg/kg bw per day on average. No high-percentile (95th–97.5th percentile) exposure data were

provided. The main foods contributing to total exposure were potato chips (23%), biscuits (14.5%) and breakfast cereals (9.5%).

7.1.8 United Kingdom

A publication from Mills et al. (2008) gives estimates of dietary exposure to acrylamide for the United Kingdom population. Acrylamide concentrations (n = 7000) used in calculations were taken from the European Union acrylamide monitoring database (EC, 2006). Dietary exposures to acrylamide were generated deterministically using mean acrylamide concentrations and individual food consumption data reported for 2000 adults in the 2000 National Diet and Nutrition Survey. The dietary exposure estimates for the adult population (19 years of age and older) were 0.6 µg/kg bw per day on average and 1.3 µg/kg bw per day for the 97.5th percentile. The main food group contributors to total exposure were potato chips (21%), white bread (13%) and potato crisps (9%).

7.1.9 Summary of national dietary exposure estimates

A summary of the results is presented in Table 13. Estimates of mean dietary exposures at the national level ranged from 0.2 to 1.0 µg/kg bw per day for the general adult population. For adult consumers at the high (95th–97.5th) percentile, the estimates of dietary exposure ranged from 0.6 to 1.8 µg/kg bw per day. Based on the few data available for children, it was noted that children had dietary exposures to acrylamide that were about twice those of adult consumers when expressed on a body weight basis. The Committee noted that these estimates were similar to those used in the assessment performed by the sixty-fourth meeting, at which a dietary exposure to acrylamide of 1 µg/kg bw per day was taken to represent the mean for the general population and a dietary exposure of 4 µg/kg bw per day was taken to represent consumers with high exposure.

The major foods contributing to the total mean dietary exposure for most countries were potato chips (USA = french fries) (10–60%), potato crisps (USA = chips) (10–22%), bread and rolls/toast (13–34%) and pastry and sweet biscuits (USA = cookies) (10–15%). Generally, other food items contributed less than 10% to the total dietary exposure. The Committee noted that these contributions to overall exposure were consistent with the major contributing foods identified by the sixty-fourth meeting.

The Committee recognized that it was difficult to have a clear picture of national trends in dietary exposures since the last evaluation and noted that this was mainly due to the lack of updated dietary exposure data from the countries evaluated at the previous meeting. Additionally, there were differences in methodologies used in evaluations within a single country for obtaining data on consumption and occurrence.

Table 13. Summary of updated dietary exposure assessments for acrylamide in various countries evaluated at this meeting

Country	Population group	Average[a] or 50th percentile exposure (μg/kg bw per day)	95th- or 97.5th-percentile exposure (μg/kg bw per day)	Reference	Comments
Brazil	Adolescents (11–17 years)	0.1	0.8 (P97.5)	Arisseto et al. (2009)	Regional 24 h dietary recall survey Deterministic modelling (mean occurrence data, <LOR = ½ LOR) Major contributing foods: potato chips (60%), bread (13%), salt biscuits (11%), coffee (9%)
China	Adults (≥15 years)	0.29	0.58 (P97.5)	Chinese CDC (2009)	2000 and 2007 Chinese total diet individual food consumption, and 2007 Chinese total diet concentration Deterministic modelling (mean occurrence data, <LOR = ½ LOR) Major contributing foods to exposure are cooked vegetables, including potato fried (48.4%), cereal (27.1%) and potato boiled and baked (8%)
	Adults (≥15 years)	0.4	1.5 (P97.5)	Chen et al. (2008)	National individual food consumption (MHPRC, 2002) Deterministic modelling (mean occurrence data, <LOR = ½ LOR) Major contributing foods to exposure are flour and flour products (55%), potato crisps and potato chips (18%) and spices (13%)

Table 13 (contd)

Country	Population group	Average[a] or 50th percentile exposure (µg/kg bw per day)	95th- or 97.5th-percentile exposure (µg/kg bw per day)	Reference	Comments
France	Adults (>15 years) Children (3–14 years)	0.4 0.7	1.0 (P95) 1.8 (P95)	AFSSA (2009)	National individual food consumption (INCA2) Deterministic modelling (mean occurrence data, <LOR = ½ LOR) Major contributing foods to total exposure are potato chips (45–60%), coffee ready to drink (29.5%), biscuits (4–11%), salted biscuits (5–8%) and bread (5%)
Ireland	Adults (18–64 years)	0.6	1.8 (P97.5)	Mills et al. (2008)	National individual food consumption survey (1997–1999) Probabilistic modelling (CREMe 2.0 food model software) Main food group contributors to total exposure are potato and potato products (48%), bread (34%) and biscits (10%)
New Zealand	Adults (>19 years)	0.7–1.0	—	Love & Grounds (2006)	National individual food consumption (1999, 2003 and 2005) Deterministic modelling (mean occurrence, <LOR = 0 and LOR) Major contributing foods to total exposure are potatoes hot chips (9–23%), potato crisps (10–22% for adolescents, preschool children and
	Adolescents (11–14 years)	1.4–1.6	—		
	Preschool children (1–6 years)	2.3	—		
	Infants (6–12 months)	1.7	—		

Table 13 (contd)

Country	Population group	Average[a] or 50th percentile exposure (µg/kg bw per day)	95th- or 97.5th-percentile exposure (µg/kg bw per day)	Reference	Comments
					infants), roasted potatoes (17–20% for adolescents and preschool children), wheat biscuits (13% for preschool children 1–3 years of age)
Norway	Pregnant women (23–44 years)	0.5 (P50)	0.9 (P95)	Brantsaeter et al. (2008)	FFQ Deterministic modelling (mean occurrence data) Major contributing foods: crispbread (22%), potato crisps (16%), snacks, peanuts and popcorn (12%) and bread (11%)
Spain	Whole population	0.2	—	Rufián-Henares, Arribas-Lorenzo & Morales (2007)	National household survey (MAPA, 2005) Deterministic modelling (mean occurrence data) Major contributing foods: potato chips (23%), biscuits (14.5%) and breakfast cereals (9.5%)
United Kingdom	Adults (≥19 years)	0.6	1.3 (P97.5)	Mills et al. (2008)	National food consumption survey (2000) Deterministic modelling (mean occurrence data, <LOR = LOR) Main food group contributors to total exposure are potato chips (21%), white bread (13%) and potato crisps (9%)

P95, 95th percentile; P97.5, 97.5th percentile

[a] Unless otherwise indicated.

7.2 Regional estimates of dietary exposure using consumption cluster diets

As acrylamide occurs in every part of the world, data on food consumption from the GEMS/Food consumption cluster diets and data on food contamination collected from the world and summarized in Table 12 have been considered for the estimation of the regional dietary exposure. The GEMS/Food consumption cluster diets derived from average food balance sheet data for the period 1997–2001 were available from 183 countries (WHO, 2006). In performing the consumption cluster analysis, which differentiates 13 regional dietary patterns of raw and semi-processed food commodities using standard Food and Agriculture Organization of the United Nations (FAO) processing factors, the average dietary exposure for each food item at the cluster level was weighted by the population size of the reporting country. Consequently, in some clusters that include large countries, the composition of the cluster diet will largely reflect the composition of the large countries. In general, the food items analysed were well characterized, and it was possible to combine them with the GEMS/Food classification.

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, available acrylamide residue data from processed foodstuffs (Table 12) were matched and combined as closely as possible to the raw and semi-processed food commodities of the GEMS/Food food consumption patterns for the 13 clusters (e.g. the consumption of cassava has been combined with mean acrylamide levels taken from cassava, raw/boiled, and from processed cassava products). The Committee noted that these estimates were more refined than those prepared at the sixty-fourth meeting, which were based on the then-available five GEMS/Food regional consumption diets. Considering the high consumption of plantain by consumers in some regions and the lack of available analytical data provided at this meeting for this commodity, the acrylamide level reported for this commodity at the sixty-fourth meeting of the Committee (Table 14) was considered in this evaluation.

The summary of the results reviewed at the present meeting is presented in Table 15. The Committee estimated the international mean dietary exposures to range between 1.1 and 4.8 µg/kg bw per day across the 13 GEMS/Food consumption cluster diets, assuming a body weight of 60 kg. Cereals and root- and tuber-based foods were the main contributors to the total dietary exposure calculations for each cluster diet. Dietary exposures from cereal-based foods are between about 0.5 and 2.8 µg/kg bw per day. Depending on the patterns of consumption in each cluster, processed foods based on wheat, maize and rice were the main commodities contributing to overall exposure from cereal-based foods. Dietary exposures from roots and tubers ranged from 0.2 to 2.2 µg/kg bw per day. Processed potato was the main contributor to overall dietary exposure in most cluster diets. Food commodities based on peas, cassava and plantain were also major contributors for some cluster diets, specifically clusters A and J. Other GEMS/Food commodities contributed less than 10% to the total dietary exposure estimations.

Table 14. Summary of international dietary exposure assessments for acrylamide according to commodities evaluated from the 13 GEMS/Food consumption cluster diets (revision June 2006) (mean body weight = 60 kg), based on the analytical occurrence data compiled from the previous meeting of the Committee

(a) Clusters A–D

Commodities	Food	Mean acrylamide concentration (µg/kg)	A			B			C			D		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Cereals	Barley	168	40.6	0.11	6.0	16.8	0.05	1.0	93.9	0.26	7.2	13.2	0.04	0.8
	Maize	133	82.7	0.18	9.7	148.4	0.33	7.3	135.9	0.30	8.2	31.8	0.07	1.5
	Oats	133	1.4	0.00	0.2	0.5	0.00	0.0	0.2	0.00	0.0	4.2	0.01	0.2
	Rice	83	91.0	0.13	6.7	31.6	0.04	1.0	94.5	0.13	3.6	33.2	0.05	1.0
	Rye	405	0.1	0.00	0.0	3.7	0.02	0.6	0.2	0.00	0.0	24.3	0.16	3.4
	Wheat	284	88.4	0.42	22.2	396.3	1.87	41.8	426.5	2.02	55.0	390.2	1.85	38.4
	Total			**0.84**	**44.8**		**2.32**	**51.7**		**2.71**	**74.0**		**2.17**	**45.2**
Roots and tubers	Cassava	46	242.8	0.19	9.9	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Potatoes	548	19.1	0.17	9.2	160.8	1.47	32.7	61.2	0.56	15.2	243.6	2.23	46.4
	Total			**0.36**	**19.1**		**1.47**	**32.7**		**0.56**	**15.2**		**2.23**	**46.4**
Pulses	Beans dry	14	15.8	0.00	0.2	6.1	0.00	0.0	1.7	0.00	0.0	6.3	0.00	0.0
	Soya bean (dry)	18	9.9	0.00	0.2	36.4	0.01	0.2	34.3	0.01	0.3	22.1	0.01	0.1

Table 14 (a) Clusters A–D (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	A			B			C			D		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
	Peas dry	17	6.8	0.00	0.1	1.3	0.00	0.0	1.0	0.00	0.0	2.3	0.00	0.0
	Soya sauce	3	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Total			**0.01**	**0.4**		**0.01**	**0.3**		**0.01**	**0.3**		**0.01**	**0.2**
Sugars and honey	Sugar refined	16	21.0	**0.01**	**0.3**	76.6	**0.02**	**0.5**	54.5	**0.01**	**0.4**	73.1	**0.02**	**0.4**
Nuts and oilseeds	Oilseed	229	20.0	0.08	4.1	50.5	0.19	4.3	35.6	0.14	3.7	32.1	0.12	2.6
	Tree nuts	97	4.2	0.01	0.4	13.2	0.02	0.5	4.1	0.01	0.2	2.0	0.00	0.1
	Total			**0.08**	**4.4**		**0.21**	**4.8**		**0.14**	**3.9**		**0.13**	**2.6**
Vegetables	Artichoke, globe		0.0	0.00	0.0	10.0	0.00	0.0	2.1	0.00	0.0	0.1	0.00	0.0
	Asparagus	4	0.0	0.00	0.0	1.1	0.00	0.0	0.6	0.00	0.0	0.2	0.00	0.0
	Beetroot	5	0.0	0.00	0.0	40.7	0.00	0.1	0.0	0.00	0.0	0.1	0.00	0.0
	Broccoli	5	0.0	0.00	0.0	0.7	0.00	0.0	1.2	0.00	0.0	0.1	0.00	0.0
	Cabbages		2.1	0.00	0.0	19.8	0.00	0.0	8.3	0.00	0.0	43.9	0.00	0.0
	Carrots	33	0.6	0.00	0.0	15.1	0.01	0.2	8.1	0.00	0.1	13.9	0.01	0.2
	Cauliflower	54	0.1	0.00	0.0	5.2	0.00	0.1	1.2	0.00	0.0	0.1	0.00	0.0
	Cucumbers/ gherkins		0.6	0.00	0.0	25.4	0.00	0.0	11.8	0.00	0.0	23.1	0.00	0.0

Table 14 (a) Clusters A–D (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	A			B			C			D		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
	Eggplant	33	1.7	0.00	0.0	17.5	0.01	0.2	12.3	0.01	0.2	1.7	0.00	0.0
	Mushrooms	28	0.0	0.00	0.0	1.5	0.00	0.0	0.1	0.00	0.0	0.2	0.00	0.0
	Onion, bulb	93	5.5	0.01	0.5	49.5	0.08	1.7	33.0	0.05	1.4	31.3	0.05	1.0
	Peppers		1.4	0.00	0.0	29.9	0.00	0.0	13.0	0.00	0.0	6.3	0.00	0.0
	Squash, pumpkins, gourds	25	16.3	0.01	0.4	12.3	0.01	0.1	14.4	0.01	0.2	21.9	0.01	0.2
	Spinach	4	0.0	0.00	0.0	5.0	0.00	0.0	1.1	0.00	0.0	0.1	0.00	0.0
	Tomato		11.8	0.00	0.0	185.0	0.00	0.0	118.0	0.00	0.0	60.7	0.00	0.0
	Total			**0.02**	**0.9**		**0.11**	**2.4**		**0.07**	**1.9**		**0.07**	**1.4**
Stimulants	Chicory roots		0.0	0.00	0.0	0.2	0.00	0.0	0.0	0.00	0.0	0.6	0.00	0.0
	Cocoa beans	104	1.5	0.00	0.1	7.1	0.01	0.3	1.3	0.00	0.1	1.7	0.00	0.1
	Coffee beans	267	3.1	0.01	0.7	12.7	0.06	1.3	3.0	0.01	0.4	1.3	0.01	0.1
	Tea	324	0.3	0.00	0.1	2.4	0.01	0.3	2.8	0.01	0.4	2.1	0.01	0.2
	Total			**0.02**	**1.0**		**0.08**	**1.8**		**0.03**	**0.8**		**0.02**	**0.4**

Table 14 (a) Clusters A–D (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	A			B			C			D		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Fish and seafood	Crustaceans	20	0.2	0.00	0.0	2.6	0.00	0.0	0.4	0.00	0.0	0.2	0.00	0.0
	Freshwater fish	38	5.4	0.00	0.2	3.0	0.00	0.0	5.3	0.00	0.1	4.2	0.00	0.1
	Marine fish	41	8.8	0.01	0.3	20.4	0.01	0.3	8.7	0.01	0.2	17.8	0.01	0.3
	Molluscs/ cephalopods	17	0.0	0.00	0.0	9.8	0.00	0.1	0.1	0.00	0.0	0.2	0.00	0.0
	Total			**0.01**	**0.5**		**0.02**	**0.4**		**0.01**	**0.3**		**0.01**	**0.3**
Eggs	Chicken eggs	2	2.2	**0.00**	**0.0**	29.5	**0.00**	**0.0**	10.6	**0.00**	**0.0**	24.0	**0.00**	**0.0**
Fruits	Apples	2	0.3	0.00	0.0	60.5	0.00	0.1	18.5	0.00	0.0	39.9	0.00	0.0
	Bananas	199	38.8	0.13	6.8	17.4	0.06	1.3	16.0	0.05	1.5	6.6	0.02	0.5
	Currants		0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0	2.2	0.00	0.0
	Dates		0.8	0.00	0.0	1.4	0.00	0.0	31.5	0.00	0.0	5.1	0.00	0.0
	Dried fruit		0.0	0.00	0.0	0.2	0.00	0.0	0.1	0.00	0.0	0.3	0.00	0.0
	Apricots		0.3	0.00	0.0	6.2	0.00	0.0	3.9	0.00	0.0	3.2	0.00	0.0
	Figs	5	0.1	0.00	0.0	2.7	0.00	0.0	4.4	0.00	0.0	0.3	0.00	0.0
	Grapes	4	3.7	0.00	0.0	128.5	0.01	0.2	27.1	0.00	0.1	33.1	0.00	0.0
	Papayas		5.1	0.00	0.0	0.1	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0

Table 14 (a) Clusters A–D (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	A			B			C			D		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
	Pineapples	8	3.8	0.00	0.0	6.2	0.00	0.0	0.6	0.00	0.0	0.9	0.00	0.0
	Plantains	80	275.7	0.37	19.4	1.7	0.00	0.0	0.0	0.00	0.0	0.1	0.00	0.0
	Plums (including prunes)	182	0.1	0.00	0.0	5.9	0.02	0.4	2.5	0.01	0.2	7.3	0.02	0.5
	Total			**0.50**	**26.3**		**0.09**	**2.0**		**0.06**	**1.7**		**0.05**	**1.0**
Milk/milk products		8		**0.00**	**0.3**	178.5	**0.02**	**0.5**	52.0	**0.01**	**0.2**	284.2	**0.04**	**0.8**
Meat and offals		34		**0.02**	**1.2**	190.0	**0.11**	**2.4**	77.1	**0.04**	**1.2**	91.0	**0.05**	**1.1**
Beverages (beer, cider, spirit, wine)		7		**0.02**	**0.8**	160.9	**0.02**	**0.4**	5.2	**0.00**	**0.0**	81.4	**0.01**	**0.2**
Total				**1.9**			**4.5**			**3.7**			**4.8**	

Table 14 (b) Clusters E–G (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	E			F			G		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Cereals	Barley	168	48.6	0.14	3.1	36.1	0.10	2.5	5.9	0.02	0.7
	Maize	133	33.3	0.07	1.7	7.5	0.02	0.4	35.2	0.08	3.4
	Oats	133	5.7	0.01	0.3	8.9	0.02	0.5	0.2	0.00	0.0
	Rice	83	12.6	0.02	0.4	12.6	0.02	0.4	376.9	0.52	22.7
	Rye	405	25.8	0.17	4.0	45.8	0.31	7.8	0.4	0.00	0.1
	Wheat	284	236.3	1.12	25.8	216.0	1.02	25.7	172.9	0.82	35.7
	Total			**1.53**	**35.4**		**1.49**	**37.4**		**1.44**	**62.7**
Roots and tubers	Cassava	46	0.0	0.00	0.0	0.0	0.00	0.0	15.6	0.01	0.5
	Potatoes	548	230.1	2.10	48.6	204.7	1.87	47.1	52.7	0.48	21.0
	Total			**2.10**	**48.6**		**1.87**	**47.1**		**0.49**	**21.5**
Pulses	Beans dry	14	1.8	0.00	0.0	5.0	0.00	0.0	3.4	0.00	0.0
	Soya bean (dry)	18	34.8	0.01	0.2	39.1	0.01	0.3	25.9	0.01	0.3
	Peas dry	17	4.6	0.00	0.0	3.4	0.00	0.0	1.8	0.00	0.0
	Soya sauce	3	0.3	0.00	0.0	0.4	0.00	0.0	6.6	0.00	0.0
	Total			**0.01**	**0.3**		**0.01**	**0.3**		**0.01**	**0.4**

Table 14 (b) Clusters E–G (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	E			F			G		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Sugars and honey	Sugar refined	16	96.7	**0.03**	**0.6**	91.9	**0.02**	**0.6**	31.1	**0.01**	**0.4**
Nuts and oilseeds	Oilseed	229	62.1	0.24	5.5	30.6	0.12	2.9	26.1	0.10	4.4
	Tree nuts	97	4.0	0.01	0.2	4.7	0.01	0.2	16.4	0.03	1.2
	Total			**0.24**	**5.6**		**0.12**	**3.1**		**0.13**	**5.5**
Vegetables	Artichoke, globe		0.8	0.00	0.0	0.1	0.00	0.0	0.1	0.00	0.0
	Asparagus	4	1.2	0.00	0.0	0.1	0.00	0.0	3.7	0.00	0.0
	Beetroot	5	6.0	0.00	0.0	0.1	0.00	0.0	0.0	0.00	0.0
	Broccoli	5	4.2	0.00	0.0	4.0	0.00	0.0	3.2	0.00	0.0
	Cabbages		29.9	0.00	0.0	28.0	0.00	0.0	23.6	0.00	0.0
	Carrots	33	27.1	0.01	0.3	28.4	0.02	0.4	5.4	0.00	0.1
	Cauliflower	54	4.2	0.00	0.1	4.0	0.00	0.1	3.2	0.00	0.1
	Cucumbers/gherkins		12.1	0.00	0.0	14.2	0.00	0.0	15.8	0.00	0.0
	Eggplant	33	0.8	0.00	0.0	0.4	0.00	0.0	20.1	0.01	0.5
	Mushrooms	28	5.3	0.00	0.1	1.4	0.00	0.0	0.4	0.00	0.0
	Onion, bulb	93	23.2	0.04	0.8	14.6	0.02	0.6	17.3	0.03	1.2

Table 14 (b) Clusters E–G (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	E			F			G		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
	Peppers		6.2	0.00	0.0	4.0	0.00	0.0	8.7	0.00	0.0
	Squash, pumpkins, gourds	25	3.2	0.00	0.0	1.0	0.00	0.0	7.1	0.00	0.1
	Spinach	4	2.6	0.00	0.0	0.1	0.00	0.0	9.4	0.00	0.0
	Tomato		31.6	0.00	0.0	40.9	0.00	0.0	23.5	0.00	0.0
	Total			**0.06**	**1.4**		**0.04**	**1.1**		**0.05**	**2.1**
Stimulants	Chicory roots		4.5	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Cocoa beans	104	11.8	0.02	0.5	10.8	0.02	0.5	0.8	0.00	0.1
	Coffee beans	267	10.1	0.04	1.0	18.0	0.08	2.0	0.3	0.00	0.1
	Tea	324	2.0	0.01	0.3	0.8	0.00	0.1	1.3	0.01	0.3
	Total			**0.08**	**1.8**		**0.10**	**2.6**		**0.01**	**0.4**
Fish and seafood	Crustaceans	20	1.6	0.00	0.0	4.3	0.00	0.0	3.6	0.00	0.1
	Freshwater fish	38	3.2	0.00	0.0	9.1	0.01	0.1	17.0	0.01	0.5
	Marine fish	41	18.7	0.01	0.3	35.0	0.02	0.6	9.4	0.01	0.3
	Molluscs/cephalopods	17	6.8	0.00	0.0	0.8	0.00	0.0	14.9	0.00	0.2
	Total			**0.02**	**0.4**		**0.03**	**0.8**		**0.02**	**1.0**

Table 14 (b) Clusters E–G (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	E			F			G		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Eggs	Chicken eggs	2	33.6	**0.00**	**0.0**	27.4	**0.00**	**0.0**	17.5	**0.00**	**0.0**
Fruits	Apples	2	50.8	0.00	0.0	39.4	0.00	0.0	14.4	0.00	0.0
	Bananas	199	21.5	0.07	1.7	33.8	0.11	2.8	21.4	0.07	3.1
	Currants		3.1	0.00	0.0	2.0	0.00	0.0	0.0	0.00	0.0
	Dates		0.3	0.00	0.0	0.2	0.00	0.0	0.9	0.00	0.0
	Dried fruit		0.2	0.00	0.0	0.3	0.00	0.0	0.2	0.00	0.0
	Apricots		2.0	0.00	0.0	0.8	0.00	0.0	0.2	0.00	0.0
	Figs	5	0.7	0.00	0.0	0.5	0.00	0.0	0.0	0.00	0.0
	Grapes	4	107.5	0.01	0.2	44.0	0.00	0.1	2.6	0.00	0.0
	Papayas		0.1	0.00	0.0	0.0	0.00	0.0	1.3	0.00	0.0
	Pineapples	8	7.6	0.00	0.0	8.0	0.00	0.0	3.9	0.00	0.0
	Plantains	80	0.3	0.00	0.0	0.0	0.00	0.0	1.8	0.00	0.1
	Plums (including prunes)	182	6.9	0.02	0.5	2.6	0.01	0.2	3.3	0.01	0.4
	Total			**0.10**	**2.4**		**0.13**	**3.2**		**0.08**	**3.7**

Table 14 (b) Clusters E–G (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	E			F			G		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Milk/milk products		8	178.6	**0.02**	**0.6**	237.1	**0.03**	**0.8**	41.9	**0.01**	**0.3**
Meat and offals		34	163.4	**0.09**	**2.1**	166.5	**0.09**	**2.4**	77.7	**0.04**	**1.9**
Beverages (beer, cider, spirit, wine)		7	311.9	**0.04**	**0.8**	186.9	**0.02**	**0.5**	22.9	**0.00**	**0.1**
Total				**4.3**			**4.0**			**2.3**	

Table 14 (c) Clusters H–J (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	H			I			J		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Cereals	Barley	168	20.5	0.06	2.5	5.9	0.02	0.9	2.5	0.01	0.7
	Maize	133	298.6	0.66	28.4	248.1	0.55	28.8	57.4	0.13	12.5
	Oats	133	2.0	0.00	0.2	0.8	0.00	0.1	0.0	0.00	0.0
	Rice	83	64.3	0.09	3.8	37.9	0.05	2.7	74.3	0.10	10.1
	Rye	405	0.0	0.00	0.0	0.2	0.00	0.1	0.1	0.00	0.1
	Wheat	284	79.0	0.37	16.0	68.1	0.32	16.9	41.9	0.20	19.5
	Total			**1.19**	**50.9**		**0.94**	**49.5**		**0.44**	**42.9**
Roots and tubers	Cassava	46	23.9	0.02	0.8	171.3	0.13	6.9	282.2	0.22	21.3
	Potatoes	548	57.1	0.52	22.4	50.1	0.46	24.0	4.3	0.04	3.9
	Total			**0.54**	**23.2**		**0.59**	**30.9**		**0.26**	**25.2**
Pulses	Beans dry	14	25.5	0.01	0.2	7.8	0.00	0.1	2.1	0.00	0.0
	Soya bean (dry)	18	59.3	0.02	0.8	11.0	0.00	0.2	11.0	0.00	0.3
	Peas dry	17	2.3	0.00	0.0	3.3	0.00	0.0	26.7	0.01	0.7
	Soya sauce	3	0.1	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Total			**0.02**	**1.0**		**0.01**	**0.3**		**0.01**	**1.1**

Table 14 (c) Clusters H–J (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	H			I			J		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Sugars and honey	Sugar refined	16	97.5	**0.03**	**1.1**	42.7	**0.01**	**0.6**	22.1	**0.01**	**0.6**
Nuts and oilseeds	Oilseed	229	17.9	0.07	2.9	21.9	0.08	4.4	35.9	0.14	13.5
	Tree nuts	97	15.0	0.02	1.0	9.8	0.02	0.8	1.9	0.00	0.3
	Total			**0.09**	**4.0**		**0.10**	**5.2**		**0.14**	**13.8**
Vegetables	Artichoke, globe		0.1	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Asparagus	4	0.3	0.00	0.0	0.2	0.00	0.0	0.0	0.00	0.0
	Beetroot	5	0.1	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Broccoli	5	7.8	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Cabbages		12.0	0.00	0.0	5.0	0.00	0.0	1.9	0.00	0.0
	Carrots	33	7.9	0.00	0.2	2.5	0.00	0.1	3.5	0.00	0.2
	Cauliflower	54	7.8	0.01	0.3	0.3	0.00	0.0	0.1	0.00	0.0
	Cucumbers/gherkins		1.3	0.00	0.0	0.3	0.00	0.0	0.0	0.00	0.0
	Eggplant	33	0.1	0.00	0.0	0.6	0.00	0.0	6.3	0.00	0.3
	Mushrooms	28	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Onion, bulb	93	27.8	0.04	1.9	7.4	0.01	0.6	16.0	0.02	2.4

Table 14 (c) Clusters H–J (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	H			I			J		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
	Peppers		22.4	0.00	0.0	8.4	0.00	0.0	9.4	0.00	0.0
	Squash, pumpkins, gourds	25	4.6	0.00	0.1	11.3	0.00	0.2	3.0	0.00	0.1
	Spinach	4	0.4	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Tomato		31.7	0.00	0.0	15.0	0.00	0.0	16.1	0.00	0.0
	Total			**0.06**	**2.5**		**0.02**	**1.0**		**0.03**	**3.1**
Stimulants	Chicory roots		0.0	0.00	0.0	1.1	0.00	0.0	0.0	0.00	0.0
	Cocoa beans	104	3.6	0.01	0.3	0.8	0.00	0.1	0.8	0.00	0.1
	Coffee beans	267	7.0	0.03	1.3	0.5	0.00	0.1	0.2	0.00	0.1
	Tea	324	0.2	0.00	0.0	0.9	0.00	0.2	0.6	0.00	0.3
	Total			**0.04**	**1.6**		**0.01**	**0.4**		**0.01**	**0.5**
Fish and seafood	Crustaceans	20	1.0	0.00	0.0	0.2	0.00	0.0	0.3	0.00	0.0
	Freshwater fish	38	2.6	0.00	0.1	4.4	0.00	0.1	4.6	0.00	0.3
	Marine fish	41	10.4	0.01	0.3	7.1	0.00	0.3	11.0	0.01	0.7
	Molluscs/cephalopods	17	2.7	0.00	0.0	0.2	0.00	0.0	0.2	0.00	0.0
	Total			**0.01**	**0.4**		**0.01**	**0.4**		**0.01**	**1.0**

Table 14 (c) Clusters H–J (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	H			I			J		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Eggs	Chicken eggs	2	28.0	**0.00**	**0.0**	6.1	**0.00**	**0.0**	5.1	**0.00**	**0.0**
Fruits	Apples	2	10.1	0.00	0.0	2.2	0.00	0.0	0.0	0.00	0.0
	Bananas	199	36.6	0.12	5.2	11.4	0.04	2.0	9.2	0.03	3.0
	Currants		0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Dates		0.1	0.00	0.0	0.1	0.00	0.0	3.8	0.00	0.0
	Dried fruit		0.0	0.00	0.0	0.2	0.00	0.0	0.1	0.00	0.0
	Apricots		0.1	0.00	0.0	0.2	0.00	0.0	0.0	0.00	0.0
	Figs	5	0.1	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Grapes	4	4.8	0.00	0.0	11.7	0.00	0.0	0.3	0.00	0.0
	Papayas		11.5	0.00	0.0	1.6	0.00	0.0	13.7	0.00	0.0
	Pineapples	8	11.7	0.00	0.1	12.6	0.00	0.1	11.1	0.00	0.1
	Plantains	80	51.2	0.07	2.9	93.3	0.12	6.5	40.6	0.05	5.3
	Plums (including prunes)	182	1.4	0.00	0.2	0.1	0.00	0.0	0.0	0.00	0.0
	Total			**0.20**	**8.4**		**0.16**	**8.6**		**0.09**	**8.5**

Table 14 (c) Clusters H–J (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	H			I			J		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Milk/milk products		8	119.6	**0.02**	**0.7**	71.5	**0.01**	**0.5**	36.6	**0.01**	**0.5**
Meat and offals		34	232.6	**0.13**	**5.6**	61.7	**0.03**	**1.8**	37.2	**0.02**	**2.1**
Beverages (beer, cider, spirit, wine)		7	103.6	**0.01**	**0.5**	117.4	**0.01**	**0.7**	55.4	**0.01**	**0.6**
Total				**2.3**			**1.9**			**1.0**	

Table 14 (d) Clusters K–M (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	K			L			M		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Cereals	Barley	168	20.2	0.06	2.5	16.8	0.05	2.1	43.76	0.12	3.3
	Maize	133	63.1	0.14	6.1	58.6	0.13	5.7	85.48	0.19	5.1
	Oats	133	3.5	0.01	0.3	0.7	0.00	0.1	7.54	0.02	0.4
	Rice	83	238.3	0.33	14.3	381.2	0.53	23.2	34.48	0.05	1.3
	Rye	405	0.1	0.00	0.0	0.9	0.01	0.3	0.73	0.00	0.1
	Wheat	284	114.1	0.54	23.5	103.4	0.49	21.6	234.17	1.11	29.7
	Total			**1.07**	**46.7**		**1.20**	**52.9**		**1.49**	**39.9**
Roots and tubers	Cassava	46	57.7	0.04	1.9	20.0	0.02	0.7	0.66	0.00	0.0
	Potatoes	548	54.7	0.50	21.8	41.0	0.37	16.5	167.98	1.53	41.1
	Total			**0.54**	**23.7**		**0.39**	**17.2**		**1.53**	**41.2**
Pulses	Beans dry	14	44.7	0.01	0.4	5.5	0.00	0.1	7.30	0.00	0.0
	Soya bean (dry)	18	109.3	0.03	1.4	51.5	0.02	0.7	123.22	0.04	1.0
	Peas dry	17	1.5	0.00	0.0	1.7	0.00	0.0	1.88	0.00	0.0
	Soya sauce	3	0.0	0.00	0.0	13.9	0.00	0.0	0.36	0.00	0.0
	Total			**0.04**	**1.9**		**0.02**	**0.8**		**0.04**	**1.1**

Table 14 (d) Clusters K–M (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	K			L			M		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Sugars and honey	Sugar refined	16	123.6	**0.03**	**1.4**	48.3	**0.01**	**0.6**	88.18	**0.02**	**0.6**
Nuts and oilseeds	Oilseed	229	7.5	0.03	1.3	62.7	0.24	10.6	29.74	0.11	3.0
	Tree nuts	97	19.2	0.03	1.3	29.0	0.05	2.1	5.14	0.01	0.2
	Total			**0.06**	**2.6**		**0.29**	**12.6**		**0.12**	**3.3**
Vegetables	Artichoke, globe		0.0	0.00	0.0	0.0	0.00	0.0	1.01	0.00	0.0
	Asparagus	4	0.0	0.00	0.0	0.5	0.00	0.0	1.12	0.00	0.0
	Beetroot	5	0.2	0.00	0.0	0.0	0.00	0.0	14.25	0.00	0.0
	Broccoli	5	0.3	0.00	0.0	0.4	0.00	0.0	6.58	0.00	0.0
	Cabbages		3.8	0.00	0.0	55.5	0.00	0.0	18.94	0.00	0.0
	Carrots	33	4.1	0.00	0.1	8.6	0.00	0.2	19.39	0.01	0.3
	Cauliflower	54	0.6	0.00	0.0	0.4	0.00	0.0	6.58	0.01	0.2
	Cucumbers/gherkins		10.9	0.00	0.0	0.8	0.00	0.0	10.57	0.00	0.0
	Eggplant	33	0.5	0.00	0.0	6.3	0.00	0.2	0.68	0.00	0.0
	Mushrooms	28	0.0	0.00	0.0	0.5	0.00	0.0	3.91	0.00	0.0
	Onion, bulb	93	22.8	0.04	1.5	34.4	0.05	2.4	30.09	0.05	1.3

Table 14 (d) Clusters K–M (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	K			L			M		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
	Peppers		3.3	0.00	0.0	5.3	0.00	0.0	8.86	0.00	0.0
	Squash, pumpkins, gourds	25	7.0	0.00	0.1	6.7	0.00	0.1	7.62	0.00	0.1
	Spinach	4	0.2	0.00	0.0	4.3	0.00	0.0	1.98	0.00	0.0
	Tomato		35.6	0.00	0.0	9.9	0.00	0.0	102.96	0.00	0.0
	Total			**0.04**	**1.8**		**0.07**	**2.9**		**0.07**	**1.9**
Stimulants	Chicory roots		0.0	0.00	0.0	0.0	0.00	0.0	0.00	0.00	0.0
	Cocoa beans	104	4.5	0.01	0.3	2.5	0.00	0.2	11.39	0.02	0.5
	Coffee beans	267	5.3	0.02	1.0	5.7	0.03	1.1	12.46	0.06	1.5
	Tea	324	0.1	0.00	0.0	1.5	0.01	0.4	0.98	0.01	0.1
	Total			**0.03**	**1.4**		**0.04**	**1.7**		**0.08**	**2.2**
Fish and seafood	Crustaceans	20	0.8	0.00	0.0	4.6	0.00	0.1	4.86	0.00	0.0
	Freshwater fish	38	4.2	0.00	0.1	5.3	0.00	0.1	2.49	0.00	0.0
	Marine fish	41	7.4	0.01	0.2	47.4	0.03	1.4	13.81	0.01	0.3
	Molluscs/cephalopods	17	1.2	0.00	0.0	11.8	0.00	0.1	2.61	0.00	0.0
	Total			**0.01**	**0.4**		**0.04**	**1.8**		**0.01**	**0.4**

Table 14 (d) Clusters K–M (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	K			L			M		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Eggs	Chicken eggs	2	16.9	**0.00**	**0.0**	33.5	**0.00**	**0.0**	34.35	**0.00**	**0.0**
Fruits	Apples	2	9.8	0.00	0.0	17.9	0.00	0.0	36.32	0.00	0.0
	Bananas	199	70.2	0.23	10.2	40.5	0.13	5.9	32.64	0.11	2.9
	Currants		0.0	0.00	0.0	0.0	0.00	0.0	0.03	0.00	0.0
	Dates		0.0	0.00	0.0	0.0	0.00	0.0	0.20	0.00	0.0
	Dried fruit		0.0	0.00	0.0	0.3	0.00	0.0	0.08	0.00	0.0
	Apricots		0.0	0.00	0.0	0.1	0.00	0.0	1.09	0.00	0.0
	Figs	5	0.2	0.00	0.0	0.0	0.00	0.0	0.39	0.00	0.0
	Grapes	4	6.7	0.00	0.0	10.9	0.00	0.0	58.66	0.00	0.1
	Papayas		14.5	0.00	0.0	1.0	0.00	0.0	0.64	0.00	0.0
	Pineapples	8	16.6	0.00	0.1	20.9	0.00	0.1	22.17	0.00	0.1
	Plantains	80	39.2	0.05	2.3	1.1	0.00	0.1	1.87	0.00	0.1
	Plums (including prunes)	182	0.5	0.00	0.1	1.5	0.00	0.2	2.21	0.01	0.2
	Total			**0.29**	**12.6**		**0.14**	**6.4**		**0.13**	**3.4**

Table 14 (d) Clusters K–M (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	K			L			M		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Milk/milk products		8	205.6	**0.03**	**1.2**	55.9	**0.01**	**0.3**	285.43	**0.04**	**1.0**
Meat and offals		34	235.3	**0.13**	**5.8**	96.4	**0.05**	**2.4**	279.07	**0.16**	**4.2**
Beverages (beer, cider, spirit, wine)		7	104.3	**0.01**	**0.5**	85.8	**0.01**	**0.4**	295.33	**0.03**	**0.9**
Total				**2.3**			**2.3**			**3.7**	

Table 15. Summary of international dietary exposure assessments for acrylamide according to commodities evaluated from the 13 GEMS/Food consumption cluster diets (revision June 2006) (mean body weight = 60 kg), based on the analytical occurrence data compiled from the present meeting of the Committee

(a) Clusters A–D

Commodities	Food	Mean acrylamide concentration (µg/kg)	A			B			C			D		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Cereals	Barley	153	40.6	0.10	5.6	16.8	0.04	0.9	93.9	0.24	6.3	13.2	0.03	0.7
	Maize	172	82.7	0.24	12.8	148.4	0.43	9.0	135.9	0.39	10.3	31.8	0.09	1.9
	Oats	142	1.4	0.00	0.2	0.5	0.00	0.0	0.2	0.00	0.0	4.2	0.01	0.2
	Rice	65	91.0	0.10	5.3	31.6	0.03	0.7	94.5	0.10	2.7	33.2	0.04	0.7
	Rye	233	0.1	0.00	0.0	3.7	0.01	0.3	0.2	0.00	0.0	24.3	0.09	1.9
	Wheat	289	88.4	0.43	22.9	396.3	1.91	40.3	426.5	2.05	54.3	390.2	1.88	38.8
	Total			**0.87**	**46.7**		**2.43**	**51.3**		**2.79**	**73.7**		**2.14**	**44.3**
Roots and tubers	Cassava	28	242.8	0.11	6.1	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Potatoes	535	19.1	0.17	9.1	160.8	1.43	30.3	61.2	0.55	14.4	243.6	2.17	44.9
	Total			**0.28**	**15.3**		**1.43**	**30.3**		**0.55**	**14.4**		**2.17**	**44.9**
Pulses	Beans dry	40	15.8	0.01	0.6	6.1	0.00	0.1	1.7	0.00	0.0	6.3	0.00	0.1
	Soya bean (dry)	63	9.9	0.01	0.6	36.4	0.04	0.8	34.3	0.04	0.9	22.1	0.02	0.5

Table 15 (a) Clusters A–D (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	A			B			C			D		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
	Peas dry	349	6.8	0.04	2.1	1.3	0.01	0.2	1.0	0.01	0.2	2.3	0.01	0.3
	Soya sauce	7	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Total			**0.06**	**3.2**		**0.05**	**1.0**		**0.04**	**1.1**		**0.04**	**0.8**
Sugars and honey	Sugar refined	86	21.0	**0.03**	**1.6**	76.6	**0.11**	**2.3**	54.5	**0.08**	**2.1**	73.1	**0.11**	**2.2**
Nuts and oilseeds	Oilseed	131	20.0	0.04	2.3	50.5	0.11	2.3	35.6	0.08	2.1	32.1	0.07	1.4
	Tree nuts	104	4.2	0.01	0.4	13.2	0.02	0.5	4.1	0.01	0.2	2.0	0.00	0.1
	Total			**0.05**	**2.7**		**0.13**	**2.8**		**0.08**	**2.2**		**0.07**	**1.5**
Vegetables	Artichoke, globe	10	0.0	0.00	0.0	10.0	0.00	0.0	2.1	0.00	0.0	0.1	0.00	0.0
	Asparagus	100	0.0	0.00	0.0	1.1	0.00	0.0	0.6	0.00	0.0	0.2	0.00	0.0
	Beetroot	15	0.0	0.00	0.0	40.7	0.01	0.2	0.0	0.00	0.0	0.1	0.00	0.0
	Broccoli	20	0.0	0.00	0.0	0.7	0.00	0.0	1.2	0.00	0.0	0.1	0.00	0.0
	Cabbages	13	2.1	0.00	0.0	19.8	0.00	0.1	8.3	0.00	0.0	43.9	0.01	0.2
	Carrots	31	0.6	0.00	0.0	15.1	0.01	0.2	8.1	0.00	0.1	13.9	0.01	0.1
	Cauliflower	28	0.1	0.00	0.0	5.2	0.00	0.1	1.2	0.00	0.0	0.1	0.00	0.0

Table 15 (a) Clusters A–D (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	A			B			C			D		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
	Cucumbers/gherkins	17	0.6	0.00	0.0	25.4	0.01	0.2	11.8	0.00	0.1	23.1	0.01	0.1
	Eggplant	12	1.7	0.00	0.0	17.5	0.00	0.1	12.3	0.00	0.1	1.7	0.00	0.0
	Mushrooms	22	0.0	0.00	0.0	1.5	0.00	0.0	0.1	0.00	0.0	0.2	0.00	0.0
	Onion, bulb	61	5.5	0.01	0.3	49.5	0.05	1.1	33.0	0.03	0.9	31.3	0.03	0.7
	Peppers	96	1.4	0.00	0.1	29.9	0.05	1.0	13.0	0.02	0.5	6.3	0.01	0.2
	Squash, pumpkins, gourds	32	16.3	0.01	0.5	12.3	0.01	0.1	14.4	0.01	0.2	21.9	0.01	0.2
	Spinach	144	0.0	0.00	0.0	5.0	0.01	0.3	1.1	0.00	0.1	0.1	0.00	0.0
	Tomato	10	11.8	0.00	0.1	185.0	0.03	0.6	118.0	0.02	0.5	60.7	0.01	0.2
	Total			**0.02**	**1.1**		**0.19**	**3.9**		**0.10**	**2.6**		**0.09**	**1.8**
Stimulants	Chicory roots	2470	0.0	0.00	0.1	0.2	0.01	0.2	0.0	0.00	0.0	0.6	0.03	0.5
	Cocoa beans	128	1.5	0.00	0.2	7.1	0.02	0.3	1.3	0.00	0.1	1.7	0.00	0.1
	Coffee beans	314	3.1	0.02	0.9	12.7	0.07	1.4	3.0	0.02	0.4	1.3	0.01	0.1
	Tea	47	0.3	0.00	0.0	2.4	0.00	0.0	2.8	0.00	0.1	2.1	0.00	0.0
	Total			**0.02**	**1.1**		**0.09**	**1.9**		**0.02**	**0.5**		**0.04**	**0.8**

Table 15 (a) Clusters A–D (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	A			B			C			D		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Fish and seafood	Crustaceans	105	0.2	0.00	0.0	2.6	0.00	0.1	0.4	0.00	0.0	0.2	0.00	0.0
	Freshwater fish	56	5.4	0.01	0.3	3.0	0.00	0.1	5.3	0.00	0.1	4.2	0.00	0.1
	Marine fish	15	8.8	0.00	0.1	20.4	0.01	0.1	8.7	0.00	0.1	17.8	0.00	0.1
	Molluscs/ cephalopods	55	0.0	0.00	0.0	9.8	0.01	0.2	0.1	0.00	0.0	0.2	0.00	0.0
	Total			**0.01**	**0.4**		**0.02**	**0.5**		**0.01**	**0.2**		**0.01**	**0.2**
Eggs	Chicken eggs	18	2.2	**0.00**	**0.0**	29.5	**0.01**	**0.2**	10.6	**0.00**	**0.1**	24.0	**0.01**	**0.1**
Fruits	Apples	12	0.3	0.00	0.0	60.5	0.01	0.3	18.5	0.00	0.1	39.9	0.01	0.2
	Bananas	117	38.8	0.08	4.1	17.4	0.03	0.7	16.0	0.03	0.8	6.6	0.01	0.3
	Currants	107	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0	2.2	0.00	0.1
	Dates	15	0.8	0.00	0.0	1.4	0.00	0.0	31.5	0.01	0.2	5.1	0.00	0.0
	Dried fruit	35	0.0	0.00	0.0	0.2	0.00	0.0	0.1	0.00	0.0	0.3	0.00	0.0
	Apricots	32	0.3	0.00	0.0	6.2	0.00	0.1	3.9	0.00	0.1	3.2	0.00	0.0
	Figs	5	0.1	0.00	0.0	2.7	0.00	0.0	4.4	0.00	0.0	0.3	0.00	0.0
	Grapes	5	3.7	0.00	0.0	128.5	0.01	0.2	27.1	0.00	0.1	33.1	0.00	0.1
	Papayas	36	5.1	0.00	0.2	0.1	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0

Table 15 (a) Clusters A–D (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	A			B			C			D		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
	Pineapples	5	3.8	0.00	0.0	6.2	0.00	0.0	0.6	0.00	0.0	0.9	0.00	0.0
	Plantains	80	275.7	0.37	19.7	1.7	0.00	0.0	0.0	0.00	0.0	0.1	0.00	0.0
	Plums (including prunes)	98	0.1	0.00	0.0	5.9	0.01	0.2	2.5	0.00	0.1	7.3	0.01	0.2
	Total			**0.45**	**24.1**		**0.07**	**1.5**		**0.05**	**1.4**		**0.04**	**0.9**
Milk/milk products		6	34.5	**0.00**	**0.2**	178.5	**0.02**	**0.4**	52.0	**0.00**	**0.1**	284.2	**0.03**	**0.6**
Meat and offals		42	39.4	**0.03**	**1.5**	190.0	**0.13**	**2.8**	77.1	**0.05**	**1.4**	91.0	**0.06**	**1.3**
Beverages (beer, cider, spirit, wine)		17	135.0	**0.04**	**2.1**	160.9	**0.05**	**1.0**	5.2	**0.00**	**0.0**	81.4	**0.02**	**0.5**
Total				**1.9**			**4.7**			**3.8**			**4.8**	

Table 15 (b) Clusters E–G (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	E			F			G		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Cereals	Barley	153	48.6	0.12	2.7	36.1	0.09	2.3	5.9	0.01	0.7
	Maize	172	33.3	0.10	2.1	7.5	0.02	0.5	35.2	0.10	4.5
	Oats	142	5.7	0.01	0.3	8.9	0.02	0.5	0.2	0.00	0.0
	Rice	65	12.6	0.01	0.3	12.6	0.01	0.3	376.9	0.41	18.1
	Rye	233	25.8	0.10	2.2	45.8	0.18	4.5	0.4	0.00	0.1
	Wheat	289	236.3	1.14	25.0	216.0	1.04	26.3	172.9	0.83	36.8
	Total			**1.48**	**32.6**		**1.37**	**34.6**		**1.36**	**60.1**
Roots and tubers	Cassava	28	0.0	0.00	0.0	0.0	0.00	0.0	15.6	0.01	0.3
	Potatoes	535	230.1	2.05	45.0	204.7	1.83	46.2	52.7	0.47	20.8
	Total			**2.05**	**45.0**		**1.83**	**46.2**		**0.48**	**21.1**
Pulses	Beans dry	40	1.8	0.00	0.0	5.0	0.00	0.1	3.4	0.00	0.1
	Soya bean (dry)	63	34.8	0.04	0.8	39.1	0.04	1.0	25.9	0.03	1.2
	Peas dry	349	4.6	0.03	0.6	3.4	0.02	0.5	1.8	0.01	0.5
	Soya sauce	7	0.3	0.00	0.0	0.4	0.00	0.0	6.6	0.00	0.0
	Total			**0.06**	**1.4**		**0.06**	**1.6**		**0.04**	**1.8**

Table 15 (b) Clusters E–G (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	E			F			G		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Sugars and honey	Sugar refined	86	96.7	**0.14**	**3.0**	91.9	**0.13**	**3.3**	31.1	**0.04**	**2.0**
Nuts and oilseeds	Oilseed	131	62.1	0.14	3.0	30.6	0.07	1.7	26.1	0.06	2.5
	Tree nuts	104	4.0	0.01	0.2	4.7	0.01	0.2	16.4	0.03	1.3
	Total			**0.14**	**3.1**		**0.08**	**1.9**		**0.09**	**3.8**
Vegetables	Artichoke, globe	10	0.8	0.00	0.0	0.1	0.00	0.0	0.1	0.00	0.0
	Asparagus	100	1.2	0.00	0.0	0.1	0.00	0.0	3.7	0.01	0.3
	Beetroot	15	6.0	0.00	0.0	0.1	0.00	0.0	0.0	0.00	0.0
	Broccoli	20	4.2	0.00	0.0	4.0	0.00	0.0	3.2	0.00	0.0
	Cabbages	13	29.9	0.01	0.1	28.0	0.01	0.2	23.6	0.01	0.2
	Carrots	31	27.1	0.01	0.3	28.4	0.01	0.4	5.4	0.00	0.1
	Cauliflower	28	4.2	0.00	0.0	4.0	0.00	0.0	3.2	0.00	0.1
	Cucumbers/gherkins	17	12.1	0.00	0.1	14.2	0.00	0.1	15.8	0.00	0.2
	Eggplant	12	0.8	0.00	0.0	0.4	0.00	0.0	20.1	0.00	0.2
	Mushrooms	22	5.3	0.00	0.0	1.4	0.00	0.0	0.4	0.00	0.0
	Onion, bulb	61	23.2	0.02	0.5	14.6	0.01	0.4	17.3	0.02	0.8

Table 15 (b) Clusters E–G (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	E			F			G		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
	Peppers	96	6.2	0.01	0.2	4.0	0.01	0.2	8.7	0.01	0.6
	Squash, pumpkins, gourds	32	3.2	0.00	0.0	1.0	0.00	0.0	7.1	0.00	0.2
	Spinach	144	2.6	0.01	0.1	0.1	0.00	0.0	9.4	0.02	1.0
	Tomato	10	31.6	0.01	0.1	40.9	0.01	0.2	23.5	0.00	0.2
	Total			**0.08**	**1.7**		**0.06**	**1.4**		**0.09**	**3.9**
Stimulants	Chicory roots	2470	4.5	0.19	4.1	0.0	0.00	0.0	0.0	0.00	0.0
	Cocoa beans	128	11.8	0.03	0.6	10.8	0.02	0.6	0.8	0.00	0.1
	Coffee beans	314	10.1	0.05	1.2	18.0	0.09	2.4	0.3	0.00	0.1
	Tea	47	2.0	0.00	0.0	0.8	0.00	0.0	1.3	0.00	0.0
	Total			**0.26**	**5.8**		**0.12**	**3.0**		**0.00**	**0.2**
Fish and seafood	Crustaceans	105	1.6	0.00	0.1	4.3	0.01	0.2	3.6	0.01	0.3
	Freshwater fish	56	3.2	0.00	0.1	9.1	0.01	0.2	17.0	0.02	0.7
	Marine fish	15	18.7	0.00	0.1	35.0	0.01	0.2	9.4	0.00	0.1
	Molluscs/cephalopods	55	6.8	0.01	0.1	0.8	0.00	0.0	14.9	0.01	0.6
	Total			**0.02**	**0.4**		**0.03**	**0.6**		**0.04**	**1.7**

Table 15 (b) Clusters E–G (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	E			F			G		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Eggs	Chicken eggs	18	33.6	**0.01**	**0.2**	27.4	**0.01**	**0.2**	17.5	**0.01**	**0.2**
Fruits	Apples	12	50.8	0.01	0.2	39.4	0.01	0.2	14.4	0.00	0.1
	Bananas	117	21.5	0.04	0.9	33.8	0.07	1.7	21.4	0.04	1.9
	Currants	107	3.1	0.01	0.1	2.0	0.00	0.1	0.0	0.00	0.0
	Dates	15	0.3	0.00	0.0	0.2	0.00	0.0	0.9	0.00	0.0
	Dried fruit	35	0.2	0.00	0.0	0.3	0.00	0.0	0.2	0.00	0.0
	Apricots	32	2.0	0.00	0.0	0.8	0.00	0.0	0.2	0.00	0.0
	Figs	5	0.7	0.00	0.0	0.5	0.00	0.0	0.0	0.00	0.0
	Grapes	5	107.5	0.01	0.2	44.0	0.00	0.1	2.6	0.00	0.0
	Papayas	36	0.1	0.00	0.0	0.0	0.00	0.0	1.3	0.00	0.0
	Pineapples	5	7.6	0.00	0.0	8.0	0.00	0.0	3.9	0.00	0.0
	Plantains	80	0.3	0.00	0.0	0.0	0.00	0.0	1.8	0.00	0.1
	Plums (including prunes)	98	6.9	0.01	0.2	2.6	0.00	0.1	3.3	0.01	0.2
	Total			**0.08**	**1.8**		**0.09**	**2.2**		**0.05**	**2.4**

Table 15 (b) Clusters E–G (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	E			F			G		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Milk/milk products		6	178.6	**0.02**	**0.4**	237.1	**0.02**	**0.6**	41.9	**0.00**	**0.2**
Meat and offals		42	163.4	**0.12**	**2.5**	166.5	**0.12**	**3.0**	77.7	**0.05**	**2.4**
Beverages (beer, cider, spirit, wine)		17	311.9	**0.09**	**2.0**	186.9	**0.05**	**1.4**	22.9	**0.01**	**0.3**
Total				**4.6**			**4.0**			**2.3**	

Table 15 (c) Clusters H–J (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	H			I			J		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Cereals	Barley	153	20.5	0.05	2.0	5.9	0.02	0.7	2.5	0.01	0.6
	Maize	172	298.6	0.86	31.9	248.1	0.71	33.7	57.4	0.16	15.2
	Oats	142	2.0	0.00	0.2	0.8	0.00	0.1	0.0	0.00	0.0
	Rice	65	64.3	0.07	2.6	37.9	0.04	1.9	74.3	0.08	7.4
	Rye	233	0.0	0.00	0.0	0.2	0.00	0.0	0.1	0.00	0.0
	Wheat	289	79.0	0.38	14.1	68.1	0.33	15.5	41.9	0.20	18.6
	Total			**1.37**	**50.8**		**1.10**	**52.0**		**0.45**	**41.8**
Roots and tubers	Cassava	28	23.9	0.01	0.4	171.3	0.08	3.8	282.2	0.13	12.2
	Potatoes	535	57.1	0.51	18.9	50.1	0.45	21.1	4.3	0.04	3.5
	Total			**0.52**	**19.4**		**0.53**	**24.9**		**0.17**	**15.7**
Pulses	Beans dry	40	25.5	0.02	0.6	7.8	0.01	0.2	2.1	0.00	0.1
	Soya bean (dry)	63	59.3	0.06	2.3	11.0	0.01	0.5	11.0	0.01	1.1
	Peas dry	349	2.3	0.01	0.5	3.3	0.02	0.9	26.7	0.16	14.3
	Soya sauce	7	0.1	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Total			**0.09**	**3.4**		**0.04**	**1.7**		**0.17**	**15.5**

Table 15 (c) Clusters H–J (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	H			I			J		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Sugars and honey	Sugar refined	86	97.5	**0.14**	**5.2**	42.7	**0.06**	**2.9**	22.1	**0.03**	**2.9**
Nuts and oilseeds	Oilseed	131	17.9	0.04	1.4	21.9	0.05	2.3	35.9	0.08	7.2
	Tree nuts	104	15.0	0.03	1.0	9.8	0.02	0.8	1.9	0.00	0.3
	Total			**0.07**	**2.4**		**0.06**	**3.1**		**0.08**	**7.5**
Vegetables	Artichoke, globe	10	0.1	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Asparagus	100	0.3	0.00	0.0	0.2	0.00	0.0	0.0	0.00	0.0
	Beetroot	15	0.1	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Broccoli	20	7.8	0.00	0.1	0.0	0.00	0.0	0.0	0.00	0.0
	Cabbages	13	12.0	0.00	0.1	5.0	0.00	0.1	1.9	0.00	0.0
	Carrots	31	7.9	0.00	0.1	2.5	0.00	0.1	3.5	0.00	0.2
	Cauliflower	28	7.8	0.00	0.1	0.3	0.00	0.0	0.1	0.00	0.0
	Cucumbers/gherkins	17	1.3	0.00	0.0	0.3	0.00	0.0	0.0	0.00	0.0
	Eggplant	12	0.1	0.00	0.0	0.6	0.00	0.0	6.3	0.00	0.1
	Mushrooms	22	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Onion, bulb	61	27.8	0.03	1.1	7.4	0.01	0.4	16.0	0.02	1.5

Table 15 (c) Clusters H–J (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	H			I			J		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
	Peppers	96	22.4	0.04	1.3	8.4	0.01	0.6	9.4	0.02	1.4
	Squash, pumpkins, gourds	32	4.6	0.00	0.1	11.3	0.01	0.3	3.0	0.00	0.1
	Spinach	144	0.4	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Tomato	10	31.7	0.01	0.2	15.0	0.00	0.1	16.1	0.00	0.2
	Total			**0.09**	**3.2**		**0.03**	**1.5**		**0.04**	**3.6**
Stimulants	Chicory roots	2470	0.0	0.00	0.0	1.1	0.05	2.2	0.0	0.00	0.0
	Cocoa beans	128	3.6	0.01	0.3	0.8	0.00	0.1	0.8	0.00	0.1
	Coffee beans	314	7.0	0.04	1.4	0.5	0.00	0.1	0.2	0.00	0.1
	Tea	47	0.2	0.00	0.0	0.9	0.00	0.0	0.6	0.00	0.0
	Total			**0.04**	**1.7**		**0.05**	**2.4**		**0.00**	**0.3**
Fish and seafood	Crustaceans	105	1.0	0.00	0.1	0.2	0.00	0.0	0.3	0.00	0.1
	Freshwater fish	56	2.6	0.00	0.1	4.4	0.00	0.2	4.6	0.00	0.4
	Marine fish	15	10.4	0.00	0.1	7.1	0.00	0.1	11.0	0.00	0.3
	Molluscs/cephalods	55	2.7	0.00	0.1	0.2	0.00	0.0	0.2	0.00	0.0
	Total			**0.01**	**0.3**		**0.01**	**0.3**		**0.01**	**0.7**

Table 15 (c) Clusters H–J (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	H			I			J		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Eggs	Chicken eggs	18	28.0	**0.01**	**0.3**	6.1	**0.00**	**0.1**	5.1	**0.00**	**0.1**
Fruits	Apples	12	10.1	0.00	0.1	2.2	0.00	0.0	0.0	0.00	0.0
	Bananas	117	36.6	0.07	2.7	11.4	0.02	1.1	9.2	0.02	1.7
	Currants	107	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Dates	15	0.1	0.00	0.0	0.1	0.00	0.0	3.8	0.00	0.1
	Dried fruit	35	0.0	0.00	0.0	0.2	0.00	0.0	0.1	0.00	0.0
	Apricots	32	0.1	0.00	0.0	0.2	0.00	0.0	0.0	0.00	0.0
	Figs	5	0.1	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
	Grapes	5	4.8	0.00	0.0	11.7	0.00	0.0	0.3	0.00	0.0
	Papayas	36	11.5	0.01	0.3	1.6	0.00	0.0	13.7	0.01	0.8
	Pineapples	5	11.7	0.00	0.0	12.6	0.00	0.0	11.1	0.00	0.1
	Plantains	80	51.2	0.07	2.5	93.3	0.12	5.9	40.6	0.05	5.0
	Plums (including prunes)	98	1.4	0.00	0.1	0.1	0.00	0.0	0.0	0.00	0.0
	Total			**0.15**	**5.7**		**0.15**	**7.1**		**0.08**	**7.6**

Table 15 (c) Clusters H–J (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	H			I			J		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Milk/milk products		6		**0.01**	**0.4**	71.5	**0.01**	**0.3**	36.6	**0.00**	**0.3**
Meat and offals		42		**0.16**	**6.1**	61.7	**0.04**	**2.1**	37.2	**0.03**	**2.4**
Beverages (beer, cider, spirit, wine)		17		**0.03**	**1.1**	117.4	**0.03**	**1.6**	55.4	**0.02**	**1.5**
Total				**2.7**			**2.1**			**1.1**	

Table 15 (d) Clusters K–M (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	K			L			M		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Cereals	Barley	153	20.2	0.05	2.1	16.8	0.04	2.0	43.76	0.11	2.8
	Maize	172	63.1	0.18	7.4	58.6	0.17	7.7	85.48	0.25	6.1
	Oats	142	3.5	0.01	0.3	0.7	0.00	0.1	7.54	0.02	0.4
	Rice	65	238.3	0.26	10.5	381.2	0.41	19.0	34.48	0.04	0.9
	Rye	233	0.1	0.00	0.0	0.9	0.00	0.2	0.73	0.00	0.1
	Wheat	289	114.1	0.55	22.3	103.4	0.50	22.9	234.17	1.13	28.2
	Total			**1.05**	**42.6**		**1.13**	**51.8**		**1.54**	**38.6**
Roots and tubers	Cassava	28	57.7	0.03	1.1	20.0	0.01	0.4	0.66	0.00	0.0
	Potatoes	535	54.7	0.49	19.8	41.0	0.37	16.8	167.98	1.50	37.5
	Total			**0.52**	**20.9**		**0.38**	**17.2**		**1.50**	**37.5**
Pulses	Beans dry	40	44.7	0.03	1.2	5.5	0.00	0.2	7.30	0.00	0.1
	Soya bean (dry)	63	109.3	0.11	4.6	51.5	0.05	2.5	123.22	0.13	3.2
	Peas dry	349	1.5	0.01	0.4	1.7	0.01	0.5	1.88	0.01	0.3
	Soya sauce	7	0.0	0.00	0.0	13.9	0.00	0.1	0.36	0.00	0.0
	Total			**0.15**	**6.2**		**0.07**	**3.2**		**0.14**	**3.6**

Table 15 (d) Clusters K–M (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	K			L			M		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Sugars and honey	Sugar refined	86	123.6	**0.18**	**7.2**	48.3	**0.07**	**3.2**	88.18	**0.13**	**3.2**
Nuts and oilseeds	Oilseed	131	7.5	0.02	0.7	62.7	0.14	6.3	29.74	0.06	1.6
	Tree nuts	104	19.2	0.03	1.4	29.0	0.05	2.3	5.14	0.01	0.2
	Total			**0.05**	**2.0**		**0.19**	**8.6**		**0.07**	**1.8**
Vegetables	Artichoke, globe	10	0.0	0.00	0.0	0.0	0.00	0.0	1.01	0.00	0.0
	Asparagus	100	0.0	0.00	0.0	0.5	0.00	0.0	1.12	0.00	0.0
	Beetroot	15	0.2	0.00	0.0	0.0	0.00	0.0	14.25	0.00	0.1
	Broccoli	20	0.3	0.00	0.0	0.4	0.00	0.0	6.58	0.00	0.1
	Cabbages	13	3.8	0.00	0.0	55.5	0.01	0.5	18.94	0.00	0.1
	Carrots	31	4.1	0.00	0.1	8.6	0.00	0.2	19.39	0.01	0.2
	Cauliflower	28	0.6	0.00	0.0	0.4	0.00	0.0	6.58	0.00	0.1
	Cucumbers/gherkins	17	10.9	0.00	0.1	0.8	0.00	0.0	10.57	0.00	0.1
	Eggplant	12	0.5	0.00	0.0	6.3	0.00	0.1	0.68	0.00	0.0
	Mushrooms	22	0.0	0.00	0.0	0.5	0.00	0.0	3.91	0.00	0.0
	Onion, bulb	61	22.8	0.02	0.9	34.4	0.04	1.6	30.09	0.03	0.8

Table 15 (d) Clusters K–M (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	K			L			M		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
	Peppers	96	3.3	0.01	0.2	5.3	0.01	0.4	8.86	0.01	0.4
	Squash, pumpkins, gourds	32	7.0	0.00	0.2	6.7	0.00	0.2	7.62	0.00	0.1
	Spinach	144	0.2	0.00	0.0	4.3	0.01	0.5	1.98	0.00	0.1
	Tomato	10	35.6	0.01	0.2	9.9	0.00	0.1	102.96	0.02	0.4
	Total			**0.05**	**1.8**		**0.08**	**3.6**		**0.10**	**2.5**
Stimulants	Chicory roots	2470	0.0	0.00	0.1	0.0	0.00	0.0	0.00	0.00	0.0
	Cocoa beans	128	4.5	0.01	0.4	2.5	0.01	0.2	11.39	0.02	0.6
	Coffee beans	314	5.3	0.03	1.1	5.7	0.03	1.4	12.46	0.07	1.6
	Tea	47	0.1	0.00	0.0	1.5	0.00	0.1	0.98	0.00	0.0
	Total			**0.04**	**1.6**		**0.04**	**1.7**		**0.09**	**2.3**
Fish and seafood	Crustaceans	105	0.8	0.00	0.1	4.6	0.01	0.4	4.86	0.01	0.2
	Freshwater fish	56	4.2	0.00	0.2	5.3	0.00	0.2	2.49	0.00	0.1
	Marine fish	15	7.4	0.00	0.1	47.4	0.01	0.5	13.81	0.00	0.1
	Molluscs/cephalods	55	1.2	0.00	0.0	11.8	0.01	0.5	2.61	0.00	0.1
	Total			**0.01**	**0.3**		**0.04**	**1.6**		**0.02**	**0.4**

Table 15 (d) Clusters K–M (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	K			L			M		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Eggs	Chicken eggs	18	16.9	**0.01**	**0.2**	33.5	**0.01**	**0.5**	34.35	**0.01**	**0.3**
Fruits	Apples	12	9.8	0.00	0.1	17.9	0.00	0.2	36.32	0.01	0.2
	Bananas	117	70.2	0.14	5.6	40.5	0.08	3.6	32.64	0.06	1.6
	Currants	107	0.0	0.00	0.0	0.0	0.00	0.0	0.03	0.00	0.0
	Dates	15	0.0	0.00	0.0	0.0	0.00	0.0	0.20	0.00	0.0
	Dried fruit	35	0.0	0.00	0.0	0.3	0.00	0.0	0.08	0.00	0.0
	Apricots	32	0.0	0.00	0.0	0.1	0.00	0.0	1.09	0.00	0.0
	Figs	5	0.2	0.00	0.0	0.0	0.00	0.0	0.39	0.00	0.0
	Grapes	5	6.7	0.00	0.0	10.9	0.00	0.0	58.66	0.00	0.1
	Papayas	36	14.5	0.01	0.4	1.0	0.00	0.0	0.64	0.00	0.0
	Pineapples	5	16.6	0.00	0.1	20.9	0.00	0.1	22.17	0.00	0.0
	Plantains	80	39.2	0.05	2.1	1.1	0.00	0.1	1.87	0.00	0.1
	Plums (including prunes)	98	0.5	0.00	0.0	1.5	0.00	0.1	2.21	0.00	0.1
	Total			**0.20**	**8.3**		**0.09**	**4.1**		**0.08**	**2.1**

Table 15 (d) Clusters K–M (contd)

Commodities	Food	Mean acrylamide concentration (µg/kg)	K			L			M		
			g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%	g/day	mg/kg bw per day	%
Milk/milk products		6	205.6	**0.02**	**0.8**	55.9	**0.01**	**0.2**	285.43	**0.03**	**0.7**
Meat and offals		42	235.3	**0.17**	**6.7**	96.4	**0.07**	**3.1**	279.07	**0.20**	**4.9**
Beverages (beer, cider, spirit, wine)		17	104.3	**0.03**	**1.2**	85.8	**0.02**	**1.1**	295.33	**0.09**	**2.1**
Total				**2.5**			**2.2**			**4.0**	

It is noted that because waste at the household or individual level is not taken into account, food balance sheet data tend to slightly overestimate consumption. Based on comparison with national food consumption surveys made by GEMS/Food, it could be considered that the per capita food consumption estimates using food balance sheet data are generally about 15% higher than actual average food consumption for many commodities (WHO, 2006). Comparing national reported dietary exposures from Table 13 and those from the sixty-fourth meeting with the corresponding reported dietary exposures found for the same countries in this evaluation, it could be considered that dietary exposures generated using the 13 GEMS/Food consumption cluster diets are closer to the high-percentile dietary exposure figures than to the average dietary exposures.

The Committee noted that when comparing international dietary exposure data with the occurrence data from the sixty-fourth and the present meetings (overall 18 000 analytical data; Tables 14 and 15), no significant differences were seen.

The Committee concluded that, overall, no major changes had occurred in dietary exposures since the last evaluation. Therefore, based on national and regional estimates, a dietary exposure to acrylamide of 1 µg/kg bw per day could again be taken to represent the mean for the general population, including children, and a dietary exposure of 4 µg/kg bw per day could again be taken to represent consumers with high exposure.

8. DOSE–RESPONSE ANALYSIS AND ESTIMATION OF CARCINOGENIC/TOXIC RISK

8.1 Identification of key data from risk assessment

8.1.1 Pivotal data from biochemical and toxicological studies

(a) Metabolism

Since the metabolism of acrylamide was last reviewed by the Committee at its sixty-fourth meeting, there have been a number of studies that have investigated acrylamide metabolism in rodents and humans (Settels et al., 2008; Doroshyenko et al., 2009; Kopp & Dekant, 2009). Both rodents and humans metabolize acrylamide to a chemically reactive epoxide, glycidamide, in a reaction catalysed by CYP2E1. PBPK modelling suggests only modest differences in biotransformation between rats and humans (see section 2.1.3). Glycidamide may be further metabolized by epoxide hydrolase to glyceramide or by conjugation to glutathione, or it may react with proteins, including haemoglobin, or with DNA. In all species examined, acrylamide is extensively conjugated with glutathione to form the mercapturic acid AAMA and finally is oxidized to its corresponding sulfoxide; this final oxidation step to the sulfoxide is not observed in mice or rats. PBPK modelling of acrylamide metabolism and disposition has provided estimates of internal exposures to both acrylamide and glycidamide that facilitate comparisons of internal dosimetry for use in risk assessment for neurotoxicity and carcinogenicity.

(b) Neurotoxicity

As described in the monograph of the sixty-fourth meeting, the principal site of toxic action of acrylamide is the nervous system. This information comes from numerous studies in a range of laboratory animal species as well as from epidemiological accounts of human industrial and accidental exposure. The observed neurotoxicity is progressive neuronal damage, because there is limited evidence that acrylamide accumulates in nerve tissue. At the sixty-fourth meeting, the lowest-observed-effect levels (LOELs) reported to be associated with degenerative peripheral nerve changes in rats exposed to acrylamide in drinking-water were 5 mg/kg bw per day for 90 days (Burek et al., 1980) and 2 mg/kg bw per day (Johnson et al., 1986) and 2–3 mg/kg bw per day (Friedman, Dulak & Stedman, 1995) for 2 years. In both long-term studies, the NOAEL for nerve damage, detected by light microscopy, was 0.5 mg/kg bw per day, whereas in the 90-day study, the NOAEL for morphological changes in nerves, detected by electron microscopy, was 0.2 mg/kg bw per day. In the new 2-year toxicity study in rats treated with acrylamide, peripheral nerve (sciatic) axonal degeneration was observed at doses of 1.36 mg/kg bw per day and higher in males and 4.09 mg/kg bw per day in females. For acrylamide-treated rats, the NOAEL for peripheral nerve axonal degeneration was 0.67 mg/kg bw per day in males and 1.88 mg/kg bw per day in females. At doses up to 9.11 and 9.97 mg/kg bw per day in males and females, respectively, no axonal degeneration was observed in mice treated with acrylamide. For glycidamide, no axonal degeneration was observed in either mice or rats at concentrations in water of 0.7 mmol/l.

Despite overt symptoms of neurotoxicity (i.e. hindlimb paralysis) at the highest oral acrylamide dose tested (44 mg/kg bw per day in drinking-water), a short-term study in adult male rats indicated that only minor changes were seen in mRNA levels of the more than 50 genes directly related to the cholinergic, noradrenergic, GABAergic or glutamatergic neurotransmitter systems in the striatum, substantia nigra or parietal cortex. No evidence of axonal, dendritic, neuronal cell body damage or microglial activation was found in the forebrain at acrylamide doses less than 44 mg/kg bw per day. In addition, levels of serotonin, dopamine and their metabolites were essentially unchanged in the striatum, substantia nigra or parietal cortex. The motor deficits observed were interpreted as being caused by damage to the brain stem, spinal cord and peripheral neurons.

The effect of orally administered acrylamide on neurodevelopment in rats was investigated following exposure during gestation and postnatally in two separate studies. In one study, food-motivated behaviour, evaluated at 6–12 weeks of exposure, was significantly changed only at the highest dose tested (5 mg/kg bw per day). In a second study in rats, oral acrylamide doses of 7.9 mg/kg bw per day and 14.6 mg/kg bw per day caused gait abnormalities in dams from PND 18 and PND 2, respectively, to PND 21. A corresponding reduction in pup body weight occurred over the same time interval. Histopathological changes were observed in ganglion cells of the trigeminal nerves at doses of 7.9 mg/kg bw per day and above. Pups from untreated dams that received acrylamide doses at 50 mg/kg bw intraperitoneally 3 times a week from PND 2 to PND 21 showed similar trigeminal nerve lesions. Morphometric data on the sciatic nerve in dams but not their pups at

14.6 mg/kg bw per day showed a significant increase in the number of degenerated small-diameter axons and myelinated nerves. Similar lesions were found in pups treated intraperitoneally. All male pups from dams treated at 14.6 mg/kg bw per day and those treated intraperitoneally showed evidence of delayed spermatogenesis.

(c) Mutagenicity and clastogenicity

The genotoxicity of acrylamide has been studied in both in vivo and in vitro testing systems. Most studies involve either mice or rats, but there are a few in vitro assays of human cells. Overall, the results show that acrylamide is genotoxic and most potent in its ability to induce clastogenic effects (including heritable translocations in offspring of acrylamide-exposed male rodents mated with untreated females), DNA damage and gene mutations, such as male germ cell–mediated dominant lethal mutations and heritable specific locus mutations.

Clastogenicity of DNA, like aneuploidy, is likely to require multiple events in order to occur successfully. It might be reasonable to expect that clastogenicity occurs at a threshold dose, an effect recently reported by Zeiger et al. (2009) for micronuclei formation in the peripheral blood of mice.

Recent mutagenesis assays in vivo have demonstrated that administration of acrylamide or glycidamide in the drinking-water increases mutant frequencies in lymphocyte *Hprt* and liver and lung *cII* genes of adult Big Blue mice by inducing primarily G:C to T:A transversions. Similarly, acrylamide and glycidamide (approximately 5–10 mg/kg bw per day) are weakly mutagenic in thyroid, but not liver or mammary gland, of male and female Big Blue rats. In addition, glycidamide, but not acrylamide, was found to be a genotoxic mutagen in neonatal Tk mice at *Hprt* and *Tk* loci.

(d) Carcinogenicity

Two previous studies investigated the chronic carcinogenicity of acrylamide in male and female F344 rats (Johnson et al., 1986; Friedman, Dulak & Stedman, 1995). Using acrylamide doses of 0, 0.01, 0.1, 0.5 and 2.0 mg/kg bw per day, Johnson et al. (1986) reported significant increases in thyroid gland follicular adenomas, peritesticular mesotheliomas and adrenal gland phaeochromocytomas in male rats. Similarly, in female F344 rats, significant increases in mammary tumours, central nervous system glial tumours, thyroid gland follicular adenomas or adenocarcinomas, oral cavity squamous papillomas, uterine adenocarcinomas, clitoral gland adenomas and pituitary adenomas were observed. In addition, in both male and female F344 rats, Johnson et al. (1986) reported significant increases in light microscopically detected degenerative nerve changes at 2.0 mg/kg bw per day.

Using acrylamide doses of 0, 0.01, 0.1, 0.5 and 2.0 mg/kg bw per day, Friedman, Dulak & Stedman (1995) reported that male rats had significant increases in peritesticular mesotheliomas and thyroid gland follicular adenomas. Similarly, in female F344 rats, Friedman, Dulak & Stedman (1995) reported significant increases in thyroid gland follicular adenomas, total follicular cell neoplasms and mammary fibroadenomas and adenocarcinomas.

In the 2-year NCTR/NTP study in mice and rats (Beland, 2010), the sites of tumours (i.e. thyroid and mammary glands and peritesticular mesothelium) observed in male and female F344 rats at a dose range up to 2.78 mg/kg bw per day in males and 4.09 mg/kg bw per day in females were reasonably concordant with those found in previous 2-year studies in rats (Johnson et al., 1986; Friedman, Dulak & Stedman, 1995). Additional tumour sites observed in the NCTR/NTP study were heart schwannomas and pancreatic islet tumours in males. However, there were no brain or spinal cord tumours of glial origin observed in the NCTR/NTP study (Beland, 2010). This confirmed the observations of Friedman, Dulak & Stedman (1995), but not those of Johnson et al. (1986).

The tumour incidences were also significantly elevated for animals treated at the same molar concentrations of glycidamide. The tumour incidences were comparable for male and female mice and female rats and slightly higher in male rats. The only exceptions were ovarian benign granulosa cell tumours in female mice and pancreatic adenomas and carcinomas in male rats. Additional sites of tumours were observed in glycidamide-treated rats and mice, including skin in mice and oral cavity and mononuclear cell leukaemia in rats (Beland, 2010).

In male and female B6C3F1 mice treated with acrylamide, the tumour-bearing tissues were lung, Harderian gland, forestomach, mammary gland and ovaries. The achieved acrylamide doses in mice were up to 9.11 mg/kg bw per day for males and 9.97 mg/kg bw per day for females. In a parallel group of mice that were treated with equimolar concentrations of glycidamide in drinking-water, similar observations were made. The concordance of tumour sites and glycidamide internal dosimetry from PBPK modelling between acrylamide- and glycidamide-treated rodents provides support for the hypothesis that glycidamide is the ultimate carcinogenic species derived from metabolism of acrylamide. Additional support for the tumorigenicity of glycidamide, but not acrylamide, was observed in livers of male Tk mice treated neonatally on PNDs 1, 8 and 15 and evaluated after 1 year of life (Beland, 2008).

(e) Non-genotoxic mode of action

On the basis of the results of in vitro studies, some non-genotoxic nechanisms for acrylamide carcinogenicity in male and female Fischer 344 rats have been suggested. These include hormonal dysregulation (Shipp et al., 2006), oxidative stress and modification of critical sulfhydryl residues on kinesin proteins that function in chromosome separation (Sickles et al., 2007). A short-term study by Bowyer et al. (2008a,b) reported hormonal changes from direct effects of acrylamide on thyroid and testes in the male F344 rat. However, these effects occurred only at relatively high doses (10 and 50 mg/kg bw per day) that exceeded the rat 2-year bioassay conditions (<4.09 mg/kg bw per day). In addition, there was no evidence observed for alterations of dopaminergic systems in the central nervous system and pituitary; the hypothalamic–pituitary–thyroid (HPT) axis; or mRNA changes in HPT axis–related genes, cell cycle–specific genes or genes associated with elevated oxidative stress. Although significantly decreased levels of testosterone and increased luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels were observed (Doerge et al., 2008), no evidence was

observed for increased cell proliferation in the peritesticular mesothelium, thyroid, pituitary or liver of male F344 rats at any dose, and significant decreases were observed in all tissues examined at the highest dose (Bowyer et al., 2008a,b; Doerge et al., 2008). These studies were interpreted as providing no evidence for disruption of the HPT or hypothalamic–pituitary–testes axes in male F344 rats by doses of acrylamide relevant to the recent carcinogenicity bioassays (Beland, 2010). Because evidence for hormonal dysregulation by acrylamide is observed only at doses above those used in the rodent chronic carcinogenicity bioassays, even less relevance is suggested for human risk assessment at exposure levels several orders of magnitude lower. Moreover, these alternative hypotheses do not account for the significant body of evidence from males and females from two rodent species supporting a glycidamide-mediated genotoxic mechanism for tumorigenesis in multiple tissues as described above.

(f) Reproductive and developmental toxicity

The results of the reproductive toxicity studies reported at the sixty-fourth meeting of the Committee indicated that rats are more sensitive than mice to the effects of acrylamide. In the two-generation reproduction study in Fischer 344 rats, Tyl et al. (2000) reported a significant reduction in live implants and increases in pre-implantation and post-implantation losses at 5 mg/kg bw per day. There were no developmental effects observed in a series of studies in mice and rats. The overall NOAEL for reproductive and developmental toxicity was 2 mg/kg bw per day. A recent study in Sprague-Dawley rats did not observe any changes in reproduction parameters such as gestation period, implantation numbers, live birth or sex ratio at doses up to an average of 14.6 mg/kg bw per day throughout gestation. However, neurotoxicity was observed in the dams at a dose of 14.6 mg/kg bw per day.

8.1.2 Pivotal data from human clinical/epidemiological studies

The updated analyses of two cohorts of exposed workers revealed much less evidence than in previous analyses of a possible relationship between acrylamide exposure and pancreatic cancer. The updated analyses are based upon both comparisons with mortality in the general population as well as comparisons of different levels of acrylamide exposure within the cohort, with control for smoking history. Taken together, in spite of high acrylamide exposure in some workers, results for these two cohorts do not provide support for any relationship between acrylamide exposure at the workplace and cancer mortality.

A summary of the results from the reviewed prospective studies with assessment of dietary exposure to acrylamide is shown in Table 16. No associations were found between acrylamide exposure and cancer of the breast, urinary bladder, colon and rectum, stomach, oesophagus, pancreas, brain, pharynx, larynx, thyroid or prostate. A statistically significant increase in risk was observed for ovarian and endometrial cancers, but only for non-smoking women in one study, whereas no association was found for these tumours in another study. An increase in risk has also been reported for the oral cavity, restricted to non-smoking women. For lung cancer, there was a significant inverse association only for women with adenocarcinoma type. None of these associations has been replicated.

Table 16. Summary of prospective studies[a] assessing the potential association with cancer based on estimated dietary exposure to acrylamide

	NLCS	SMC	CSM	SWLHC	NHS-II
Population	The Netherlands, approximately 5000 men and women 59–69 years of age	Sweden, approximately 61 000 women, with mean age 54 years	Sweden, approximately 45 000 men, 45–79 years	Sweden, approximately 49 000 women, 30–49 years	USA, approximately 90 000 women, 25–42 years
Acrylamide exposure, µg/day (µg/kg bw per day)	All 21.8 µg/day (0.30 µg/kg bw per day) Men 22.5 µg/day (0.29 µg/kg bw per day) Women 21.0 µg/day (0.32 µg/kg bw per day)	24.6 µg/day (0.38 µg/kg bw day)	36.1 µg/day (0.48 µg/kg bw per day) (assuming 75 kg body weight)	25.9 µg/day (0.40 µg/kg bw per day) (assuming 65 kg body weight)	20.2 µg/day (0.32 µg/kg bw per day)
Ovary	HR 1.99 (95% CI 1.12–3.52) per 10 µg acrylamide, non-smokers	NA	—	—	—
Endometrium	HR 1.17 (95% CI 1.01–1.21) per 10 µg acrylamide, non-smokers	NA	—	—	—
Breast	NA	NA	—	NA	NA
Renal cell	HR 1.10 (95% CI 1.01–1.21) per 10 µg acrylamide	—	—	—	—
Urinary bladder	NA	—	—	—	—

Table 16 (contd)

	NLCS	SMC	CSM	SWLHC	NHS-II
Colorectal	NA	NA	—	—	—
Stomach	NA	—	—	—	—
Oesophagus	NA	—	—	—	—
Pancreas	NA	—	—	—	—
Lung	HR 0.61 (95% CI 0.45–0.81) per 10 µg acrylamide, only women with adenocarcinoma	—	—	—	—
Brain	NA	—	—	—	—
Oral cavity	HR 1.28 (95% CI 1.01–1.62) per 10 µg acrylamide, only non-smoking women	—	—	—	—
Pharynx	NA	—	—	—	—
Larynx	NA	—	—	—	—
Thyroid	NA	—	—	—	—
Prostate	NA	—	NA	—	—

NA, no association

[a] A full description of the studies may be found in the text (section 2.3.3).

Two studies on cancer risk have used acrylamide–haemoglobin adducts to assess acrylamide exposure; they are summarized in Table 17. No association was found with prostate cancer in relation to AA-Val concentration in a population-based case–control study. In a prospective study, there was no association between AA-Val or GA-Val concentration and risk of breast cancer in postmenopausal women. A significantly increased risk was reported for smokers only after adjusting for duration and intensity of smoking, and this association was even stronger when the analysis focused on ER+ tumours. The role of smoking in breast cancer has not been clearly elucidated yet, and this result needs replication. In contrast, the lack of association with GA-Val may suggest that some mechanisms other than glycidamide genotoxicity could be involved.

Compared with the studies reviewed in the previous evaluation, the quality of epidemiological studies available to the present meeting has improved substantially. They are prospective studies specifically designed to assess any effects of acrylamide. The assessment of dietary exposure is also better than in previous studies, and relevant confounders for each cancer investigated have been included. However, most of the evidence still relies on studies in which dietary

Table 17. Summary of prospective studies assessing the potential association with cancer based on acrylamide–haemoglobin adducts

	Cancer of the Prostate in Sweden	Danish Diet, Cancer and Health
Population	Sweden, 170 cases and 161 controls, non-smoking men aged 35–76 years	Denmark, 372 case–control pairs of premenopausal women
Acrylamide adducts (controls)	53.7 pmol/g globin (mean) Medians of 1st and 4th quartiles: 32 and 56 pmol/g globin	AA-Val: 47 (18–205) pmol/g globin; GA-Val: 28 (9–99) pmol/g globin (median, 5th and 95th percentiles)
Acrylamide exposure (controls)	44.5 µg/day (0.56 µg/kg bw per day) (mean)	—
Prostate	No association: RR 1.00 (95% CI 0.86–1.16) per 10-unit increase in level of adducts (pmol/g)	—
Breast (postmenopausal)	—	Overall no association: RR (95% CI), per $\log_{10}$ pmol/g: AA-Val: 1.05 (80.66–1.69); GA-Val: 0.88 (0.51–1.52) *Only for AA-Val (no GA-Val)*: Among smokers, adjusted by duration and time smoked, RR = 3.1 (1.0–9.7) As above, restricted to cases ER+: RR = 4.9 (1.2–2.0)

exposure was assessed by means of questionnaires, which have known limitations. Validation studies comparing dietary exposure to acrylamide with adduct levels in subjects without other sources of acrylamide exposure found moderate correlations between both. It is well known that FFQs usually have good ranking validity (i.e. the ability to rank subjects correctly according to exposure), but they have measurement errors when assessing the specific amount consumed of a particular component of diet. This measurement error may challenge the ability to detect a true cancer risk related to such exposure. Furthermore, this prevents a valid analysis of the exposure–response when an association is found.

In the prospective studies included in this review, the average daily exposure ranged from 22 to 36 µg, or 0.30–0.50 µg/kg bw. The exposures are substantially lower than the 1 µg/kg bw assumed to represent the average exposure for the general population in the previous report. They are also slightly lower than the estimates from studies applying probabilistic models in some European countries, where the estimates were in the range 0.5–0.6 µg/kg bw. This disagreement may be an indication of the poor performance of some questionnaires or reflect the limitations of the database on acrylamide in foods. An issue of further concern is that, in addition to underestimation of overall consumption, there may also be an underestimation of variability of exposure, thus reducing the chance to detect a true association with the outcome of interest. It must be recalled that the only study that found a positive association with cancer risk compared subjects with a 10-fold different level of exposure, which roughly corresponded to levels in the 5th and 95th percentiles of the distribution.

This fact emphasizes the need for large prospective studies using biomarkers of long-term exposure (e.g. acrylamide–haemoglobin adduct) to assess the potential risk of acrylamide. Unfortunately, only one study of this type has been published to date. Furthermore, simultaneous assessment of dietary exposure in the same studies would be useful for two purposes: first, it would help elucidate the type of relationship between exposure and adduct levels in the target population; second, as the measurement errors of these two methods are most likely not related, they can be used together to improve the assessment of true exposure.

In addition, as acrylamide adducts may have sources other than diet, it is important to know the background level of acrylamide adducts in the population. A summary of results from studies showing levels of AA-Val and GA-Val adducts in subjects without evidence of occupational exposure, taking into account their smoking habits, is presented in Table 18. The AA-Val levels ranged between 27 and 54 pmol/g globin, with 40 pmol/g globin as a good overall average value. The GA-Val levels ranged from 21 to 50 pmol/g globin, with 30 pmol/g globin as an approximate average estimate. The GA-Val to AA-Val ratio ranged from 0.51 to 1.13, with the best estimate around 0.85. Compared with non-smokers, smokers had 2- to 5-fold higher levels of AA-Val and 1.5- to 4-fold higher levels of GA-Val. Using toxicokinetic parameters for detoxification and adduct formation, it has often been quoted that an estimated dietary exposure of 1.2 µg/kg bw per day would correspond to an adduct level of 30 pmol/g globin. This exposure is substantially higher than that observed in most studies included in this review, and it could be even higher, considering that the actual background level could be 40 pmol/g globin

Table 18. Background levels of acrylamide–haemoglobin adducts

Reference	No.	AA-Val, mean or median level (pmol/g globin)			GA-Val, mean or median level (pmol/g globin)			Ratio of GA-Val to AA-Val	
		Smoker	Non-smoker	Ratio S/NS	Smoker	Non-smoker	Ratio S/NS	Smoker	Non-smoker
Studies on background adduct levels									
Chevolleau et al. (2007)	68	53.5	27.0	1.98	34.0	22.0	1.55	0.64	0.81
Vesper et al. (2008)	510	137	48.4	2.83	101.0	43.3	2.33	0.74	0.89
Kütting et al. (2009)	898	83.2	27.1	3.07	—	—	—	—	—
Hartmann et al. (2008)	91	—	30.0	—	—	34.0	—	—	1.13
Validation studies of dietary exposure									
Hagmar et al. (2005)	40	152	31.0	4.90	—	—	—	—	—
Urban et al. (2006)	120	81.8	27.6	2.96	—	—	—	—	—
Bjellaas et al. (2007b)	50	154	38.4	4.01	76.5	19.6	3.90	0.50	0.51
Wilson et al. (2009c)	332 (f)	93.7	43.9	2.13	137.5	49.4	2.78	1.47	1.13
Association studies									
Olesen et al. (2008)	372 (f)	122	35.0	3.49	60.0	21.0	2.86	0.49	0.60
Wilson et al. (2009b)	161 (m)	—	53.7	—	—	—	—	—	—

f, female; m, male; NS, non-smoker; S, smoker

instead of the 30 pmol/g globin used. Thus, the relationship between background adduct levels in the population and estimates of acrylamide exposure needs to be clarified. An example of potential discrepancies between these two ways of measuring exposure (assuming accuracy of toxicokinetic parameters) is presented in Table 19, showing results from studies in which data on both adduct levels and acrylamide exposure were assessed.

In conclusion, most of the evidence available from epidemiological studies does not support the hypothesis that dietary acrylamide exposure is associated with cancer in humans, although there is some suggestion of a possible association with hormone-related tumours in women that needs replication. Prospective studies measuring acrylamide exposure by means of acrylamide–haemoglobin adducts are still lacking; the only study of this type suggests that further research is needed using this approach to assess any potential association with cancer risk. These studies should be large enough to achieve good statistical power. Apart from proper epidemiological design (i.e. avoid selection bias and control of confounding), these prospective studies should validly assess (or rule out) occupational exposure to acrylamide and include detailed information on smoking habits. Simultaneous accurate assessment of acrylamide dietary exposure would be desirable as well.

8.2 Estimates of BMDs and BMDLs

In the dose–response analysis using the USEPA BMD software (BMDS version 2.1.1), the nine different dichotomous models were fitted to the unadjusted data for all end-points for which there was a statistically significant dose–response trend. Those resulting in acceptable fits based on statistical considerations were selected to derive BMDs for a 10% response (BMD_{10}) and $BMDL_{10}$ values. For most data sets, a *P*-value of 0.10 was used as an exclusion criterion. However, there were a few data sets where none of the models resulted in a *P*-value in excess of 0.5. For those data sets, a model was excluded only if the *P*-value was less than 10% of the value from the best-fitting model (i.e. the exclusion criterion was relative rather than absolute). The resulting ranges are provided in Table 20.

The end-points in mice and rats with the lowest $BMDL_{10}$s were the male mouse Harderian gland adenoma or carcinoma and female rat mammary gland fibroadenoma. The modelling results for these end-points are presented in Tables 21 and 22. The model resulting in both the best fit and the lowest $BMDL_{10}$ for the male Harderian gland data was the log-logistic model. This model and the underlying data are presented in Figure 2. The model resulting in both the best fit and the lowest $BMDL_{10}$ for the female rat mammary gland fibroadenoma was also the log-logistic model. This model and the underlying data are presented in Figure 3.

Table 19. Studies with data on background levels of acrylamide–haemoglobin adducts and dietary exposure

References	Country	Population	Background level of adducts (non-smokers)		Dietary exposure to acrylamide	
			AA-Val	GA-Val	µg/day	µg/kg bw per day
Hagmar et al. (2005); Wirfält et al. (2008)	Sweden	40	31.0 (median)	—	25.0 (median)	0.36 (median)[a]
Bjellaas et al. (2007b)	Norway	50	38.0 (median)	19.6 (median)	18.3 (median)	0.26 (median)[a]
Wilson et al. (2009c)	USA	332 (f)	43.9 (median)	49.0 (median)	19.3 (mean)	0.27 (mean)
Wilson et al. (2009b)	Sweden	161 (m)	53.7 (mean)	—	44.5 (mean)	0.56 (mean)

f, female; m, male

[a] Not given in the study, estimated assuming average body weight of 70 kg.

Table 20. BMD_{10} and $BMDL_{10}$ values for mice and rats dosed with acrylamide

Neoplastic finding	BMD_{10} range	$BMDL_{10}$ range
Male B6C3F1 mice		
Harderian gland adenoma	0.36–0.67	0.18–0.56
Harderian gland adenoma or carcinoma	0.37–0.66	0.18–0.55
Lung alveolar/bronchiolar adenoma	2.14–4.15	1.29–2.84
Lung alveolar/bronchiolar adenoma or carcinoma	2.13–4.07	1.28–2.78
Forestomach squamous cell papilloma	4.82–8.09	3.18–6.02
Forestomach squamous cell papilloma or carcinoma	3.96–6.82	2.68–5.36
Female B6C3F1 mice		
Harderian gland adenoma	0.43–0.63	0.31–0.53
Lung alveolar/bronchiolar adenoma	1.95–4.00	1.29–2.84
Lung alveolar/bronchiolar adenoma or carcinoma	2.02–3.84	1.28–2.78
Mammary gland adenocarcinoma	1.61–4.08	1.19–3.41
Mammary gland adenoacanthoma	10.92–11.12	6.39–8.19
Mammary gland adenocarcinoma or adenoacanthoma	2.91–9.04	2.06–5.22
Ovarian benign granulosa cell tumour	9.450–11.45	6.51–7.83

Table 20 (contd)

Neoplastic finding	BMD_{10} range	$BMDL_{10}$ range
Male F344 rats		
Testicular mesothelioma	2.14–2.26	1.25–1.73
Heart malignant schwannoma	2.48–2.77	1.29–1.92
Pancreas islet adenoma	2.82–3.52	1.60–2.20
Pancreas islet adenoma or carcinoma	2.84–3.11	1.46–2.01
Thyroid gland follicular cell carcinoma	2.03–2.62	1.11–1.83
Thyroid gland follicular cell adenoma or carcinoma	3.65–4.67	2.31–2.54
Female F344 rats		
Clitoral gland carcinoma	4.31–5.19	1.55–3.11
Mammary gland fibroadenoma	0.58–1.35	0.31–0.87
Mammary gland fibroadenoma or adenocarcinoma	0.62–1.41	0.33–0.90

BMD_{10}, benchmark dose for 10% extra risk of tumours; $BMDL_{10}$, 95% lower confidence limit on the benchmark dose for 10% extra risk of tumours. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls.

Table 21. Individual model results for male mouse Harderian gland adenoma or carcinoma

Model name	*P*-value	BMD_{10}[a]	$BMDL_{10}$[a]
Gamma	0.024	*0.41*	*0.34*
Logistic	0.000	*1.00*	*0.84*
Log-logistic	0.257	0.37	0.18
Log-probit	0.049	0.66	0.55
Multistage	0.024	*0.41*	*0.34*
Multistage cancer	0.024	*0.41*	*0.34*
Probit	0.000	*1.03*	*0.89*
Weibull	0.024	*0.41*	*0.34*
Quantal linear	0.024	*0.41*	*0.34*

[a] BMD values in italics were excluded on the basis of fit.

Table 22. Individual model results for female rat mammary gland fibroadenoma

Model name	*P*-value	BMD_{10}	$BMDL_{10}$
Gamma	0.68	0.73	0.46
Logistic	0.66	0.94	0.67
Log-logistic	0.68	0.58	0.31
Log-probit	0.52	1.35	0.87
Multistage	0.68	0.73	0.46
Multistage cancer	0.68	0.73	0.46
Probit	0.66	0.93	0.67
Weibull	0.68	0.73	0.46
Quantal linear	0.68	0.73	0.46

Figure 2. Log-logistic model for male mouse Harderian gland adenoma or carcinoma

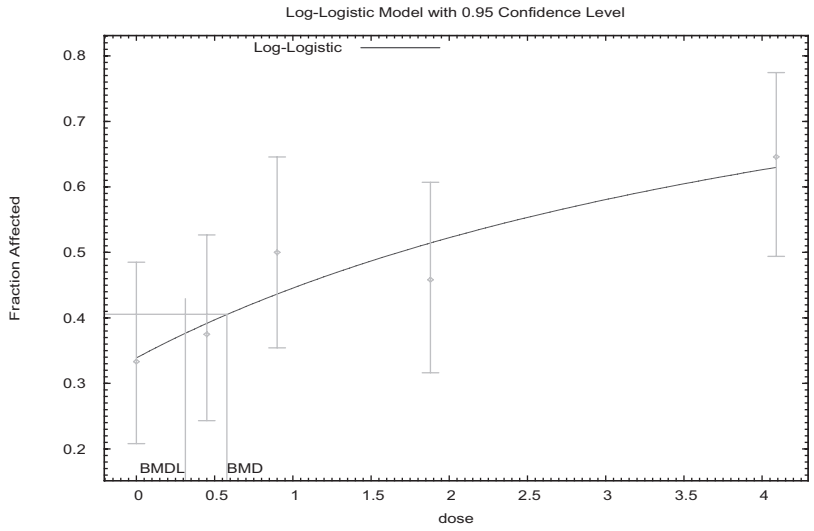
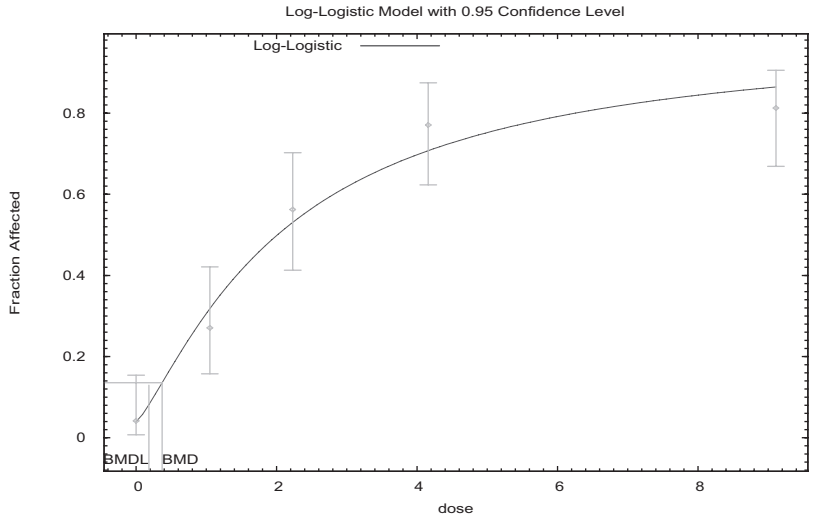


Figure 3. Log-logistic model for female rat mammary gland fibroadenoma



9. COMMENTS

9.1 Absorption, distribution, metabolism and excretion

Since the metabolism of acrylamide was last reviewed by the Committee at its sixty-fourth meeting, a number of studies have compared acrylamide metabolism in rodents and humans. Rodents and humans metabolize acrylamide to a chemically reactive epoxide, glycidamide, in a reaction catalysed by CYP2E1. In humans, there is considerable variability in the extent of acrylamide conversion to glycidamide. This difference appears to be related to interindividual variability in the amount of CYP2E1 rather than to an enzyme polymorphism. Although there are species differences in hepatic CYP2E1 activity, PBPK modelling suggests only modest differences in biotransformation between rats and humans. Glycidamide may be further metabolized by epoxide hydrolase to glyceramide or by conjugation to glutathione, or it may react with proteins, including haemoglobin, or with DNA. Acrylamide is extensively conjugated with glutathione to form a mercapturic acid, *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine, in all species examined and is oxidized to its corresponding sulfoxide in humans only. PBPK modelling of acrylamide metabolism and disposition has provided estimates of internal exposure to both acrylamide and glycidamide that facilitate comparisons of internal dosimetry for use in risk assessment for neurotoxicity and carcinogenicity.

9.2 Toxicological data

Despite overt symptoms of neurotoxicity (i.e. hindlimb paralysis) at the highest oral acrylamide dose tested (44 mg/kg bw per day in drinking-water), a short-term study in adult male rats indicated that only minor changes were seen in mRNA

levels of the more than 50 genes directly related to the cholinergic, noradrenergic, GABAergic or glutamatergic neurotransmitter systems in the striatum, substantia nigra or parietal cortex. No evidence of axonal, dendritic or neuronal cell body damage or microglial activation was found in the forebrain at acrylamide doses below 44 mg/kg bw per day. In addition, levels of serotonin, dopamine and their metabolites were essentially unchanged in the striatum, substantia nigra or parietal cortex. The motor deficits observed were interpreted as being caused by damage to the brain stem, spinal cord and peripheral neurons.

The effect of orally administered acrylamide on neurodevelopment in rats was investigated following exposure during gestation and postnatally in two separate studies. In one study, food-motivated behaviour, evaluated at 6–12 weeks of exposure, was significantly changed only at the highest dose tested (5 mg/kg bw per day).

In a second study in rats, oral acrylamide doses of 7.9 mg/kg bw per day and 14.6 mg/kg bw per day caused gait abnormalities in dams from PND 18 and PND 2, respectively, to PND 21. A corresponding reduction in pup body weight occurred over the same time interval. Histopathological changes were observed in ganglion cells of the trigeminal nerves at doses of 7.9 mg/kg bw per day and above. Pups from untreated dams that received acrylamide intraperitoneally at a dose of 50 mg/kg bw 3 times a week from PND 2 to PND 21 showed similar trigeminal nerve lesions. Morphometric data of the sciatic nerve in dams but not their pups at 14.6 mg/kg bw per day showed a significant increase in the number of degenerated small-diameter axons and myelinated nerves. Similar lesions were found in pups treated intraperitoneally. All male pups from dams treated at 14.6 mg/kg bw per day and those treated intraperitoneally showed evidence of delayed spermatogenesis.

Significantly increased incidences of neurotoxicity, measured as peripheral nerve (sciatic) axon degeneration by microscopic histopathology, were observed in a 2-year NCTR/NTP bioassay (Beland, 2010) with F344 rats treated with acrylamide in drinking-water. The NOAELs were 0.67 mg/kg bw per day in males and 1.88 mg/kg bw per day in females.

9.3 Genotoxicity

In accord with the previously reported findings, the new in vitro genotoxicity studies indicate that acrylamide in the absence of activation is a weak mutagen but an effective clastogen. In contrast, glycidamide is a mutagen and clastogen. Assays of mutagenicity in vivo have demonstrated that administration of acrylamide or glycidamide in the drinking-water increases mutant frequencies in lymphocyte *Hprt* and liver and lung *cII* genes of adult Big Blue mice by inducing primarily G:C to T:A transversions. Similarly, acrylamide and glycidamide (approximately 3–5 mg/kg bw per day) are mutagenic in thyroid, but not liver or mammary gland, of male and female Big Blue rats. In addition, glycidamide, but not acrylamide, was found to be a DNA-reactive mutagen in neonatal Tk mice at *Hprt* and *Tk* loci. In mice treated with acrylamide for 28 days, there was a linear increase in the number of micronuclei that achieved significance at 6 mg/kg bw per day in erythrocytes and at 4 mg/kg bw per day in reticulocytes. Use of an internal marker of acrylamide

exposure, such as concentrations of haemoglobin adducts (GA-Val, AA-Val) or DNA adducts (N7-GA-Gua), gave a better fit than the external dose for modelling micronuclei frequency. The fitted model gave a threshold at adduct levels equivalent to an external dose of 1–2 mg/kg bw per day.

9.4 Carcinogenicity

In the recently completed 2-year NCTR/NTP studies in which mice and rats were treated with acrylamide in drinking-water (Beland, 2010), the sites of tumours (thyroid and mammary gland, peritesticular mesothelium) induced in male and female F344 rats at a dose range up to 2.78 mg/kg bw per day in males and 4.09 mg/kg bw per day in females were concordant with those found in previous 2-year studies in rats. Additional tumour sites observed in the new study were heart schwannomas and pancreatic islet tumours in males. A notable absence in the new study was the lack of significantly elevated incidences of brain and spinal cord tumours of glial origin. The new study also reported the tumorigenesis of acrylamide in multiple tissues of male and female B6C3F1 mice (lung, Harderian gland, forestomach, mammary, ovary) using the same drinking-water concentrations as used in the rat study. The achieved acrylamide doses in mice were up to 9.11 mg/kg bw per day for males and 9.97 mg/kg bw per day for females. These findings were further supported by results from parallel groups of animals that were treated with equimolar concentrations of glycidamide in drinking-water. Most tumour sites at which the incidence was significantly elevated in rats and mice exposed to acrylamide were also significantly increased by glycidamide, with glycidamide-induced tumour incidences being either similar or higher. The only exceptions were ovarian benign granulosa cell tumours in female mice and pancreatic adenomas and carcinomas in male rats. Tumours in other tissues were observed to be significantly increased in glycidamide-treated rats and mice, including skin in mice and oral cavity and mononuclear cell leukaemia in rats. The concordance of tumour sites and glycidamide internal dosimetry from PBPK modelling between acrylamide- and glycidamide-treated rodents provides strong support for the hypothesis that glycidamide is the ultimate carcinogenic species derived from metabolism of acrylamide. Additional support for the tumorigenicity of glycidamide, but not acrylamide, was observed in livers of male Tk mice treated neonatally on PNDs 1, 8 and 15 and evaluated after 1 year of life.

9.5 Observations in humans

The updated analyses of workers exposed to acrylamide by inhalation revealed considerably lower relative risks for mortality from pancreatic cancer than in previous analyses of the same cohorts, and the results were not statistically significant. The updated analyses are based upon comparisons with mortality in the general population as well as comparisons of different levels of acrylamide exposure within the cohort, with control for smoking history. Taken together, in spite of high acrylamide exposure in some workers, results for these two cohorts do not provide support for any relationship between acrylamide exposure at the workplace and cancer mortality.

The potential association between dietary exposure to acrylamide and cancer has been assessed in five prospective studies. Without taking into account subgroup analyses (i.e. different histological types of tumour in a particular organ, different stage at diagnosis, stratified analysis by smoking), these cohorts provided 23 estimates of relative risk for 16 tumour sites. No statistically significant associations were found between dietary acrylamide exposure and the following cancers: breast (four studies), ovary (two), endometrium (two), prostate (two), urinary bladder, colon and rectum (two), stomach, oesophagus, pancreas, lung (men), brain, oral cavity, pharynx, larynx and thyroid. Statistically significant associations were found in some studies for some cancers, including renal cell cancer, when adjusted for smoking, and for ovarian and endometrial cancers among non-smokers. A significant increase in risk was also reported for cancer of the oral cavity, but this was restricted to female non-smokers. For lung cancer, there was a significant inverse association among women; this association was stronger among non-smokers and for adenocarcinomas. To date, none of these associations between acrylamide exposure and cancer at particular sites have been confirmed.

No association was found between concentrations of the biomarker AA-Val haemoglobin adduct and prostate cancer in a population-based case–control study. In a prospective study, no association between AA-Val/GA-Val concentrations and risk of breast cancer in postmenopausal women was found. However, a significantly increased risk was reported in smokers after adjusting for duration and intensity of smoking. This effect was even stronger when the analysis was restricted to cases with ER+ tumours. These associations were found for AA-Val adducts but not for GA-Val adducts.

Overall, the epidemiological studies do not provide any consistent evidence that occupational exposure or dietary exposure to acrylamide is associated with cancer in humans. Although some studies indicate an association with some tumour types, particularly the hormone-related cancers in women, this needs confirmation. While the epidemiological investigations have not shown an increased cancer risk from acrylamide exposure, the statistical power and potential for misclassification of acrylamide dietary exposure in these studies are of concern. The reviewed studies, including those with a relatively large sample size, had low power (always below 50%) to detect an increased risk of small magnitude. Data from FFQs, which are used to estimate the extent of dietary exposure to acrylamide in population-based studies, have been shown to correlate poorly with biomarkers of acrylamide and glycidamide exposure. Dietary exposure estimates derived from FFQs cannot readily capture the inherent variability of acrylamide concentrations in individual foods (see section 2.3). Consequently, epidemiological studies that use FFQs have a limited ability to detect an association between the surrogate measure of dietary acrylamide exposure and a modest increase in cancer risk.

9.6 Analytical methods

Reliable methods for the determination of acrylamide in all relevant foods are available, as demonstrated both by collaborative validation trials of single methods as well as by proficiency tests with a variety of methods. Analytical laboratories are enabled to demonstrate and maintain measurement quality through

the availability of certified reference materials and proficiency testing schemes. Isotope-labelled acrylamide for use as an internal standard is commercially available. A majority of validated and fit-for-purpose methods are isotope dilution mass spectrometric procedures, most commonly LC-MS/MS and, after derivatization, GC-MS or GC-MS/MS. Development of simpler, inexpensive and quick methods (e.g. immunoassays) has been reported, but validated methods of this type are still not available.

9.7 Formation during cooking and heat processing

The main route for acrylamide formation in foods is the Maillard reactions. Upon heating, the free amino acid asparagine is decarboxylated and deaminated to form acrylamide via routes involving initial reaction with reducing sugars or other carbonyl compounds. The Maillard reactions are also responsible for the flavour and colours typical of fried foods; unlike acrylamide formation, these processes also involve amino acids other than asparagine.

Other formation mechanisms have been identified; for example, acrylamide can be formed through pyrolysis of the wheat protein gluten or via initial enzymatic decarboxylation of asparagine in raw potatoes. Although these routes are believed to be of minor importance, the degree to which they contribute to acrylamide formation in different foods has not yet been thoroughly investigated.

9.8 Prevention and control

Reduction and control of acrylamide in foods have relied mainly on voluntary actions by the food industry to reduce the acrylamide levels in their products. Many national authorities provide information to consumers on how to reduce the formation of acrylamide in home cooking; to some extent, dietary advice is also given. A Code of Practice for the Reduction of Acrylamide in Foods has recently been adopted by the Codex Alimentarius Commission. The European Commission, in cooperation with the food industry, has initiated several measures on acrylamide mitigation. These were to a large extent based on the more extensive “toolbox for acrylamide mitigation” produced by the food industry.

Although a large and growing number of mitigation methods are being published, there is still no single method that can efficiently lower the levels of acrylamide in all foods. The food industry toolbox lists a number of measures that may be introduced at the various stages: agronomical, recipe, processing and final preparation. Only a limited number of measures have been implemented at an industrial production scale so far, including control of sugar levels in potatoes, treatment with the enzyme asparaginase, addition of various salts and acids, control of thermal input and cooking profile, and control of moisture and browning in the final product.

Significant mitigation achievements were reported by producers of potato crisps (USA = chips) and potato chips (USA = french fries) in some countries during the first years after the discovery of acrylamide in foods in 2002, but fewer achievements have been reported in recent years. Average acrylamide levels in German potato crisps produced from stored potatoes were in the range of

800–1000 µg/kg in 2002–2003 and 400–600 µg/kg in 2004–2009. In general, mitigation efforts have had limited success when applied to bread and other cereal products, although significant reductions in acrylamide levels have been reported more recently for some specific products. Mitigation after 2003 has been reported mainly for food types with comparably high acrylamide levels or single products that are at the high end of contamination within their food type. Although this might significantly reduce the exposure for some individuals or population subgroups, it will have little effect on the dietary exposure for the general population in most countries.

9.9 Levels and patterns of contamination in food commodities

At the current meeting, the Committee reviewed data from 31 countries on the occurrence of acrylamide in different foods analysed between 2004 and 2009. The total number of analytical results (single or composite samples) was 12 582, with 61% coming from Europe, 28% from Asia, 9% from North America, 1% from the Pacific and 1% from Latin America. No data were received from Africa. The Committee noted that the occurrence data evaluated at its present meeting were more comprehensive than the data submitted at the sixty-fourth meeting. Most countries used validated analytical methods and employed quality control programmes to ensure the reliability of the data.

National mean concentrations of acrylamide in major foods were found to range from 399 to 1202 µg/kg for potato crisps (USA = chips); from 159 to 963 µg/kg for potato chips (USA = french fries); from 169 to 518 µg/kg for biscuits (USA = cookies); from 87 to 459 µg/kg for crispbread and crackers; and from 3 to 68 µg/l for coffee (ready to drink). The Committee noted that the mean concentration ranges of acrylamide in the above foods are similar to those considered in its previous evaluation at the sixty-fourth meeting. In comparing global mean acrylamide levels for commodity groups with the levels obtained at the sixty-fourth meeting, the Committee noted that acrylamide levels in rye products had decreased significantly. No significant differences were observed for products made from potato, barley, rice, wheat, maize or oats.

9.10 Food consumption and dietary exposure assessment

Data on dietary exposure for eight countries were evaluated at this meeting. All regions were represented, except for Africa, for which no dietary exposure data were available. National dietary exposures were calculated mainly using a deterministic assessment. The modelling combined national individual consumption data with mean occurrence data obtained from national monitoring surveys and with the consumer body weights reported in consumption surveys.

Estimates of mean dietary exposures at the national level ranged from 0.2 to 1.0 µg/kg bw per day for the general adult population. For adult consumers at the high (95th–97.5th) percentile, the estimates of dietary exposure ranged from 0.6 to 1.8 µg/kg bw per day. Based on the few data available for children, it was noted that children had dietary exposures to acrylamide that were about twice those of adult consumers when expressed on a body weight basis. The Committee noted

that these estimates were similar to those used in the assessment performed by the sixty-fourth meeting, at which a dietary exposure to acrylamide of 1 µg/kg bw per day was taken to represent the mean for the general population and a dietary exposure of 4 µg/kg bw per day was taken to represent consumers with a high dietary exposure.

The major foods contributing to the total mean dietary exposures for most countries were potato chips (USA = french fries) (10–60%), potato crisps (USA = chips) (10–22%), bread and rolls/toast (13–34%) and pastry and sweet biscuits (USA = cookies) (10–15%). Generally, other food items contributed less than 10% to the total dietary exposures. The Committee noted that these contributions to overall exposures were consistent with the major contributing foods identified by the sixty-fourth meeting.

International estimates of dietary exposure were prepared by combining the international means of contamination levels reviewed at this meeting with food consumption data from the GEMS/Food consumption cluster diets, which differentiate 13 regional dietary patterns for food commodities (e.g. the consumption of cassava has been combined with mean acrylamide levels taken from cassava, raw/boiled, and from processed cassava products). The Committee noted that these estimates were more refined than those prepared at the sixty-fourth meeting, which were based on the then-available five GEMS/Food regional consumption diets.

The Committee estimated the international mean dietary exposures to range between 1.1 and 4.8 µg/kg bw per day across the 13 GEMS/Food consumption cluster diets, assuming a body weight of 60 kg. Cereals and root- and tuber-based foods were the main contributors to the total dietary exposure calculations for each cluster diet. Dietary exposures from cereal-based foods are between about 0.5 and 2.8 µg/kg bw per day. Depending on the patterns of consumption in each cluster, processed foods based on wheat, maize and rice were the main commodities contributing to overall exposure from cereal-based foods. Dietary exposures from roots and tubers ranged from 0.2 to 2.2 µg/kg bw per day. Processed potato was the main contributor to overall dietary exposure in most cluster diets. Food commodities based on peas, cassava and plantain were also major contributors for some cluster diets, specifically clusters A and J. Other GEMS/Food commodities contributed less than 10% to the total dietary exposure estimations.

The Committee recognized that it was difficult to have a clear picture of national trends in dietary exposures since the last evaluation and noted that this was mainly due to the lack of updated dietary exposure data from the countries evaluated at the previous meeting. Additionally, there were differences in methodologies used in evaluations within a single country for obtaining data on consumption and occurrence. Nevertheless, when comparing international dietary exposure data with the occurrence data from the sixty-fourth and the present meetings (overall 18 000 analytical data), no significant differences were seen.

The Committee concluded that, overall, no major changes in dietary exposures had occurred since the last evaluation. Therefore, based on national and regional estimates, a dietary exposure to acrylamide of 1 µg/kg bw per day could again be taken to represent the mean for the general population, including children,

and a dietary exposure of 4 µg/kg bw per day could again be taken to represent consumers with a high dietary exposure.

9.11 Dose–response analysis

At its sixty-fourth meeting, the Committee noted that the lowest NOAEL for a non-carcinogenic end-point was 0.2 mg/kg bw per day. This end-point was based on the induction of morphological nerve changes in rats following administration of acrylamide in drinking-water. There were no new studies in laboratory animals in which non-carcinogenic effects were observed at a dose below 0.2 mg/kg bw per day.

The Committee considered that the pivotal effects of acrylamide were its genotoxicity and carcinogenicity. As expressed in the previous evaluation, the Committee considered that the available epidemiological data were not suitable for a dose–response analysis. Therefore, the assessment was based on the available studies in laboratory animals. In the dose–response analysis using the USEPA BMD software (BMDS version 2.0), the nine different statistical models were used to fit the new experimental data in mice and rats from the NCTR/NTP studies (Beland, 2010). Those models resulting in acceptable fits, based on biological and statistical considerations, were selected to derive a BMD and a BMDL for a 10% extra risk of tumours (i.e. a BMD_{10} and a $BMDL_{10}$).

This process resulted in a range of BMD_{10} and $BMDL_{10}$ values for each end-point considered (see Table 20 in section 8.2). The Committee noted that the $BMDL_{10}$ values from the NCTR/NTP 2-year bioassay of acrylamide in male and female F344 rats (Beland, 2010) were similar to those reported at the sixty-fourth meeting for the earlier rat bioassays of carcinogenicity. However, the lowest range of $BMDL_{10}$ values was observed for the Harderian gland in B6C3F1 mice treated with acrylamide. As humans have no equivalent organ, the significance of these benign mouse tumours in the Harderian gland is difficult to interpret with respect to humans. However, in view of acrylamide being a multisite carcinogen in rodents, the Committee was unable to discount the effect in the Harderian gland.

The Committee considered it appropriate to use 0.18 mg/kg bw per day (the lowest value in the range of $BMDL_{10}$ values) for tumours in the Harderian gland of male mice and 0.31 mg/kg bw per day for mammary tumours in female rats as the points of departure.

10. EVALUATION

The Committee noted that mitigation after 2003 has been reported for food types with high acrylamide levels or single products that contain higher levels within their food type. Although this might significantly reduce the exposure for some individuals or population subgroups, the Committee noted that this will have little effect on the dietary exposure of the general population in all countries. In line with this, neither the estimated average acrylamide exposure for the general population (0.001 mg/kg bw per day) nor the exposure for consumers in the high percentile (0.004 mg/kg bw per day) had changed since the sixty-fourth meeting. The MOE calculated relative to the NOAEL of 0.2 mg/kg bw per day for the most sensitive

non-carcinogenic end-point—namely, morphological changes in nerves, detected by electron microscopy, in rats—therefore remains unchanged. For the general population and consumers with high exposure, the MOE values are 200 and 50, respectively. Consistent with the conclusion made at the sixty-fourth meeting, the Committee noted that while adverse neurological effects are unlikely at the estimated average exposure, morphological changes in nerves cannot be excluded for individuals with a high dietary exposure to acrylamide.

When average and high dietary exposures are compared with the $BMDL_{10}$ of 0.31 mg/kg bw per day for the induction of mammary tumours in rats, the MOE values are 310 and 78, respectively. For Harderian gland tumours in mice, the $BMDL_{10}$ is 0.18 mg/kg bw per day, and the MOE values are 180 and 45 for average and high exposures, respectively.

The Committee considered that for a compound that is both genotoxic and carcinogenic, these MOEs indicate a human health concern. The Committee recognized that these MOE values were similar to those determined at the sixty-fourth meeting and that the extensive new data from cancer bioassays in rats and mice, PBPK modelling of internal dosimetry, a large number of epidemiological studies and updated dietary exposure assessments support the previous evaluation.

The Committee noted that there was a poor correlation between the estimated dietary exposure and internal biological markers of acrylamide exposure (AA-Val and GA-Val adducts) in humans and that worker cohort epidemiological studies did not provide any evidence that exposure to acrylamide resulted in an increase in the incidence of cancer. To better estimate the risk from acrylamide in food for humans, the Committee recommended that longitudinal studies on intra-individual levels of acrylamide and glycidamide haemoglobin adducts be measured over time in relation to concurrent dietary exposure. Such data would provide a better estimate of acrylamide exposure for epidemiological studies designed to assess risk from the diet.

10.1 Recommendation

The Committee recommends further efforts on developing and implementing mitigation methods for acrylamide in foods of major importance for dietary exposure.

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ARSENIC (addendum)

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1. EXPLANATION

Arsenic is a metalloid that occurs in different inorganic and organic forms, which are found in the environment both from natural occurrence and from anthropogenic activity. Arsenic was previously evaluated by the Committee at its tenth, twenty-seventh and thirty-third meetings (Annex 1, references *13*, *63* and *84*). At its twenty-seventh meeting (in 1983), it was concluded that "on the basis of the data available the Committee could arrive at only an estimate of 0.002 mg/kg b.w. as a provisional maximum tolerable daily intake for ingested inorganic arsenic; no figure could be arrived at for organic arsenicals in food" (Annex 1, reference *63*). This was based on the observation that arsenicism can be associated with water supplies containing an upper arsenic concentration of 1 mg/l or greater and that a concentration of 0.1 mg/l may give rise to presumptive signs of toxicity. Assuming a daily water consumption of 1.5 litres, the Committee concluded that inorganic arsenic intakes of 1.5 mg/day were likely to result in chronic arsenic toxicity and that daily intakes of 0.15 mg may also be toxic in the long term to some individuals. The Committee noted that the International Programme on Chemical Safety (IPCS) had estimated that an arsenic concentration of 0.2 mg/l in drinking-water would lead to a 5% lifetime risk of skin cancer, but that skin cancer did not occur in the absence of other toxic effects due to arsenic. The Committee also noted a need for information on:

- arsenic accumulation in humans exposed to various forms of arsenic in the diet and drinking-water;
- the identification, absorption, elimination and toxicity of arsenic compounds in food, with particular reference to arsenic in fish;
- the contribution of arsenic in fish to human body burden of arsenic;
- epidemiological studies on populations exposed to elevated intakes of arsenic of known speciation.

At its thirty-third meeting (in 1988), the Committee considered information relevant to assessing the significance of organoarsenicals in fish. The previous evaluation was confirmed by assigning a provisional tolerable weekly intake (PTWI) of 0.015 mg/kg body weight (bw) for inorganic arsenic, "with the clear understanding that the margin between the PTWI and intakes reported to have toxic effects in

epidemiological studies was narrow" (Annex 1, reference *84*). The Committee noted that the organic forms of arsenic present in seafood needed different consideration from the inorganic arsenic in water. It concluded that there had been no reports of ill-effects among populations consuming large quantities of fish that result in organoarsenic intakes of about 0.05 mg/kg bw per day, but further investigation would be desirable to assess the implications for human health of exposure to naturally occurring organoarsenic compounds in marine products.

Inorganic arsenic has also been evaluated on a number of occasions by the International Agency for Research on Cancer (IARC). In 1973, IARC concluded that there was a causal relationship between skin cancer and exposure to inorganic arsenic in drugs, in drinking-water with a high arsenic content or in the occupational environment and that the risk of lung cancer was clearly increased in certain smelter workers who inhaled high levels of arsenic trioxide. However, the causative role of arsenic was uncertain, as the influence of other constituents of the working atmosphere could not be determined. In 1980, IARC concluded that there was sufficient evidence that inorganic arsenic compounds are skin and lung carcinogens in humans (Group 1). In 2004, IARC concluded that there was sufficient evidence in humans that arsenic in drinking-water causes cancers of the urinary bladder, lung and skin, whereas the evidence for carcinogenicity in experimental animals was limited. In 2009, IARC again concluded that arsenic in drinking-water causes cancers of the urinary bladder, lung and skin and that the evidence was "limited" for cancers of the kidney, liver and prostate (Straif et al., 2009).

At its present meeting, the Committee was asked to consider all information related to the toxicology and epidemiology, exposure assessment, including biomarker studies, analytical methodology, speciation and occurrence in food and drinking-water, in order to re-evaluate and review the PTWI for inorganic arsenic. The literature relating to arsenic is extensive, and the present Committee used three recent reviews—ATSDR (2007), EFSA (2009) and IARC (in press)—as the starting point for its evaluation and also took into account newer studies that were considered to be informative for the evaluation. The arsenic-containing compounds found in water, foods and biological samples are shown in Table 1.

Table 1. Arsenic compounds found in water, foods and biological samples

Name	Synonyms and abbreviations	Chemical Abstracts Service Registry No.
Arsenate	As^{V}	—
Arsenite	As^{III}	—
Methylarsonic acid	Monomethylarsonic acid, methylarsonate, MMA^{V}	124-58-3
Dimethylarsinic acid	Dimethylarsinite, cacodylic acid, DMA^{V}	75-60-5
Methylarsonous acid	Monomethylarsonous acid, MMA^{III}	—

Table 1 (contd)

Name	Synonyms and abbreviations	Chemical Abstracts Service Registry No.
Dimethylarsinous acid	DMA^{III}	—
Arsenobetaine	AB	64436-13-1
Arsenocholine	AC	39895-81-3
Trimethylarsine oxide	TMAO	4964-14-1
Tetramethylarsonium ion	TMA^{+}	27742-38-7
Dimethylarsionylethanol	DMAE	—
Trimethylarsoniopropionate	TMAP	—
Dimethylarsionylribosides	Oxo-arsenosugars	—
Dimethylmonothioarsinic acid	$DMMTA^{V}$	—
Dimethyldithioarsinic acid	$DMDTA^{V}$	—

Note: Except for biochemical and toxicological studies of specific arsenic compounds, the valency of MMA and DMA is usually not specified. The analysis of MMA^{III} and DMA^{III} has become possible only recently. In this monograph, the terms MMA and DMA are used as cited in the original papers. Where MMA and DMA are measured in foods, they have been measured as the pentavalent form. Where biological samples have been analysed, it is assumed that MMA and DMA refer to total [MMA^{III} + MMM^{V}] and total [DMA^{III} + DMM^{V}], respectively.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

Pentavalent and trivalent arsenicals are readily absorbed via the gastrointestinal tract. The absorption of arsenic is in the range 0.70–0.98 (Owen, 1990), indicating that soluble arsenicals (in water) are highly bioavailable for absorption. The degree of bioavailability of arsenic is variable, depending on the matrix. For example, about 33% of arsenic in rice with a relatively high content of dimethylarsinic acid (DMA^{V}) is available for absorption, compared with 89% from rice containing mainly sodium arsenate with a low DMA^{V} content and cooked in arsenic-contaminated water (Juhasz et al., 2006). It is difficult to determine if this higher bioavailability of arsenic is from arsenic accumulated in the rice grain or the arsenic absorbed by rice from the water during the cooking process. The variation in absorption of arsenic from soil covers a wider range, from a few per cent to about 70% (Freeman et al., 1993, 1995; Ng & Moore, 1996; Ng et al., 1998; Bruce et al., 2003; Bruce, 2004; Diacomanolis, Ng & Noller, 2007; Juhasz et al., 2007, 2008). Generally, soil contains insoluble arsenic sulfide forms such as galena and arsenopyrite, which would have a lower absorption.

The absorption depends on the arsenic species and its solubility. For example, Ng & Moore (1996) demonstrated differences in the absorption of sodium arsenite, sodium arsenate and calcium arsenite in a rodent model. Arsenite has been shown to be more extensively absorbed from the gastrointestinal tract of mice compared with arsenate when given lower doses (0.4 mg/kg bw as arsenic), whereas the reverse is true at higher doses (4.0 mg/kg bw as arsenic). Fasting and food restriction can increase arsenic absorption. Other dietary factors can also influence the absorption. Much less pentavalent arsenic was absorbed from the gastrointestinal tract of mice following oral administration in a study by Odanaka, Matano & Goto (1980) (48.5% of the 5 mg/kg bw dose excreted in urine) than in a study by Vahter & Norin (1980) (89% of the 4 mg/kg bw dose excreted in urine). This difference can be explained by the fact that mice in the Vahter & Norin (1980) study were not fed for at least 2 h before and 48 h after dosing, whereas mice in the Odanaka, Matano & Goto (1980) study were not food restricted. Kenyon, Hughes & Levander (1997) reported that feeding a diet lower in fibre or "bulk" to female B6C3F1 mice increased the absorption of pentavalent arsenic by about 10% compared with standard rodent chow diet.

Inorganic arsenic is rapidly cleared from blood in humans and most experimental animal species that have been tested. The exception is rats, in which arsenic binds to erythrocytes, delaying clearance (IPCS, 1981, 2001). Accumulation of arsenic in tissues increases with age. In a study conducted in Glasgow, Scotland, arsenic levels in liver, lung and spleen from adults were higher than those from infants (Raie, 1996), and this is consistent with observations in laboratory animals (Marafante et al., 1982).

Most ingested arsenic is rapidly excreted via the kidney within a few days (Tam et al., 1979; Vahter, 1994). For example, healthy male volunteers excreted 62.3% ± 4.0% of a 0.06 ng dose of arsenic acid (As^{V}) in urine over a period of 7 days, whereas only 6.1% ± 2.8% of the dose was excreted in the faeces (Pomroy et al., 1980). Several other studies reported that between 45% and 75% of the dose of various trivalent forms of arsenic are excreted in the urine within a few days, which suggests that gastrointestinal absorption is both relatively rapid and extensive. No quantitative data were available that directly addressed biliary excretion of trivalent or pentavalent arsenic in humans.

Ingested inorganic arsenic is excreted in human urine as inorganic arsenate and arsenite (10–15%) and its methylated metabolites, including monomethylarsonic acid (MMA^{V}) (10–15%) and DMA^{V} (60–80%) (Tam et al., 1979; Foa et al., 1984; Vahter et al., 1995a; Hopenhayn-Rich et al., 1996). Monomethylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}) have been detected in human urine at relatively lower levels (Aposhian et al., 2000a,b; Le et al., 2000a,b; Del Razo et al., 2001; Mandal, Ogra & Suzuki, 2001).

Arsenic is also excreted in human milk, although the levels are low. In a study of Andean women in Argentina, the average concentration of arsenic in breast milk was quite low (3.1 µg/l), even when urinary arsenic excretion was high (230–300 µg/l) (Concha, Nermell & Vahter, 1998).

High levels of arsenic are retained for a longer period in bone, skin, hair and nails compared with other tissues of exposed humans (Karagas et al., 2000; Mandal, Ogra & Suzuki, 2003). Hence, arsenic levels in hair and nails have been used as biomarkers reflecting longer-term exposure than those in blood or urine (see section 2.3.1).

2.1.2 Biotransformation

Unlike inorganic arsenic, ingested organic arsenicals, such as "fish arsenic" and arsenosugars, undergo very little biotransformation and are excreted almost entirely unchanged. However, it has been reported that urinary DMA^{V} is increased after consumption of DMA^{V}-containing seafood or from metabolism of arsenosugars (IPCS, 2001). Arsenolipids present in cod liver can also be metabolized into DMA^{V} (Schmeisser, Goessler & Francesconi, 2006). Organoarsenicals can be metabolized into DMA^{V}, although the mechanism underpinning this biotransformation is not clear.

Inorganic arsenic undergoes stepwise reduction of pentavalent arsenic to trivalent arsenic followed by oxidative addition of a methyl group to the trivalent arsenic. The sequential reduction and oxidation pathway shown in Figure 1 is generally believed to be the biotransformation pathway for inorganic arsenic in humans and experimental animals (Aposhian et al., 2000b).

The methylation of arsenite is catalysed by a specific methyltransferase with *S*-adenosylmethionine (SAM) as a methyl group donor (Zakharyan et al., 1995). Both in vivo and in vitro studies have shown that SAM and glutathione (GSH) are essential cofactors in enzymatic arsenic methylation (Hirata et al., 1989; Styblo, Delnomdedieu & Thomas, 1996).

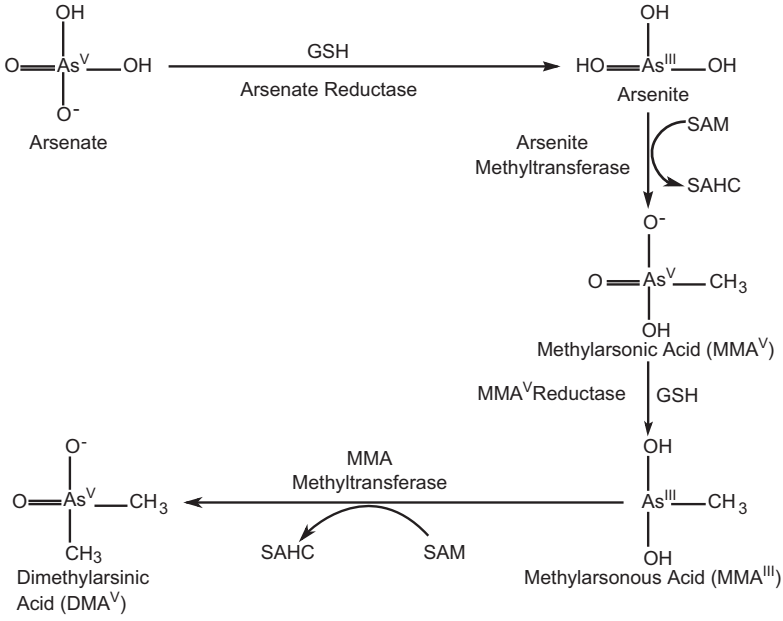
Besides the repeated reduction and oxidative methylation reactions in the arsenic metabolic pathway, the function of conjugation reaction involving GSH, resulting in excretion from the liver, has been discussed by Suzuki (2005).

An alternative, but controversial, metabolic pathway of arsenic via arsenic–GSH complexes was proposed by Hayakawa et al. (2005). The authors claimed that metabolism of inorganic As^{III} to methylated arsenicals by human recombinant Cyt19 was via arsenic triglutathione and monomethylarsonic diglutathione rather than by oxidative methylation of inorganic As^{III} and MMA^{III}.

In a study by Naranmandura, Suzuki & Suzuki (2006), it was proposed that inorganic arsenic was successively methylated reductively in the presence of GSH, rather than by a stepwise oxidative methylation, and pentavalent arsenicals (MMA^{V} and DMA^{V}) were present as end products of metabolism, rather than intermediates.

The recent study by Naranmandura et al. (2007) demonstrated the presence of monomethylthioarsonic acid ($MMMTA^{V}$), dimethylmonothioarsinic acid ($DMMTA^{V}$) and dimethyldithioarsinic acid ($DMDTA^{V}$) in the urine of hamsters and $MMMTA^{V}$ and $DMMTA^{V}$ in the urine of rats administered a single oral dose of arsenite (inorganic As^{III}) at 5.0 mg/kg bw.

Figure 1. Pathway for biotransformation of arsenic (GSH, glutathione; SAHC, S-adenosylhomocysteine; SAM, S-adenosylmethionione) (adapted from Aposhian et al., 2000b)



In another study, it was shown that the protein Cyt19 can completely methylate inorganic arsenic to trimethyl species (Thomas et al., 2007). This is unlike what Aposhian et al. (2000b) proposed, which involves two separate enzymes. However, all these studies support the view that arsenic biotransformation is via oxidative methylation, whereas an alternative reductive methylation pathway has also been proposed by other researchers (Hayakawa et al., 2005; Naranmandura, Suzuki & Suzuki, 2006). It would appear that there are two or more competing proposed pathways, and additional studies are needed.

As MMA^{III} and DMA^{III} are more toxic than inorganic arsenic and have high affinity for thiols and cellular proteins (Styblo, Hughes & Thomas, 1996; Styblo & Thomas, 1997), the hypothesis of methylation as a detoxification pathway needs to be re-evaluated.

2.1.3 Effects on enzymes and other biochemical parameters

Arsenic has higher affinity for binding to dithiol (vicinal sulfhydryl group) compared with monothiol groups of a variety of essential enzymes and proteins. When arsenic binds to critical dithiols, it can interfere with the activity of many enzymes and inhibit important biochemical events, resulting in cell damage and toxicity (Hughes, 2002). For example, arsenic is known to affect enzymes involved in haem synthesis and alter porphyrin profiles in experimental animals and humans

(Fowler & Mahaffey, 1978; Garcia-Vargas et al., 1994; Ng et al., 2005; Krishnamohan et al., 2007a,b).

Impacting on functions of some enzymes, such as glutathione peroxidase, catalase and superoxide dismutase and particularly glutathione *S*-transferase (GST), arsenic affects malondialdehyde production (Delnomdedieu et al., 1993, 1994). Malondialdehyde is a by-product of lipid oxidation. Yamanaka et al. (1990) showed a metabolic pathway of arsenic during oxidative stress in which a reactive oxidative species ($(CH_3)_2As\bullet$ radical) can be produced within the body. Other reports (Kitchin & Ahmad, 2003; Shi et al., 2004) have also provided evidence of oxidative damage induced by arsenic exposure in both experimental animals and humans. Similarly, 8-hydroxydeoxyguanine, an oxidative stress biomarker of deoxyribonucleic acid (DNA) damage, is induced by arsenic (Yamauchi et al., 2004).

Chronic arsenic exposure has been associated with type II diabetes. Wang et al. (2009b) studied biochemical parameters, including urinary *N*-acetyl-β-glucosaminidase (NAG), and blood biochemistry in humans with and without type II diabetes in an arsenic-endemic area of Xinjiang, China. They reported elevated NAG in all patients with diabetes compared with those without diabetes. Further, NAG levels in patients with diabetes from the endemic area were higher than those from the control area. NAG is a lysosomal enzyme involved in the metabolism of glycoproteins. Increased NAG levels in the urine are an early indication of renal disease and can serve as a valuable renal function test in disorders such as nephritis syndrome and other diseases associated with nephropathy (Price, 1992).

2.2 Toxicological studies

2.2.1 Acute toxicity

Inorganic arsenic can be lethal to experimental animals and humans. Arsenic toxicity depends on its solubility, chemical form and route of administration and varies among experimental animals (Table 2). Generally, trivalent arsenic is more toxic than the pentavalent forms. For example, the more soluble sodium arsenite is more toxic than arsenic trioxide (Done & Peart, 1971). Also, the inorganic arsenicals are more toxic than MMA^V and DMA^V.

More details on the acute oral toxicity of inorganic arsenic are given in ATSDR (2007). Reported lowest-observed-adverse-effect levels (LOAELs) for inorganic arsenic causing gastrointestinal irritation are 0.05 mg/kg bw per day for humans, 6 mg/kg bw per day for monkeys and 11 mg/kg bw per day for rats (ATSDR, 2007).

Although inorganic arsenic is more toxic than its major metabolites MMA^V and DMA^V and other organic arsenic, MMA^{III} was found to be more cytotoxic than inorganic arsenite in Chang human hepatocytes (Petrick et al., 2000). In several cell lines, MMA^{III} was more cytotoxic than inorganic As^{III}, whereas DMA^{III} was at least as toxic as inorganic As^{III} for most of the cell types examined, but the pentavalent arsenicals were significantly less cytotoxic (Styblo et al., 1999, 2000). These results show the following order of toxicity: $MMA^{III} > DMA^{III} \approx AsI^{II} > AsV > MMAV > DMAV$.

Table 2. LD_{50} values of different arsenic species in various experimental animal species

Chemical	Species (sex)	Route	LD_{50} (mg/kg bw as arsenic)	Reference
Arsenic trioxide	Mouse (m)	Oral	26	Kaise, Watanabe & Itoh (1985)
Arsenic trioxide	Mouse (m)	Oral	26–48	Harrison, Packman & Abbott (1958)
Arsenic trioxide	Rat (m/f)	Oral	15	Harrison, Packman & Abbott (1958)
Arsenite	Mouse (m)	Intramuscular	8	Bencko et al. (1978)
Arsenite	Hamster (m)	Intraperitoneal	8	Petrick et al. (2001)
Arsenite	Mouse (m)	Intramuscular	22	Bencko et al. (1978)
MMA^{III}	Hamster (m)	Intraperitoneal	2	Petrick et al. (2001)
MMA^{V}	Mouse (m)	Oral	916	Kaise, Watanabe & Itoh (1985)
DMA^{V}	Mouse (m)	Oral	648	Kaise, Watanabe & Itoh (1985)
TMAO	Mouse (m)	Oral	10 600	Kaise et al. (1989)
AB	Mouse (m)	Oral	>10 000	Kaise, Watanabe & Itoh (1985)

f, female; LD_{50}, median lethal dose; m, male

2.2.2 Short-term studies of toxicity

There are very few short-term studies of the toxicity of arsenic reported in the literature. Respiratory effects were observed in rats and mice exposed to very high levels of DMA^{V} (2172 mg/m^3 as arsenic) and MMA^{V} (≤2485 mg/m^3 as arsenic) (Stevens, DiPasquale & Farmer, 1979). DMA^{V} and MMA^{V} at high concentrations are considered to be respiratory irritants.

In a 28-day study (Hughes & Thompson, 1996) in which mice were exposed to sodium arsenate (0.025 and 2.5 mg/l as arsenate), hepatic vacuolar degeneration was observed in a dose–response manner, but no effect was observed in the kidney. Short-term exposure of guinea-pigs to arsenic trioxide resulted in a significant decrease in total hepatic carbohydrates (Reichl et al., 1988). This observation is thought to be due to inhibition of gluconeogenesis and may lead to serious toxic effects (Reichl et al., 1988; Szinicz & Forth, 1988).

2.2.3 Long-term studies of toxicity and carcinogenicity

Oral exposure to inorganic arsenicals has a number of effects, including effects on the cardiovascular, respiratory, gastrointestinal, haematological, immune, reproductive and nervous systems (reviewed in IPCS, 2001; ATSDR, 2007).

In 2-year feeding studies, there was evidence of gastrointestinal injury in dogs exposed to arsenite at 2.4 mg/kg bw per day, but not in rats at doses of arsenate or arsenite up to 30 mg/kg bw per day (ATSDR, 2007).

MMA^{V} has been shown to have effects on the gastrointestinal tract, kidney, thyroid and reproductive system (ATSDR, 2007). The most sensitive effect is diarrhoea, which has been reported in rats, mice, rabbits and dogs, occurring at decreasing doses with increasing duration of treatment. Histological alterations in the gastrointestinal tract generally occurred at higher doses than the lowest dose resulting in diarrhoea. The lowest no-observed-adverse-effect level (NOAEL) following dietary administration was 3.0 mg/kg bw per day in a 2-year dietary study in rats in which the LOAEL for diarrhoea was 25.7 mg/kg bw per day (Arnold et al., 2003).

DMA^{V} has effects on the urinary bladder, kidneys, thyroid and fetal development (ATSDR, 2007).

The evidence for the carcinogenicity of arsenical compounds has been reviewed in detail by IARC (in press). Most studies in experimental animals have not shown increased tumour incidences following chronic oral exposure to inorganic arsenic. Arsenic trioxide, various arsenate salts and sodium arsenite were not carcinogenic when administered via the oral route in mice and rats, and sodium arsenite and arsenate were not carcinogenic in dogs (IARC, 1973, 1980).

There are two exceptions to this general observation. Administration of sodium arsenate in the drinking-water (0, 1, 10 and 100 mg/l) to groups of 30 male A/J mice for 18 months resulted in a dose-related increase in lung tumour multiplicity and lung tumour size. In this study, some mice of all except the highest dose group died from 10 months onwards. The survival at the end of the study was 19/30, 14/30, 16/30 and 30/30 at 0, 1, 10 and 100 mg/l, respectively (Cui et al., 2006).

In a study for which detailed results are so far reported only in a PhD thesis, groups of 70 C57BL/6J mice were given drinking-water containing arsenic at concentrations of <0.0001 (controls, $n = 105$), 0.1, 0.25 or 0.5 mg/l in the form of sodium arsenate (As^{V}) or MMA^{III} ad libitum for 24 months. There were no significant differences in body weight, feed or water consumption in the treatment groups compared with controls. Some animals died suddenly, some for unknown reasons and some from the bursting of a blood-filled ovarian cyst or from tumorous lesions. Treatment with both sodium arsenate and MMA^{III} resulted in a dose-related, statistically significant increased incidence of lymphoma. At the highest dose, MMA^{III} treatment resulted in a higher lymphoma incidence than sodium arsenate, but this difference was not seen at the other doses (Krishnamohan, 2007). DMA^{III} has not been tested for carcinogenicity.

MMA^{V} was not carcinogenic in 2-year cancer bioassays when administered to male rats at concentrations up to 200 mg/l in drinking-water (Shen et al., 2003a) or to mice or rats at dietary concentrations up to 400 mg/kg feed (Arnold et al., 2003). The dietary concentrations were comparable to doses in the region of 100 mg/kg bw per day.

Trimethylarsine oxide (TMAO) (200 mg/l in drinking-water for 2 years) induced hepatocellular adenomas in rats, possibly by a mechanism involving oxidative damage and cell proliferation (Shen et al., 2003b).

DMA^{V} (≥50 mg/l in drinking-water) was carcinogenic in the urinary bladder of rats but not in the urinary bladder of mice. The NOAEL was 10 mg/l in drinking-water, equivalent to 0.73 mg/kg bw per day. The mode of action is considered to involve cytotoxicity and sustained increased cell proliferation, and the rat is considered to be particularly sensitive to DMA^{V}, owing to slower elimination and greater potential for metabolism to DMA^{III} compared with other species, including humans (Cohen et al., 2006, 2007; ATSDR, 2007). Similarly, DMA^{V} administered in drinking-water for 50 weeks or more increased the incidence and multiplicity of lung adenoma or carcinoma in A/J mice at 400 mg/l (Hayashi et al., 1998) and increased lung tumours in mutant Ogg–/– mice (which cannot repair certain types of oxidative DNA damage) but not Ogg+/+ mice at 200 mg/l (Kinoshita et al., 2007). Furthermore, DMA^{V} has been reported to promote carcinogenesis in the urinary bladder (≥10 mg/l), kidney (≥200 mg/l), liver (≥200 mg/l) and thyroid gland (≥400 mg/l) (Yamamoto et al., 1995; Wanibuchi et al., 1996).

In addition, studies in mice have shown evidence of transplacental carcinogenesis (Waalkes, Liu & Diwan, 2007; Liu & Waalkes, 2008). Sodium arsenite (0, 42.5 and 85 mg/l) was administered in drinking-water to pregnant mice during days 8–18 of gestation, and the offspring were observed for up to 2 years. There were dose-related increases in hepatocellular carcinoma and adrenal cortical carcinoma in male offspring and in ovarian tumours, lung adenocarcinomas and proliferative lesions of the uterus and oviduct of female offspring (Waalkes, Liu & Diwan, 2007). Combined prenatal exposure to the tumour promoter 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) (Waalkes, Ward & Diwan, 2004), diethylstilbestrol (DES) or tamoxifen (Waalkes et al., 2006a,b) enhanced the carcinogenic response of prenatal arsenic exposure in a variety of mouse tissues. Arsenic exposure in utero did not cause skin cancer, but exacerbated the skin cancer response after TPA exposure, possibly by altering tumour stem cell response (Waalkes et al., 2008).

Sodium arsenite (≥1.25 mg/l in drinking-water) was co-carcinogenic with solar ultraviolet (UV) light (Rossman et al., 2001; Burns et al., 2004), and arsenate (25 mg/l in drinking-water ad libitum for a period of 25 weeks) was co-carcinogenic with 9,10-dimethyl-1,2-benzanthracene (Motiwale, Ingle & Rao, 2005).

A recent study that evaluated the impact of early life stage and prolonged arsenic exposure on arsenic-induced proliferative lesions and neoplasia reported that C3H mice treated for 1 year with inorganic arsenic at 85 mg/l in drinking-water (~8 mg/kg bw per day) during gestation, pre-pubescence and post-pubescence exhibited differential proliferative lesions and tumour outcomes (Ahlborn et al.,

2009). The authors observed that urinary bladder hyperplasia incidence was significantly increased in female mice chronically exposed to arsenic from either gestational day (GD) 8 or postnatal day (PND) 21 through 1 year. In contrast, male mice continuously exposed to arsenic from GD 8 through 1 year had significantly decreased incidence of liver and adrenal tumours, in comparison with both mice exposed in utero only and untreated control mice. These results suggest that continuous inorganic arsenic exposure at 85 mg/l from gestation through 1 year increases the incidence and severity of urogenital proliferative lesions in female mice and decreases the incidence of liver and adrenal tumours in male mice. The paradoxical nature of these effects may be related to altered lipid metabolism, the effective dose in each target organ or the shorter 1-year observational period (Ahlborn et al., 2009).

Nelson et al. (2009) investigated the hepatic gene expression patterns that may lead to the apparent protective effect of continuous arsenic treatment of C3H mice seen at 1 year, using liver tissue samples taken from the different treatment regimens of the companion study (Ahlborn et al., 2009). The authors found that continuous arsenic treatment altered expressions of genes involved in cellular growth and proliferation, cell death, oxidative stress, protein ubiquitination and mitochondrial dysfunction, and many of these genes are known to be involved in liver carcinogenesis. Furthermore, the study demonstrated a marked reduction in stearoyl-coenzyme A desaturase-1 messenger ribonucleic acid (mRNA) in mice continuously exposed to arsenic for 1 year compared with controls and the in utero–only treatment group. The authors concluded that the unexpected liver tumour–protective effect of continuous arsenic exposure from GD 8 until 1 year in C3H mice can most likely be attributed to gene pathways involving gene expression, oxidative stress and cell death.

In previous experimental studies, DES was shown to enhance the carcinogenic response of prenatal arsenic exposure in a variety of mouse tissues. A new study by Liu et al. (2009) examined interactions of in utero arsenic exposure and postnatal DES treatment in the neonatal adrenal gland and evaluated the resulting gene expression related to estrogen signalling and steroid metabolism. Pregnant CD1 mice were exposed to drinking-water containing sodium arsenite at an arsenic concentration of 85 mg/l from day 8 to day 18 of gestation and were allowed to deliver normally. The offspring were subsequently injected subcutaneously on postpartum days 1–5 with DES (2 µg/pup per day) and killed on PND 12. The study found that fetal arsenic exposure greatly enhanced DES-induced, estrogen-linked gene expression (such as estrogen receptor-α and trefoil factors), as well as the expression of genes involved with steroid metabolism and/or methionine metabolism, including genes encoding for 17β-hydroxysteroid dehydrogenase type 5 (*HSD17β5*) and androstenedione 15α-hydroxylase (*Cyp2a4*). In addition, the transcripts for homocysteine cycling genes (betaine-homocysteine methyltransferase and thioether *S*-methyltransferase) and developmental marker genes (α-fetoprotein, insulin-like growth factor [IGF] 2 and IGF binding protein-1) were higher with arsenic plus DES than with either treatment alone. The authors concluded that exposure of the mice to arsenic during a critical period of fetal development may potentially alter adrenal genetic programming,

leading to endocrine disruption and potentially enhancing tumour formation together with DES at other sites much later in life.

In its most recent evaluation, IARC concluded that there is sufficient evidence for carcinogenicity of inorganic arsenic compounds in experimental animals and sufficient evidence for carcinogenicity of DMA^V in experimental animals (IARC, in press).

2.2.4 Genotoxicity

Evidence from a wide range of studies has led to the conclusion that arsenic compounds do not react directly with DNA (EFSA, 2009; IARC, in press). Inorganic arsenic does not covalently bind to DNA (Kitchin & Wallace, 2008). It does not induce point mutations in bacterial or mammalian test systems, and it has been shown to be an extremely weak (or insignificant) mutagen at single gene loci, such as thymidine kinase (*TK*) or hypoxanthine guanine phosphoribosyltransferase (*HPRT*) (ATSDR, 2007; EFSA, 2009). However, as a secondary result of genomic instability, chronic exposure to low, non-cytotoxic concentrations of arsenite (≥0.1 µmol/l) has been shown to induce delayed mutagenesis at the *HPRT* locus and cell transformation after 20–30 generations in cultured human osteogenic sarcoma cells (Mure et al., 2003). At higher concentrations, arsenite (≥7 µmol/l) induced large deletion (multilocus) mutations in hamster human hybrid cells (Hei, Liu & Waldren, 1998), micronuclei and chromosomal aberrations, aneuploidy and sister chromatid exchanges in various mammalian cells (ATSDR, 2007). In vivo, oral treatment with arsenite induced chromosomal aberrations in mouse peripheral blood lymphocytes and in mouse bone marrow (IPCS, 2001; USEPA, 2007).

Studies in mammalian cells have shown the induction of DNA damage (strand breaks, oxidative base modifications, apurinic/apyrimidinic sites, DNA–protein crosslinks) by non-cytotoxic (nanomole per litre to micromole per litre) arsenite concentrations (≥0.1 µmol/l, Wang et al., 2002; ≥10 nmol/l, Schwerdtle et al., 2003). Thus, chromosomal alterations may be a secondary result of arsenite-induced DNA damage and interference with DNA damage response pathways. Li & Broome (1999) proposed that arsenite crosslinks tubulin and inhibits guanosine triphosphate binding, resulting in disturbed tubulin polymerization, and mitosis, which may contribute to micronuclei formation. Additionally, inorganic arsenic can cause gene amplification in mouse 3T6 cells (Lee et al., 1988).

Inorganic arsenic increases the genotoxicity, mutagenicity and clastogenicity of other DNA-damaging agents, including UV light, benzo[*a*]pyrene and alkylating agents (Okui & Fujiwara, 1986; Rossman, Molina & Klein, 1986), which may be mediated via interference with DNA damage response processes. Arsenite strongly increased micronuclei induced by benzo[*a*]pyrene in mouse bone marrow (sodium arsenite at 50 mg/l, 7 days; Lewinska et al., 2007) and increased the mutagenicity of benzo[*a*]pyrene in mouse skin (sodium arsenite at 10 mg/l, 10 weeks; Fischer et al., 2005).

DMA^V and MMA^V were not mutagenic in the Ames test, but a number of studies have shown that they can cause chromosomal aberrations and mutations at cytotoxic (high micromole per litre) concentrations (ATSDR, 2007; EFSA, 2009).

In subcellular and cellular systems, MMA^{III} and DMA^{III} induced DNA strand breaks and oxidative base lesions generally at lower concentrations than were required for inorganic arsenic and the pentavalent metabolites (EFSA, 2009). $DMMTA^{V}$ induced aneuploidy, structural chromosomal aberrations and abnormalities of spindle organization and centrosome integrity, starting at micromole per litre (≥10 µmol/l) concentrations (Ochi et al., 2008).

Oral administration of DMA^{V} to mice caused DNA strand breaks in the lung (1500 mg/kg bw, a single dose; Yamanaka et al., 1989; Yamanaka & Okada, 1994) and increased the urinary level of 8-hydroxy-2α-deoxyguanosine (8-OHdG) lesions (50 mg/kg bw, a single dose; Yamanaka et al., 2001) and the 8-OHdG DNA levels in the lung and liver (400 mg/l in drinking-water, 4 weeks; Yamanaka et al., 2001), but not in the bladder, skin, spleen or kidney. In contrast, DMA^{V} administered to rats significantly increased the level of 8-OHdG in the bladder (200 mg/l in drinking-water, 2 weeks or 20 days; Wei et al., 2002; Kinoshita et al., 2007) and kidney (10 mg/kg bw, 4 weeks, every 5 days; Vijayaraghavan et al., 2001). Following an intraperitoneal injection, DMA^{V} induced aneuploidy (300 mg/kg bw, a single injection), but no chromosomal aberrations, in mouse bone marrow cells (Kashiwada, Kuroda & Endo, 1998) and an increase of *lacZ* mutations in the lung, but not in the bladder or bone marrow, in Muta™Mouse (10.6 mg/kg bw per day, 6 days; Noda et al., 2002). After TMAO exposure (200 mg/l in drinking-water, 15 days; Kinoshita et al., 2007), a significant increase in 8-OHdG was observed in the rat liver.

The major underlying mechanisms of the genotoxic effects of arsenic compounds include the rapid induction of oxidative DNA damage and DNA repair inhibition and slower changes in DNA methylation patterns, aneuploidy and gene amplification. Gene amplification, altered DNA methylation and aneuploidy lead to altered gene expression and genomic instability. Inhibition of DNA repair leads to co-mutagenicity as well. These effects are consistent with the experimental animal carcinogenicity data, in which arsenite is a transgenerational carcinogen, with exposure being present during many cell generations, and with co-carcinogenicity (EFSA, 2009; IARC, in press).

There is very limited information relating to the genotoxicity of other arsenic compounds. One paper has been identified relating to the genotoxicity of arsenosugars. The trivalent and pentavalent arsenosugars that were investigated were not mutagenic in *Salmonella* strain TA104 (Andrewes et al., 2004). Arsenobetaine (AB) at concentrations up to 10 mg/ml did not induce mutations in bacterial or mammalian cell assays and did not induce sister chromatid exchanges or metabolic cooperation in V79 Chinese hamster cells. Unlike inorganic and methylated arsenic species, AB had no synergistic or antagonistic effects on the action of benzo[*a*]pyrene and TPA (Jongen et al., 1985).

2.2.5 Reproductive and developmental toxicity

As discussed in EFSA (2009), inorganic arsenic has been shown to be embryotoxic and teratogenic in experimental animals; however, most studies have used high parenteral arsenic dosing, which might have involved maternal toxicity

(Golub, Macintosh & Baumrind, 1998; Wang et al., 2006). Recently, experimental studies without maternal toxicity have shown fetal growth retardation, neurotoxicity and alteration in pulmonary structure following oral dosing at relevant exposure levels, often in the form of arsenate (Wang et al., 2006; Hill, Wlodarczyk & Finnell, 2008). Using a mouse model, in utero and early postnatal exposures to arsenic (100 µg/l or less in drinking-water in the form of arsenite) were found to alter airway reactivity to methacholine challenge in 28-day-old pups (Lantz et al., 2009). The functional changes were correlated with protein and gene expression changes as well as morphological structural changes around the airways.

During its development, the brain is particularly vulnerable, and fetal arsenic exposure and exposure soon after birth cause neurotoxicity, resulting in behavioural changes (Rodriguez, Jimenez-Capdeville & Giordano, 2003; Wang et al., 2006). Rats exposed to high concentrations of arsenite (37 mg/l) in drinking-water from GD 15 until 4 months of age showed increased spontaneous locomotor activity and alterations in a spatial learning task compared with control rats (Rodriguez et al., 2002). The latter effects were also found in rats exposed from PND 1. Exposure of pregnant rats and offspring to high inorganic arsenic (sodium arsenite at 100 mg/l in drinking-water from GD 6 to PND 42) also caused alterations in learning and memory behaviour and some reflex responses (Xi et al., 2009).

Exposure of mouse dams to relatively low levels of arsenic (50 µg/l as arsenate) during pregnancy and lactation resulted in changes in the neuroendocrine markers associated with depression and altered behaviour indicative of depression (learned helplessness and immobility during forced swim) in affected adult C57BL/6J mouse offspring (Martinez et al., 2008). The results suggested that perinatal arsenic exposure may disrupt the regulatory interactions between the hypothalamic–pituitary–adrenal axis and the serotonergic system in the dorsal hippocampal formation in a manner that predisposes affected offspring towards depressive-like behaviour.

Neural tube defects have been observed in experimental studies, with a dose-related increase at inorganic arsenic doses of 4.8–14.4 mg/kg bw per day administered to mice by oral gavage as sodium arsenate (Hill, Wlodarczyk & Finnell, 2008).

In summary, studies in experimental animals demonstrate that in utero exposure to inorganic arsenic via oral administration to the dam causes neural tube defects, fetal growth retardation and neurotoxicity, including alterations in locomotor activity and spatial learning and changes in neuroendocrine markers associated with depressive-like behaviours in the offspring. Inhibition of arsenic methylation has been shown to increase its developmental toxicity (EFSA, 2009).

Little information exists on early-life toxicity of DMA^V and MMA^V. Developmental toxicity studies of orally administered DMA^V and MMA^V in the Sprague-Dawley rat and New Zealand White rabbit have shown an absence of dose-related effects at exposure levels that were not maternally toxic. MMA^V at doses of 0, 10, 100 and 500 mg/kg bw per day (rat) and 0, 1, 3, 7 and 12 mg/kg bw per day (rabbit) and DMA^V at doses of 0, 4, 12 and 36 mg/kg bw per day (rat) and 0, 3, 12 and 48 mg/kg bw per day (rabbit) were administered by oral gavage daily

during organogenesis (GDs 6–15 in rats and GDs 7–19 in rabbits), and the litters were examined at maternal sacrifice (GD 20 in rats; GD 29 in rabbits). After treatment with MMA^V, both maternal toxicity and fetal toxicity were observed at the highest doses of 500 mg/kg bw per day (rat) and 12 mg/kg bw per day (rabbit), but no treatment-related developmental toxicity was found at the lower doses. There was no evidence of teratogenicity associated with MMA^V treatment. With DMA^V, maternal toxicity and developmental toxicity were observed in the rat at 36 mg/kg bw per day. In the rabbits at 48 mg/kg bw per day, there was marked maternal toxicity, culminating in maternal death or abortion, and there were no surviving fetuses for evaluation. There was no treatment-related maternal or developmental toxicity in the rat or rabbit at 12 mg/kg bw per day or below (Irvine, Boyer & DeSesso, 2006).

Groups of 12 time-mated pregnant Sprague-Dawley rats were given AB in aqueous solution by oral gavage at doses of 0, 0.1, 1.0 and 10 mg/kg bw per day from GD 8 until sacrifice on GD 20 (6 dams), PND 13 (3 dams plus pups) and PND 21 (3 dams), at which time pups were weaned and allowed to reach 90 days of age without further dosing. Reproductive and developmental parameters were monitored. There were no differences in maternal body weight or organ weights or in sex ratio or litter size of the offspring. In the pups, there were no treatment-related differences in body weight or organ weights. Preliminary analysis indicated a small, significant increase in crown–rump length in the pups of the highest dose group. In male pups, preputial separation was delayed slightly by the low dose of AB, and in females, vaginal opening was delayed by both the low and high doses of AB. A small advancement in the day of eye opening was also observed. Clinical chemistry and haematology showed some minor differences (Cooke, 2009).

2.2.4 Special studies

(a) Immunotoxicity

EFSA (2009) described studies demonstrating effects of arsenicals on the immune system. Arsenate at concentrations of 0.5, 5 and 50 mg/l in drinking-water administered to female mice for 12 weeks resulted in decreased production of nitric oxide and superoxide in stimulated peritoneal macrophages (Arkusz et al., 2005). In male mice, 3 weeks of exposure to arsenite in drinking-water (0.5, 2.0 and 10 mg/l) resulted in immunosuppression of the humoral response, suppressing both the primary and secondary immune responses (Blakley, Sisodia & Mukkur, 1980). In day-old chicks, inorganic arsenic at 3.7 mg/l in drinking-water for up to 60 days suppressed the cellular and humoral immune response (Aggarwal et al., 2008). Suppression of the immune system has also been reported in zebrafish embryos exposed to inorganic arsenic at 2 and 10 µg/l in egg water for several days (Nayak, Lage & Kim, 2007) and in mice given arsenite at 10 or 100 µg/l in drinking-water or at 10 µg/kg in food for 5–6 weeks (Kozul et al., 2009). In male mice exposed to arsenite at 0.1, 1.0 and 50 µg/l in drinking-water for 5 weeks, there was a decrease in expression of transcripts involved in the immune response (Andrew et al., 2007).

Oral administration of MMA^V to nestling finches at 4–72 mg/kg bw per day for 20 days resulted in no effects on immune function. No further studies were found

regarding immune function or immunological or lymphoreticular effects following oral exposure to organic arsenic. No histological alterations were observed in immunological tissues following exposure of rats and mice to high doses of DMA^V (7.8 and 94 mg/kg bw per day), MMA^V (67.1 and 72.4 mg/kg bw per day) or roxarsone (4 and 43 mg/kg bw per day) (ATSDR, 2007).

In addition, Singh et al. (2010) investigated the adverse health effects of inorganic arsenic administered in the diet as sodium arsenite at low (0.05 mg/kg) and high (5 mg/kg) doses in Swiss male albino mice, alone and in combination with jaggery (a natural sweetener made from sugarcane juice) feeding (250 mg/mouse), consecutively for 180 days. Arsenic treatment resulted in substantially reduced total antioxidant levels, inhibition of pro-inflammatory cytokine activity, induction of DNA single-strand breaks and necrotic and degenerative changes in bronchiolar epithelium with emphysema and thickening of alveolar septa in the lung, in a dose-dependent manner, compared with the groups treated with both arsenic and jaggery. The authors concluded that chronic exposure to arsenic induced dose-dependent toxicity via oxidative stress with immunotoxicity and pathomorphological lesions to the respiratory system and that jaggery feeding antagonized the arsenic-induced negative effects.

(b) Neurotoxicity

A number of studies in rats and mice have reported no symptoms of overt systemic toxicity from inorganic arsenic (ATSDR, 2007; EFSA, 2009), but more subtle neurobehavioural effects have been observed (Rodriguez, Jimenez-Capdeville & Giordano, 2003). In rats, the most consistent change in behaviour after high oral inorganic arsenic administration (10 and 20 mg/kg bw per day by gavage for 2–4 weeks) was a decrease in locomotor activity. Additionally, rats showed a delay in the execution of various task tests reflecting learning and memory after oral exposure to arsenic (Rodriguez et al., 2001, 2002). Effects on locomotor activity, grip strength and rota rod performance were also observed recently in rats exposed orally to arsenite at 20 mg/kg bw per day for 28 days (Yadav et al., 2009). Mice were exposed to arsenic trioxide at 1 and 4 mg/l in the drinking-water subchronically for 60 days, and significant dose-dependent neurobehavioural changes associated with memory (Morris Water Maze test) were observed. In addition, the critical gene expression profiles related to the Creb-dependent phase of cerebellar long-term depression were analysed by GeneChip and showed downregulated expression of Ca^{2+}/calmodulin-dependent protein kinase IV (*Camk4*). Finally, antioxidants such as taurine and vitamin C did not prevent the downregulation of *Camk4*, indicating that such downregulation may be via an oxidation-independent mechanism (Y. Wang et al., 2009). Additionally, rats exposed to inorganic arsenic in drinking-water at 68 mg/l for 3 months showed a significant decrease in their spatial memory, whereas neurons and endothelial cells presented pathological changes, and the gene expression of aspartate receptors in the hippocampus was downregulated. These effects were not seen at 2.72 or 13.6 mg/l (Luo et al., 2009).

In mice, inorganic arsenic in drinking-water (0.05–5 mg/l, 4 months) led to sex-dependent alterations in dopaminergic markers, spontaneous locomotor

activity and downregulation of the antioxidant capacity of the brain (Bardullas et al., 2009).

Dietary organoarsenicals, including AB and arsenocholine (AC), have not been associated with peripheral or central neurotoxicity. MMA^{V} did not result in clinical signs of neurotoxicity or brain lesions following chronic dietary exposure of rats at doses up to 70.4 mg/kg bw per day or of mice at doses up to 67.1 mg/kg bw per day (Arnold et al., 2003). A similar outcome for DMA^{V} was reported; no clinical signs or histological alterations were observed after chronic exposure to 7.6 or 42.6 mg/kg bw per day (Arnold et al., 2006). Hippocampal slices of young (14–21 days old) and adult (2–4 months old) rats were treated with MMA^{V} and MMA^{III}, and evoked synaptic field potentials from the Schaffer collateral–CA1 (the excitatory cornu ammoni, a specific anatomic area in the hippocampus) synapse were measured under control conditions and during and after 30 and 60 min of application of the arsenic compounds. MMA^{V} had no effect on the synapse functions either in slices from adult rats or in those from young rats, whereas MMA^{III} strongly depressed the synaptic transmission at concentrations of 50/25 µmol/l (adult/young rats) and long-term potentiation amplitudes at concentrations of 25/10 µmol/l (adult/young rats). In contrast, application of MMA^{III} at 1 µmol/l led to an enhancement of the long-term potentiation amplitude in young rats, which was interpreted as an enhancing effect on *N*-methyl-D-aspartate receptors and a lack of blocking effect on α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptors. These impairments of the CA1 synapse were interpreted as being more likely caused by the action of methylarsonite on post-synaptic glutamatergic receptors and may be jointly responsible for dysfunctions of cognitive effects in arsenic toxicity (Krüger et al., 2009).

A recent study reported a link between disruption of the synthesis and assembly of myelin, an essential element for neural transmission, and a deficient production of methylated compounds in an in vivo model of prolonged arsenic exposure. Adult female Wistar rats exposed to arsenic (3 and 36 mg/l in drinking-water) from gestation throughout lactation and development until 1, 2, 3 and 4 months of age suffered myelin damage reflected as empty spaces in fibre tracts. The 3 mg/l (approximately 0.4 mg/kg bw per day) group did not present myelin damage during the first 2 months, with only moderate alterations in the third and fourth months. By contrast, animals exposed to 36 mg/l (approximately 4 mg/kg bw per day) showed moderate to severe damage to nerve tracts from the first month of age. The myelin alterations were followed by significantly lower levels of dimethyl arginine in the third and fourth months of age and exposure, compared with the controls, suggesting that myelin composition is a target of arsenic through interference with arginine methylation and that disturbances in nervous transmission through myelinated fibres are an important component of arsenic neurotoxicity (Zarazúa et al., 2010).

(c) Cardiovascular effects

Arsenate and arsenite have been shown to alter cardiovascular response in studies in rats and rabbits. Rats given arsenite or arsenate at 50 mg/l in drinking-water for 200 days showed an elevation in blood pressure up to day 80, with the

effects of arsenite being more marked than those of arsenate. The most common marker of hypertension, the angiotensin-converting enzyme (ACE), showed no significant change in either arsenic group, whereas cytochrome P450 4A (CYP4A) was highly expressed in both groups. The authors concluded that CYP4A might be more important than ACE in contributing to arsenic-induced hypertension (Yang et al., 2007; EFSA, 2009).

Sodium arsenite (50 µg/ml as arsenic) administered in drinking-water to rats (18 months) or rabbits (10 months) was associated with decreased cardiac stroke volume and output and increased vascular resistance (IPCS, 2001). Changes in blood cell counts, enzymes associated with haem synthesis and anaemia have been reported in a number of studies. The lowest arsenite doses (administered in drinking-water) associated with altered haemotocrit were 0.9 mg/kg bw per day in rats and 0.7 mg/kg bw per day in guinea-pigs (ATSDR, 2007).

Unlike inorganic arsenic, MMA^V and DMA^V have not been found to cause cardiovascular effects (ATSDR, 2007; EFSA, 2009).

(d) Nephrotoxicity

A short-term study in which Kunming mice were treated for 60 days with arsenic trioxide at 1, 2 or 4 mg/l in drinking-water showed pathological changes, such as cellular swelling, tubular dilatation and lymphocytic infiltration, as well as a significant increase in the level of 8-OHdG expression ($P < 0.01$) in the kidney tissues, suggesting that these changes may be related to arsenic-induced increases in oxidative stress (Li et al., 2010). A dose-dependent increase in renal damage and stronger immunoactivity of 8-OHdG were observed, mainly concentrated in the Bowman's capsule and renal tubules.

2.3 Observations in humans

2.3.1 Biomarkers of exposure

Biomarkers for assessing the exposure to arsenic from all sources are arsenic concentrations in urine, blood, hair and nails (Klaassen, 2001; Hughes, 2006). Perhaps the most commonly used biomarker is measurement of total arsenic in urine; ingested arsenic compounds are excreted with a short half-time of a few days (Buchet, Lauwerys & Roels, 1981; Vahter, 2002; Hughes, 2006). However, exposure to arsenic in fish or seafood commodities that contain organic arsenic in the form of AB has been observed to vastly increase the measurement of total arsenic in urine (Arbouine & Wilson, 1992; Buchet, Pauwels & Lauwerys, 1994; Heitland & Koster, 2008). Thus, measurement of total arsenic in urine may lead to an overestimation of exposure if ingestion of AB is not taken into account (Caldwell et al., 2009; Sirot et al., 2009a). Intake of certain seafood, such as mussels that contain DMA^V or seaweed containing arsenosugars, can also interfere with the interpretation of exposure when total arsenic is measured in urine (Hakala & Pyy, 1995; Ma & Le, 1998).

Urine samples may vary in dilution owing to differences in fluid intake; thus, urinary arsenic concentrations may be normalized to urinary specific gravity or, in some instances, to creatinine concentration (ACGIH, 2008; Nermell et al., 2008).

Because of the organic arsenic in fish, shellfish and seaweed and the need for determination of the level of inorganic arsenic in urine, specific measurements of inorganic arsenic and its methylated metabolites in urine are preferred (Buchet, Lauwerys & Roels, 1981; Farmer & Johnson, 1990; Hakala & Pyy, 1995; Verdon et al., 2009). High-throughput analytical methods developed for the population biomonitoring programme of the United States Centers for Disease Control and Prevention can provide determination of seven separate arsenic species in human urine: AB, AC, TMAO, arsenate, arsenite, MMA^{V} and DMA^{V} (Verdon et al., 2009). Together, arsenate and arsenite constitute excreted total inorganic arsenic, whereas MMA^{V} and DMA^{V} constitute total excreted methylated metabolites using standard analytical methods. Urinary concentrations of inorganic arsenic and methylated metabolites in the general population vary in different locations given differences in arsenic concentrations in primary foodstuffs and drinking-water, among other exposure sources. Inorganic arsenic and methylated metabolite concentrations in the urine of the general population are about 10 µg/l in European countries, approximately 9 µg/l in the USA (Caldwell et al., 2009) and up to 50 µg/l in Japan (Foa et al., 1984, 1987; Aizawa & Takata, 1990; Aitio, Hakala & Pyy, 1997; Klaassen, 2001). A reference value of 15 µg/l is reported for German children (Schulz et al., 2009).

AB, an organic arsenic compound not readily bioavailable, is excreted in urine following dietary exposure principally via ingestion of fish and seafood commodities. AB accounts for an increasing median percentage of total arsenic in urine as total arsenic in urine increases (Caldwell et al., 2009). For example, for urine samples with a total arsenic concentration below 20 µg/l, AB accounts for 16.2% of the total arsenic in the urine; for urine samples with a total arsenic concentration of 20–49 µg/l, AB accounts for 43.4% of the total; and for urine samples with a total arsenic concentration above 50 µg/l, AB accounts for 62.7% of the total.

A number of studies have indicated a roughly 1:1 ratio between the sum of the concentrations of inorganic arsenic, MMA and DMA in urine and the concentration of inorganic arsenic in drinking-water, where arsenic intake from water exceeds that from food (Hopenhayn-Rich et al., 1996; Calderon et al., 1999; Concha, Nermell & Vahter, 2006; Lindberg et al., 2006, 2008; Vahter et al., 2006). Accordingly, if drinking-water arsenic levels are low relative to those in food, the ratio of the sum of inorganic arsenic, MMA and DMA in urine to that in water may be greater than 1 (EFSA, 2009).

Following exposure, arsenic is cleared rapidly from the blood; for low and intermittent environmental or occupational exposures, arsenic blood concentration generally has not been considered a reliable indicator of exposure (NRC, 1999; ACGIH, 2008). In the instance of chronic high exposure to inorganic arsenic, however, it appears that arsenic in blood reaches a steady state and therefore may well reflect exposure in these circumstances (Hall et al., 2006, 2007).

Intake of arsenic compounds results in accumulation of arsenic in hair and nails due to binding to sulfhydryl groups in keratin; measurement of arsenic in hair and nails is considered a reasonable reflection of exposure over a period of the previous months (NRC, 1999; Hughes, 2006; ATSDR, 2007). Arsenic contamination of hair and nail samples due to adsorption from external sources is not distinguished from arsenic from internal sources using standard analytical methods (Hindmarsh, 2002; Hughes, 2006). More recent analytical work on hair has shown that arsenic from internal sources resides at the periphery of the strand (Nicolis et al., 2009). Hair can adsorb relatively more exogenous arsenic (Mandal, Ogra & Suzuki, 2003); therefore, nails are the preferred sample, although either can be contaminated from contact with exogenous sources, including water or soil. Significant correlations have been observed between exposure to arsenic in drinking-water and arsenic in hair (Kurttio et al., 1998) and toenails (Karagas et al., 2000; Slotnick et al., 2007; Slotnick, Meliker & Nriagu, 2008). A regression model that used total daily arsenic exposure from food and drinking-water explained the most variability in toenail arsenic concentrations ($R^2 = 0.71$) when the median daily arsenic dose was 1.0 µg/kg bw per day from food and 0.1 µg/kg bw per day from drinking-water (Kile et al., 2007a). Arsenic measured in toenails at 3- to 6-year intervals has been shown to yield a consistent correlation with arsenic in water, indicating stability of the measurement over time (Garland et al., 1993; Karagas et al., 2001a). Generally, the principal compound in hair and nails is inorganic arsenic; measurement of total arsenic in hair and nails is considered useful as a biomarker of exposure.

Toenail arsenic concentrations between 0.07 and 0.45 µg/g reflect arsenic water concentrations between 1 and 100 µg/l ($r = 0.65$, $P < 0.0001$); a toenail arsenic concentration of 0.326 µg/l approximates a concentration of 50 µg/l in water. The correlation of toenail arsenic with drinking-water arsenic at water concentrations above 1 µg/l is $r = 0.64$ (Karagas et al., 2000). Toenail arsenic concentrations were correlated with a food frequency questionnaire ($r = 0.33$, $P < 0.0001$), but not with published food content information (MacIntosh et al., 1997).

2.3.2 Biomarkers of effect

A number of biomarkers of effect have been reported; however, none are attributed specifically to arsenic. Urine proteomics have been applied to identify an increased level of human β-defensin-1 (HBD-1) in urine of highly exposed men (arsenic concentration in drinking-water >500 µg/l), but not women (Hegedus et al., 2008). HBD-1 was also 1 of 33 proteins identified in urine of eight patients with blackfoot disease typical of the high-arsenic areas of south-western Taiwan, China (Tan et al., 2008). It has been suggested that urine proteomics be further explored as a promising arsenic biomarker of effect (Navas-Acien & Guallar, 2008). Biomarkers of effect that include those for oxidative stress and damage related to arsenic exposure, such as urinary excretion of 8-OHdG, have been recently reviewed (De Vizcaya-Ruiz et al., 2009). Malondialdehyde levels in urine are increased in people who have been chronically exposed to arsenic and is thought to be a useful biomarker of effect (Wang et al., 2009a). Some additional nonspecific biomarkers of effect, such as high pulse pressure, increased carotid

artery intima-medial thickness, proteinuria, presence of transforming growth factor-alpha (TGFα) in urine and increased serum level of Clara cell protein, have been correlated with arsenic exposure and proposed as nonspecific biomarkers of chronic effect (Chen et al., 2010b).

2.3.3 Clinical observations

Signs and symptoms of acute illness may include anorexia, hepatomegaly, cardiac arrhythmia, respiratory tract symptoms, peripheral neuropathy and gastrointestinal, cardiovascular and haematopoietic effects (Klaassen, 2001). Other acute symptoms include muscular cramps, facial oedema and cardiac abnormalities. The fatal dose of ingested arsenic trioxide for humans ranged from 1 to 3 mg/kg bw (Vallee, Ulmer & Wacker, 1960).

Chronic exposure may lead to dose-related neurotoxicity, including sensory changes and paraesthesia, as well as progressive peripheral neuropathy. Clinical effects, including liver injury, peripheral vascular and cardiovascular effects, diabetes and cancer (see section 2.3.4), have been observed following chronic environmental or occupational exposure to various forms of arsenic. Arsenicosis is a term ascribed to a multisystem disorder, including skin manifestations, related to long-term chronic exposure to high concentrations of arsenic, principally in drinking-water (Ghosh et al., 2008; Sengupta, Das & Datta, 2008). Predominant clinical manifestations are related to cutaneous involvement, such as pigmentary changes, hyperkeratosis and skin cancers (Bowen disease, squamous cell carcinoma and basal cell epithelioma), as well as other clinical effects on the circulatory, neurological, haematological, respiratory and renal systems.

2.3.4 Epidemiological studies

(a) Cancer

(i) Skin cancer

The classification of arsenic as a carcinogen was originally based on evidence of skin cancers in patients treated with arsenic-containing solutions and in occupational settings (IARC, 1987). Subsequently, ecological studies in the blackfoot disease–endemic region of Taiwan, China, where high exposures to arsenic in drinking-water occurred, indicated a causal relationship with skin cancer alone (Tseng et al., 1968). These studies and others from the region confirmed the relationship (IARC, 2004). Studies at lower levels of arsenic exposure in drinking-water have been conducted in the USA and Denmark to examine increased risk for non-melanoma skin cancer (NMSC).

Skin cancer case–control studies

Analysis of NMSC data using alternative statistical approaches in a case–control study in New Hampshire, USA, indicated that for squamous cell carcinoma (SCC), a two-segment regression model identified a maximum likelihood change point of 0.105 µg of arsenic per gram of toenails (95% confidence interval [CI]

0.068–0.115), after which the increasing trend of 0.61% increase in risk of SCC associated with a 1% increase in toenail arsenic was statistically significant (Karagas, Stukel & Tosteson, 2002). For SCC, the 95% CI fell within the exposure range of the control group, and both the quadratic and two-segment models produced relatively consistent results. The change point for SCC was at arsenic concentrations corresponding to about 1–2 µg/l in water, with the 95% CI spanning from below 1 µg/l up to 10–20 µg/l. The two-segment regression analysis could not be estimated reliably for basal cell carcinoma (BCC). The authors pointed out that "change points" need to be interpreted with caution, as they rely on the appropriate model fit as well as statistical precision.

A case–control study in Iowa, USA, examined risk of melanoma skin cancer in relation to arsenic exposure and found a significantly increased trend for risk of melanoma with elevated toenail arsenic concentration, particularly among those with self-reported prior skin cancer diagnosis (Beane Freeman et al., 2004). A study limitation is that melanoma cases were compared with colon cancer controls.

Skin cancer cohort study

A prospective cohort study on the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort was conducted in Denmark using a geographic information system (GIS) to estimate individual exposure to arsenic in drinking-water, which ranged from 0.05 to 25.3 µg/l (mean 1.2 µg/l). After adjustment for enrolment area, no significant increase in NMSC was found.

Results of key studies are summarized in Table 3.

(ii) Bladder cancer

Significant associations between exposure to high levels of ingested arsenic in drinking-water and bladder cancer have been observed in ecological studies from Chile, Argentina and Taiwan, China, and case–control studies in Taiwan, China (IARC, 2004). A number of more recent bladder cancer studies of populations exposed to drinking-water arsenic concentrations at or below 100 µg/l have used total arsenic toenail concentration as an exposure biomarker, as it may integrate exposure from all routes and reflect exposure over a longer period than either blood or urinary levels of arsenic (see section 2.3.1).

Bladder cancer ecological study

An ecological study was conducted on all 44 counties in Idaho, USA, grouping counties into three categories of exposure based on groundwater arsenic measurements (Han et al., 2009). In total, 3530 bladder cancer cases (960 cases in 23 Low counties, defined as <2 µg/l in groundwater; 1895 cases in 16 Intermediate counties, defined as 2–10 µg/l; and 675 cases in 5 High counties, defined as >10 µg/l) were included in the study. After adjustment for race, sex, population density, smoking prevalence and body mass index, no relationship between arsenic level in groundwater and cancer incidence was shown.

Table 3. Epidemiological case–control studies on skin cancer in humans in relation to ingested inorganic arsenic exposure below 100 µg/l in drinking-water[a]

Design Study population Reference(s)	Outcome definition	Population size (*n*)	Arsenic exposure	Results		Additional information
Ecological study South-west Taiwan, China Tseng et al. (1968)	Skin cancer prevalence	40 421 (428 skin cancers)	Concentration in water (µg/l)	Prevalence rate (/1000)		Used in earlier risk assessments with extrapolation to lower levels of exposure
			<300	2.6		
			300–600	10.1		
			>600	21.4		
Case–control study New Hampshire, USA Karagas et al. (2001b); Karagas, Stukel & Tosteson (2002)	Histologically confirmed incident BCC and SCC	BCC/SCC/ controls	Concentration in toenail (µg/g)	OR (95% CI)		Maximum likelihood estimate of the point at which the dose–response began to increase for SCC by 0.61% with a 1% increase in toenail arsenic: 0.105 µg/g (95% CI = 0.093–0.219). In total, 587 BCC cases and 284 SCC cases
				BCC	SCC	
		281/155/263	0.009–0.089	1.00 (reference)	1.00 (reference)	
		156/64/136	0.090–0.133	1.01 (0.76–1.35)	0.93 (0.64–1.34)	
		92/33/73	0.134–0.211	1.06 (0.74–1.51)	0.98 (0.61–1.58)	
		22/14/26	0.212–0.280	0.72 (0.40–1.31)	1.10 (0.55–2.21)	
		10/5/11	0.281–0.344	0.75 (0.31–1.81)	1.00 (0.33–3.01)	
		26/13/15	0.345–0.81	1.44 (0.74–2.81)	2.07 (0.92–4.66)	
Case–control study Iowa, USA Beane Freeman et al. (2004)	Histologically confirmed incident melanoma of the skin	Cases/ controls	Concentration in toenail (µg/g)	OR (95% CI) (*P* for trend = 0.001)		Estimated 12% equal to or above 10 µg/l in the population, highest level 80 µg/l. Note: finding was regarded
		52/82	≤0.020	1.0		
		58/83	0.021–0.039	1.0 (0.6–1.6)		

Design Study population Reference(s)	Outcome definition	Population size (*n*)	Arsenic exposure	Results				Additional information
		95/82	0.04–0.083	1.7 (1.1–2.7)				as preliminary because controls were colon cancer cases. In total, 363 melanoma cases and 373 controls (colon cancer)
		121/82	≥0.084	2.1 (1.4–3.3)				
Cohort study Denmark Baastrup et al. (2008)	First NMSC	1010 NMSC cases, 147 melanomas, cohort size = 57 053		Adjusted analysis		Further adjustment for area of enrolment		GIS analysis based on "Diet, Cancer and Health" cohort, exposure range 0.05–25.3 µg/l (mean = 1.2 µg/l). No designation of histological type of NMSC; most could be BCC
			Time-weighted average exposure from water (µg/l)	NMSC	Melanoma	NMSC	Melanoma	
				P-value = 0.99	*P*-value = 0.80	*P*-value = 0.85	*P*-value = 0.14	
				0.88 (0.81–0.94)	0.89 (0.73–1.07)	0.99 (0.94–1.06)	0.80 (0.59–1.08)	
			Cumulative exposure (5 mg)	*P*-value = <0.0001	*P*-value = 0.35	*P*-value = 0.35	*P*-value = 0.32	
				0.95 (0.92–0.97)	0.80 (0.59–1.08)	0.99 (0.97–1.01)	0.96 (0.89–1.04)	

OR, odds ratio

[a] Excludes Knobeloch, Zierold & Anderson (2006) study of skin cancer (no histological type specified) based on self-report; excludes Guo et al. (2001) ecological study.

Source: Adapted from Table 33 in EFSA (2009).

Bladder cancer case–control studies

Case–control studies in Utah, USA (Bates, Smith & Cantor, 1995), showed a statistically significant trend for smokers for cumulative exposure to arsenic in water (arsenic concentrations 0.5–160 µg/l). In Finland (Kurttio et al., 1999), an increase was also seen in smokers with relatively short latency at drinking-water concentrations up to 64 µg/l.

Three case–control studies have found increased risk for bladder cancer principally in ever smokers at moderately high arsenic exposures. Steinmaus et al. (2003) showed excess bladder cancer risk for smokers with a 40-year lag period and a median arsenic exposure of 177 µg/day, but not for shorter lag times or for never smokers. Karagas et al. (2004) likewise found an elevated odds ratio (OR) for bladder cancer (OR 2.17, 95% CI 0.92–5.11) for toenail arsenic category above 0.330 µg/g compared with below 0.06 µg/g. Among never smokers, a significant association was not found. Bates et al. (2004) found no evidence of bladder cancer associations with exposure estimates based on arsenic concentrations in drinking-water; however, when well water consumption was used as the exposure measure, time window analyses suggested that use of well water with arsenic levels above 50 µg/l more than 50 years before the interview was associated with increased bladder cancer risk in ever smokers only (OR 2.5, 95% CI 1.1–5.5).

A case–control study of incident bladder cancer risk conducted in Finland in which toenail arsenic (0.02–17.5 µg/g) was used as a biomarker of exposure found no association between inorganic arsenic concentration and bladder cancer risk (OR 1.13, 95% CI 0.70–1.81, for the highest versus the lowest tertile) (Michaud et al., 2004).

Bladder cancer cases (n = 832) in a case–control study in which individual exposure to arsenic was determined in home drinking-water and toenail samples were evaluated for survival with a median duration of follow-up of 9.3 years (Kwong et al., 2010). Comparisons of survival time with various percentiles of arsenic exposure were conducted—for example, lowest quartile exposure (0.057 µg/g toenail or 0.11 µg/l drinking-water) versus highest quartile exposure (0.12 µg/g toenail or 0.74 µg/l drinking-water). Results showed that overall survival was significantly prolonged for the highest arsenic exposure group using either measure of exposure after adjustment for age, sex, smoking status, stage, grade and therapy.

Bladder cancer cohort studies

A cohort study of 8086 subjects conducted in north-eastern Taiwan, China, found a nonsignificant increase in relative risk (RR) for exposure between 10.1 and 50 µg/l in several multivariate-adjusted models with an approximately 5-year follow-up (Chiou et al., 2001). A subsequent study at 12 years of follow-up was conducted (Chen et al., 2010a). Forty-five incident urinary cancer cases were ascertained through linkage with a national cancer registry and showed a significant positive trend with increasing arsenic concentration in drinking-water. For exposures above 100 µg/l, the RR was increased 5-fold, whereas the risk was elevated but not significant for low exposure (<100 µg/l).

A cohort standardized mortality ratio (SMR) study conducted in Utah, USA, on 2203 deceased individuals found no excess risk for bladder cancer (drinking-water arsenic range 14–166 µg/l) in this predominantly Mormon population (Lewis et al., 1999). Exposure misclassification is a consideration, and the number of bladder cancer cases observed was small ($n = 5$).

A prospective cohort study of 57 053 persons was conducted using the Danish Cancer Registry to identify cancer cases, including bladder cancer cases (n = 214 cases) (Baastrup et al., 2008). Individual exposure to arsenic was estimated to range between 0.05 and 25.3 µg/l. No significant association was found between exposure to arsenic and risk for a number of cancers, including bladder cancer.

Literature reviews on arsenic and bladder cancer have been conducted by Cantor & Lubin (2007) and Mink et al. (2008). It is conjectured that inconsistencies in arsenic bladder cancer study results may be related to low statistical power to detect modest effects at lower levels of exposure, among other factors. In general, bladder cancer risks at lower levels of exposure (e.g. 100 µg/l) appear to be below predictions based on high-exposure studies from Taiwan, China, and other high-exposure areas (Steinmaus et al., 2003).

Results of key studies are summarized in Table 4. As shown in Table 4, six bladder cancer case–control studies evaluated RR for never smokers and ever smokers. Of those, two studies (Bates, Smith & Cantor, 1995; Kurttio et al., 1999) showed significantly increased RR for ever smokers with cumulative arsenic exposure. One study (Bates et al., 2004) showed significantly increased RR after 50 years of arsenic exposure in ever smokers only, and three studies (Steinmaus et al., 2003; Karagas et al., 2004; Michaud et al., 2004) found non-significant increases in trend for RR for ever smokers only. Chiou et al. (2001), Chen et al. (2010a) and Baastrup et al. (2008) reported RR adjusted for age, sex and smoking in multivariate models. Of those studies, only Chen et al. (2010a) reported a significant trend ($P < 0.0001$) for RR with increasing arsenic concentrations.

(iii) Lung cancer

Exposure to arsenic at high concentrations in drinking-water has been shown to be associated with lung cancer in studies from Japan, Chile, Argentina, the USA and Taiwan, China (IARC, 2004). There are fewer studies at drinking-water exposures at and below 100 µg/l.

Lung cancer ecological study

An ecological study was conducted on all 44 counties in Idaho, USA, in which counties were grouped into three categories of exposure based on groundwater arsenic measurements (Han et al., 2009). A total of 9291 lung and bronchus cancer cases (2471 cases in 23 Low counties, defined as <2 µg/l in groundwater; 4910 cases in 16 Intermediate counties, defined as 2–10 µg/l; and 1910 cases in 5 High counties, defined as >10 µg/l) were included in the study. After adjustment for race, sex, population density, smoking and body mass index, no relationship between arsenic level in groundwater and lung and bronchus cancer incidence was shown.

Table 4. Epidemiological studies on bladder cancer and arsenic exposure informing dose–response at arsenic levels below 100 µg/l in drinking-water[a]

Design Study population Reference(s)	Outcome definition	Population size (*n*)	Smoking status	Arsenic exposure	Results: OR/RR (95% CI)	Additional information
Case–control study Utah, USA Bates, Smith & Cantor (1995)	Histologically confirmed bladder cancer	Bladder cancer cases =177; controls = 266 Cases/controls		Cumulative exposure (mg)	All subjects	Range 0.5–160 µg/l; recorded daily total fluid intake in litres. Statistically significant trend observed for ever smokers with 10–19 years' exposure, but not for shorter or longer exposure or for any exposure period for never smokers
		14/47		<19	1.00	
		21/36		19–<33	1.56 (0.8–3.2)	
		17/39		33–<53	0.95 (0.4–2.0)	
		19/38		>53	1.41 (0.7–2.9)	
				(mg/l)-years, latency 10–19 years	All subjects	
		18/42		<33	1.00	
		16/42		33–<53	0.69 (0.3–1.5)	
		16/40		53–<74	0.54 (0.3–1.2)	
		21/36		≥74	1.00 (0.5–2.1)	
					10–19 years' exposure	
		9/23	Never smoked	<8	1.00	
		8/19		8–<10	0.99 (0.3–2.9)	
		6/20		10–<13	0.67 (0.2–2.2)	

Design Study population Reference(s)	Outcome definition	Population size (*n*)		Smoking status	Arsenic exposure	Results: OR/RR (95% CI)		Additional information
		6/17			≥13	0.79 (0.2–2.6)		
		8/21		Ever Smoked	<8	1.00		
		12/19			8–<10	1.36 (0.5–3.9)		
		12/19			10–<13	1.57 (0.5–4.5)		
		17/18			≥13	2.92 (1.1–8.0)		
						P for trend < 0.05		
Case–control study Finland Kurttio et al. (1999)	Bladder cancer	Cases			Concentration in water (μg/l)	Short latency	Long latency	Maximum = 64 μg/l, and 1% exceeded 10 μg/l
		Short latency	Long latency					
		23	26		<0.1	1.00	1.00	Bladder cancer cases = 61, controls = 275
		19	18		0.1–0.5	1.53 (0.75–3.09)	0.81 (0.41–1.63)	
		19	17		0.5–64	2.44 (1.11–5.37)	1.51 (0.67–3.38)	
					(log) continuous	1.37 (0.95–1.96)	0.96 (0.59–1.55)	
		Smoker	Never/ ex-smoker		Concentration in water (μg/l)	Smoker	Never/ex-smoker	
		8	8	See results	<0.1	1.00	1.00	
		3	4		0.1–0.5	1.10 (0.19–6.24)	0.95 (0.25–3.02)	
		7	5		0.5–64	10.3 (1.16–92.6)	0.87 (0.25–3.02)	

Design Study population Reference(s)	Outcome definition	Population size (*n*)		Smoking status	Arsenic exposure	Results: OR/RR (95% CI)		Additional information
Case–control study Nevada, California, USA Steinmaus et al. (2003)	Primary bladder cancer	5-year lag	40-year lag		Cumulative exposure from water (mg)	5-year lag	40-year lag	Bladder cancer cases = 181, controls = 238
		58/63	130/189	Ever smokers	<6.4	1.00	1.00	
		46/79	6/8		6.4–82.8	0.69 (0.40–1.18)	1.06 (0.34–3.33)	
		48/66	16/11		>82.8	0.76 (0.44–1.30)	2.25 (0.97–5.20)	
		8/38	23/92	Never Smokers	<6.4	1.00	1.00	
		11/32	3/5		6.4–82.8	1.55 (0.51–4.72)	2.65 (0.49–14.24)	
		10/49	3/22		>82.8	0.83 (0.28–2.49)	0.50 (0.12–2.05)	
Case–control study New Hampshire, USA Karagas et al. (2004)	Incident TCC (96%)	Never smoker	Ever smoker	See results	Toenail arsenic (µg/g)	Never smoker	Ever smoker	Maximum likelihood estimate change point of 0.326 µg/g (95% CI 0.121–0.446), which equates to approximately 50 µg/l, with a 1.1% increase in ORs for 1% increase in toenail arsenic concentration
		15/41	75/121		0.009–0.059	1.00	1.00	
		20/56	99/105		0.060–0.086	0.85 (0.38–1.91)	1.53 (1.02–2.29)	
		22/48	66/109		0.087–0.126	1.18 (0.53–2.66)	1.02 (0.66–1.56)	
		11/29	37/67		0.127–0.193	1.10 (0.42–2.90)	1.00 (0.60–1.67)	
		3/14	18/18		0.194–0.277	0.49 (0.12–2.05)	1.78 (0.86–3.67)	
		0/3	3/10		0.278–0.330	—	0.50 (0.1–1.88)	
		0/8	14/11		0.331–2.484	—	2.17 (0.92–5.11)	
		7/4			0.331–2.484, <15 years	3.09 (0.80–11.96)		

Design Study population Reference(s)	Outcome definition	Population size (*n*)		Smoking status	Arsenic exposure	Results: OR/RR (95% CI)		Additional information
		7/6			0.331–2.484, ≥15 years	1.86 (0.57–6.03)		above change point ($P = 0.10$) TCC cases = 383, controls = 641
Case–control study Argentina Bates et al. (2004)	Incident transitional bladder cell cancer cases	Never smoker	Ever smoker		Concentration in water (µg/l)	Never smoker	Ever smoker	Possible latency effects: statistically significant associations among smokers with more than 50 years of exposure: 51–60 years: OR = 2.65 (1.2–5.8) 61–70 years: OR = 2.54 (1.0–6.4) for well water use TCC cases = 114, controls = 114
		22/37	65/45		0–50	1.00	1.00	
		2/4	7/4		51–100	1.05 (0.2–6.9)	1.29 (0.3–5.0)	
		3/5	10/8		101–200	1.10 (0.2–6.3)	0.96 (0.3–3.0)	
		1/4	2/6		>200	0.58 (0.1–6.2)	0.17 (0.0–1.0)	

Design Study population Reference(s)	Outcome definition	Population size (*n*)			Smoking status	Arsenic exposure	Results: OR/RR (95% CI)			Additional information
Case–control study Finland Michaud et al. (2004)	All cases of bladder cancer as defined by the Finnish Cancer Registry	Years of smoking Cases				Concentration in toenail (μg/g)	Years of smoking			Male smokers aged 50–60, range 0.02–17.5 μg/g Choice of the concentration intervals was based on tertiles or quartiles Total bladder cancer cases = 280, controls = 280
		≤35	36–45	>45			≤35	36–45	>45	
		16	57	11		0.017–0.070	1.0	1.0	1.0	
		21	50	18		0.071–0.137	1.14 (0.45–2.93)	0.90 (0.53–1.53)	1.46 (0.52–4.13)	
		30	60	17		>0.137	1.30 (0.55–3.06)	1.16 (0.69–1.95)	2.30 (0.77–6.88)	
		65/74				<0.050	1.0			
		71/73				0.050–0.105	1.09 (0.68–1.74)			
		73/73				0.106–0.161	1.13 (0.71–1.80)			
		71/73				>0.161	1.13 (0.70–1.81)			
							P trend = 0.65			
Cohort study North-east Taiwan, China Chiou et al. (2001); Chen et al. (2010a)	Area endemic for arseniasis	Number of cases/person-years of observation				Concentration in well water (μg/l)	All urinary cancers			Chen et al. (2010a) is a follow-up study to Chiou et al. (2001). Adjusted for age, sex,
		5/26 609				<10.0	1.0			
		8/24 247				10–49.9	1.66 (0.53–5.21)			

Design Study population Reference(s)	Outcome definition	Population size (*n*)	Smoking status	Arsenic exposure	Results: OR/RR (95% CI)		Additional information
		5/10 359		50–99.9	2.42 (0.69–8.54)		smoking and duration of drinking well water. Duration of the study: 12-year follow-up Sample size: 8086 (*n* = 45 urinary cancers, *n* = 8 urothelial cancers)
		8/10 416		100.0–299.9	4.15 (1.32–12.91)		
		11/7799		≥300	7.80 (2.64–23.1) *P* for trend < 0.001		
				Concentration in well water (µg/l)	Urothelial carcinoma		
		2/26 609		<10.0	1.0		
		2/24 247		10.1–49.9	1.54 (0.20–12.0)		
		2/10 359		50.1–99.9	3.44 (0.45–26.5)		
1/10 416			100.0–299.9	1.69 (0.14–20.0)			
1/7799			≥300	2.40 (0.20–28.4) *P* for trend = 0.504			
Cohort study Denmark Baastrup et al. (2008)	First bladder cancer	214 bladder cancer cases out of total sample size of 57 053			IRR (95% CI)		GIS analysis based on "Diet, Cancer and Health" cohort of 57 053, exposure range 0.05–25.3 µg/l (mean = 1.2 µg/l)
				Time-weighted average exposure (µg/l)	Adjusted analysis	Further adjustment for area of enrolment	
					P-value = 0.75	*P*-value = 0.93	
					1.01 (0.93–1.11)	1.00 (0.91–1.11)	

Design Study population Reference(s)	Outcome definition	Population size (*n*)	Smoking status	Arsenic exposure	Results: OR/RR (95% CI)		Additional information
				Cumulative exposure	*P*-value = 0.55 1.0 (0.98–1.04)	*P*-value = 0.69 1.01 (0.98–1.04)	

IRR, incidence rate ratio; TCC, transitional cell carcinoma
[a] Excludes Lewis et al. (1999), Han et al. (2009) and Kwong et al. (2010).
Source: Adapted from Table 34 in EFSA (2009).

Lung cancer case–control studies

A hospital-based case–control study in Chile found evidence of a significant exposure-related increase in lung cancer with an OR of 3.9 (95% CI 1.2–12.3) beginning at an average arsenic water concentration of 30–49 µg/l (Ferreccio et al., 2000). There was evidence of synergy between cigarette smoking and ingestion of arsenic in drinking-water. Potential control selection bias in this study is a limitation due to complex recruitment of hospital-based control groups in which the target distribution of control groups between hospitals was not achieved. Controls for the highest exposure category (<400 µg/l) were overrepresented, leading to underestimation of ORs, whereas for the 100–300 µg/l exposure category, controls were markedly underrepresented, leading to bias towards overestimation of ORs. The authors stated that it is possible that some underascertainment of cases would have occurred in the same cities in which controls were underselected. Because of the above issues and the availability of a more recent study with a prospective cohort study design (Chen et al., 2010b), the above case–control study was not selected as a pivotal study upon which to model lung cancer risk.

In a case–control study, newly diagnosed lung cancer cases among 2503 residents in south-western Taiwan, China, and 8088 in north-eastern areas in Taiwan, China, were followed up for an average of 8 years (C.L. Chen et al., 2004). A significant trend for increased lung cancer risk was observed, with a synergistic effect of ingested arsenic and cigarette smoking on lung cancer.

Arsenic exposure was estimated by average concentrations for each of 64 districts in a clinic-based case–control study conducted in Bangladesh (Mostafa, McDonald & Cherry, 2008). ORs were increased with mean arsenic concentration, but were significant only for exposures to arsenic above 100 µg/l for both male and female smokers; no significant trends for lung cancer risk with arsenic exposure were seen in non-smokers. A study limitation was selection of patients with suspicious lung lesions on chest X-ray as controls.

A case–control study using toenail arsenic as a biomarker of exposure conducted in the USA found evidence of an exposure-related risk of small cell carcinoma and SCC of the lung for toenail arsenic concentrations of and above 0.114 µg/g compared with below 0.05 µg/g (OR 2.75, 95% CI 1.00–7.57) and for individuals with chronic lung disease, but no association for lung cancers overall (Heck et al., 2009). Toenail arsenic concentration was positively associated with number of fish servings per week.

Lung cancer cohort studies

An SMR cohort study conducted in Utah, USA, on 2203 deceased individuals found no excess risk for respiratory tract cancer (drinking-water arsenic concentration range 14–166 µg/l) in this predominantly Mormon population (Lewis et al., 1999); 34 respiratory tract cancer cases were observed.

A prospective cohort study of 57 053 persons was conducted in Denmark to identify cancer cases, including primary lung cancer cases (n = 402 cases) (Baastrup et al., 2008). Individual exposure to arsenic was estimated to range

between 0.05 and 25.3 µg/l. No significant association was found between exposure to arsenic and risk for a number of cancers, including lung cancer.

A prospective cohort study of 6888 residents (n = 178 incident lung cancer cases) with 11 years of follow-up in north-eastern Taiwan, China, showed a significant exposure–response trend of lung cancer risk at 100–300 µg/l (RR 1.54, 95% CI 0.97–2.46), but not between 10 and 100 µg/l (Chen et al., 2010b). In total, 3901 water samples were collected for arsenic analysis from individual wells (85.1% of 4586 households) during the personal home interview. A synergistic effect between cigarette smoking was observed for squamous and small cell lung carcinomas, but not for adenocarcinomas.

Results of key studies are summarized in Table 5. Four lung cancer studies considered the effect of smoking in combination with that of arsenic exposure via drinking-water (Ferreccio et al., 2000; C.L. Chen et al., 2004, 2010b; Mostafa, McDonald & Cherry, 2008). Studies by Ferreccio et al. (2000) and Mostafa, McDonald & Cherry (2008) were of case–control design, whereas those by C.L. Chen et al. (2004, 2010b) were cohort studies. In general, exposed smokers exhibited higher risk for lung cancer than never smokers.

(iv) Other cancers

Cancers at other sites implicated in exposure to arsenic, which include prostate, liver and kidney, have fewer studies and less conclusive results (IARC, 2004). An excess of prostate cancer was found in a study in Utah, USA (SMR 1.5, 95% CI 1.7–1.9) (Lewis et al., 1999); however, no excess of prostate cancer was seen in another study in Australia (Hinwood, Jolley & Sim, 1999). A recent study conducted in Chile found an approximately 25-year latency pattern for kidney cancer mortality following a 13-year period of high exposure (>850 µg/l) in drinking-water (Yuan et al., 2010). A recent IARC assessment found the evidence “limited” for cancers of the kidney, liver and prostate (Straif et al., 2009; IARC, in press).

(b) Effects other than cancer

(i) Skin lesions

Epidemiological studies in different regions of the world have consistently demonstrated a strong association between long-term inorganic arsenic ingestion and skin lesions, typically in the form of hyperkeratosis, hyperpigmentation or hypopigmentation. These studies have been extensively reviewed by NRC (2001) and ATSDR (2007). More recently, human studies relating to low-level inorganic arsenic exposure (<100 µg/l in drinking-water) have been reported, and the European Food Safety Authority (EFSA) has summarized them in its scientific opinion (Section 8.3.3.2 and Table 36 of EFSA, 2009). The observations of skin lesions following low-level exposure have suggested that these characteristic dermal changes are sensitive indications of the toxic effects of inorganic arsenic.

Table 5. Epidemiological studies on lung cancer in humans in relation to ingested inorganic arsenic exposure[a]

Design Study population Reference	Outcome definition	Population size (*n*)	Smoking status	Arsenic exposure	Results	Additional information
Case–control study Chile Ferreccio et al. (2000)	Lung cancer	Cases = 151, controls = 419		Average concentration in water 1930–1994 (µg/l)	OR age and sex adjusted	Potential control selection bias. Hospital-based study; two control groups: i) excludes cancers of liver, skin, kidney, bladder or prostate; ii) shared control group for bladder cancer study; excludes cardiovascular disease, skin and neurological diseases
			Total	0–10	1.00	
				10–29	1.6 (0.5–5.3)	
				30–49	3.9 (1.2–12.3)	
				50–199	5.2 (2.3–11.7)	
				200–400	8.9 (4.0–19.6)	
				Peak years average concentration in water 1958–1970 (µg/l)	OR age and sex adjusted	
				0–10	1.00	
				10–29	0.3 (0.1–1.2)	
				30–59	1.8 (0.5–6.9)	
				60–89	4.1 (1.8–9.6)	
				90–199	2.7 (1.0–7.1)	
				200–399	4.7 (2.0–11.0)	
				400–699	5.7 (1.9–16.9)	
				700–999	7.1 (3.4–14.8)	

Design Study population Reference	Outcome definition	Population size (*n*)	Smoking status	Arsenic exposure	Results	Additional information
				Average concentration in water 1930–1994 (μg/l)	OR age and sex adjusted	
			Never smoked	≤49	1.00	
				50–199	5.9 (1.2–40.2)	
				≥200	8.0 (1.7–52.3)	
			Ever smoked	≤49	6.1 (1.31–39.2)	
				50–199	18.6 (4.13–116.4)	
				≥200	32.0 (7.22–198.0)	
Case–control study Taiwan, China C.L. Chen et al. (2004)	Newly diagnosed lung cancer	2503 in south-west, 8088 in north-east (*n* = 139 lung cancer cases)		Average concentration in well water (μg/l)	Multivariate-adjusted RR (95% CI)	
			Overall	<10	1.00	
				10–99	1.09 (0.63–1.91)	
				100–299	2.28 (1.22–4.27)	
				300–699	3.03 (1.62–5.69)	
				≥700	3.29 (1.60–6.78)	
				Unknown	1.10 (0.60–2.03)	
			Non-smoker	<10	1.00	
				10–699	1.24 (0.53–2.91)	

Design Study population Reference	Outcome definition	Population size (*n*)	Smoking status	Arsenic exposure	Results	Additional information
				≥700	2.21 (0.71–6.86)	
			<25 pack-years[b]	<10	2.55 (0.68–9.52)	
				10–699	5.50 (1.96–15.5)	
				≥700	6.28 (1.53–25.7)	
			≥25 pack-years	<10	3.80 (1.29–11.2)	
				10–699	5.93 (2.19–16.1)	
				≥700	11.10 (3.32–37.2)	
Case–control study Bangladesh Mostafa, McDonald & Cherry (2008)	Primary lung cancer	Cases = 3223, controls = 1588		Average concentration in well water (µg/l)	OR	Clinic-based study; people drank from tube wells and lived in a village for 10 years; controls = patients referred for lung cytology and found not to have cancer
			Overall	0–10	1.00	
				>100	Men (all): 1.45 (1.16–1.80)	
					Women (smokers): 2.64 (0.65–10.73)	
			Non-smoker	0–≤10	1.00	No significant trends in non-smokers
				11–≤50	0.90 (0.62–1.33)	
				51–≤100	1.10 (0.62–1.96)	
				101–400	0.94 (0.62–1.41)	
			Smoker	0–≤10	1.00	

Design Study population Reference	Outcome definition	Population size (*n*)	Smoking status	Arsenic exposure	Results	Additional information
				11 –≤50	1.25 (0.96–1.62)	
				51–≤100	1.37 (0.92–2.03)	
				101–400	1.65 (1.25–2.18)	
Case–control study USA		Cases/controls		Average concentration in toenail (μg/g)	OR (95% CI)	
Heck et al. (2009)	All lung cancer	65/69		<0.05	1.00	
		58/66		0.05–<0.0768	1.34 (0.71–2.53)	
		58/44		0.0768–<0.1137	1.10 (0.55–2.20	
		58/44		≥0.1137	0.89 (0.46–1.75)	
	Small cell and squamous cell	65/17		<0.05	1.00	
		58/24		0.05–<0.0768	2.99 (1.12–7.99)	
		58/13		0.0768–<0.1137	1.86 (0.62–5.58)	
		57/21		≥0.1137	2.75 (1.00–7.57)	
	Lung disease:					
	No	52/57		<0.05	1.00	
		121/164		≥0.05	1.02 (0.62–1.69)	
	Yes	17/8		<0.05	1.31 (0.45–3.84)	
		33/9		≥0.05	4.78 (1.87–12.2)	

Design Study population Reference	Outcome definition	Population size (*n*)	Smoking status	Arsenic exposure	Results		Additional information
Cohort study Millard County, Utah, USA Lewis et al. (1999)	Respiratory tract cancer mortality	28 cases in men, 6 cases in women		Concentration in water, (μg/l)-years	SMR Women	Men	Mormon population abstains from smoking; median drinking-water concentration of towns ranged from 14 to 166 μg/l
				<1000	0.44	0.32	
				1000–4999	0.66	0.96	
				>5000	0.22	0.44	
Cohort study Denmark Baastrup et al. (2008)	Primary lung cancer	402 cases out of total sample size of 57 053			0.99 (0.92–1.07)		See notes on bladder cancer table (Table 4)
Cohort study North-eastern Taiwan, China Chen et al. (2010b)	Incident lung cancer (11-year follow-up)	6888 (*n* = 178 lung cancer cases)		Average concentration in well water (μg/l)	Multivariate-adjusted RR (95% CI)		No apparent increase in lung cancer risk observed between 10 and 100 μg/l Increased risk for squamous cell and small cell carcinoma lung cancer (but not adenocarcinoma) between 100 and 300 μg/l (RR 2.25, 95% CI 1.43–3.53) Individual well water arsenic
			Overall	<10	1.00		
				10–49.9	1.10 (0.74–1.63)		
				50–99.9	0.99 (0.59–1.68)		
				100–299.9	1.54 (0.97–2.46)		
				≥300	2.25 (1.43–3.55)		
			Non-smoker	<10	1.00		
				10–99.9	1.22 (0.64–2.32)		
				≥100	1.32 (0.64–2.74)		

Design Study population Reference	Outcome definition	Population size (*n*)	Smoking status	Arsenic exposure	Results	Additional information
			<25 pack-years	<10	2.14 (0.79–5.79)	concentrations measured
				10–99.9	1.52 (0.56–4.15)	Personal interview included smoking history and information to estimate latency, recency and cumulative arsenic exposure
				≥100	5.30 (2.19–12.8)	
			≥25 pack-years	<10	4.08 (1.83–9.10)	
				10–99.9	4.19 (1.92–5.14)	
				≥100	8.17 (3.74–17.9)	

[a] Excludes Han et al. (2009) ecological study.
[b] Packs per day × duration.
Source: Adapted from Table 35 in EFSA (2009).

In most epidemiological studies, the prevalence or odds ratio of skin lesions was associated with inorganic arsenic exposure in a dose-dependent manner. In three large-scale drinking-water studies conducted in Bangladesh (Ahsan et al., 2006; Rahman et al., 2006) and India (Guha Mazumder et al., 1998), males seemed to be more sensitive than females to inorganic arsenic–related skin lesions. Recent findings from the Health Effects of Arsenic Longitudinal Study in Bangladesh suggested that smoking, body mass index and the nutritional status of folate and selenium could influence the susceptibility to inorganic arsenic–induced skin lesions (Chen et al., 2009). Metabolism may also play a role in the dermal effects of arsenic. Elevated fractions of excreted monomethylarsenic species (MMA^{III} + MMA^{V}) and the concentration of urinary MMA^{III} have been linked to a higher risk of arsenic-related skin lesions (EFSA, 2009). Genetic polymorphism of arsenic metabolic enzymes, such as GST-1 and methylenetetrahydrofolate reductase (Ahsan et al., 2007), as well as arsenic (As^{III}) methyltransferase (Valenzuela et al., 2009), may contribute to individual variations in arsenic metabolic capacity. Epidemiological studies with larger sample size are needed to confirm the effects of the above-mentioned risk-modifying factors.

Table 6 summarizes selective large-scale epidemiological studies relating low-level drinking-water arsenic exposure to skin lesions. Ecological studies and studies with small sample sizes are not listed. A more complete study list can be found in the EFSA opinion (Table 36 of EFSA, 2009). The studies of Rahman et al. (2006) and Ahsan et al. (2006) include low-level inorganic arsenic exposure and also analysed the risk of skin lesions in males and females separately. They provide useful dose–response information that the Committee considered as a possible basis for a reference point.

Guha Mazumder et al. (1998) conducted a cross-sectional study in West Bengal, India, in which keratosis and hyperpigmentation were analysed separately. Water arsenic level was strongly related to the age-adjusted prevalence of both types of skin lesion. Calculation by dose per body weight showed that men had roughly 2–3 times the prevalence of both keratosis and hyperpigmentation compared with women ingesting the same dose of arsenic. However, the questionable disease diagnosis and the possibility of other sources of arsenic exposure have limited the validity of this study.

Rahman et al. (2006) reported a case–control study conducted in Matlab, Bangladesh, to study the dose–response relationship between skin lesions and inorganic arsenic level in drinking-water. Among residents aged 4 years and older, 504 cases were identified, and 1830 randomly selected controls were recruited. Inorganic arsenic exposure, represented by the level in drinking-water (μg/l) and cumulative arsenic exposure (μg/l × years), was measured for each participant. The OR for skin lesions, both in males and in females, increased along with arsenic exposure, with a trend of $P < 0.0001$.

Table 6. Selective large-scale cross-sectional and case–control studies relating low-level drinking-water arsenic exposure to skin lesions

Design Study population Reference	Population size (*n*)		Arsenic exposure (total arsenic in well water, µg/l)	Results (OR* or % case prevalence**)				Additional information
Cross-sectional study West Bengal, India Guha Mazumder et al. (1998)	Keratosis F: 48/4093 (cases/total) M:108/3590 (cases/total)	Hyperpigmentation F: 127/4093 (cases/total) M: 234/3590 (cases/total)		Keratosis		Hyperpigmentation		A combination of high-exposure and reference exposure areas Possible misdiagnosis of cases (EFSA, 2009)
				F	M	F	M	
			<50	0**	0.2**	0.3**	0.4**	
			50–99	0.4	1.5	0.8	3.2	
			100–149	1.2	1.6	5.7	11.0	
			150–199	2.3	4.7	5.1	7.8	
			200–349	2.0	4.9	6.5	13.1	
			350–499	2.7	9.0	9.5	15.7	
			500–799	3.1	8.9	5.3	13.8	
			>800	8.3	10.7	11.5	22.7	
Case–control study Matlab, Bangladesh Rahman et al. (2006)	F: 272/833 (cases/controls) M: 232/997 (cases/controls)			F		M		High-arsenic exposure area The controls were randomly selected from the same study area
			<10	Ref*		Ref*		
			10–49	1.66		3.25		
			50–149	3.06		2.28		
			150–299	4.08		5.41		
			>300	6.88		9.56		

Table 6 (contd)

Design Study population Reference	Population size (*n*)	Arsenic exposure (total arsenic in well water, µg/l)	Results (OR* or % case prevalence**)		Additional information
Cross-sectional study Araihazar, Bangladesh Ahsan et al. (2006)	F: 130/6562 (cases/total) M: 584/4876 (cases/total)	(Time-weighted) 0.1–8.0 8.1–40.0 40.1–91.0 91.1–175.0 175.1–864.0	F Ref* 1.59 2.82 2.53 4.81	M 3.61 6.88 11.30 14.04 19.04	Area of a full range of exposure
Cross-sectional study Inner Mongolia, China Xia et al. (2009)	622/11 416 (cases/total)	0–5 5.1–10 10.1–20 20.1–50 50.1–100 100.1–300 >300	Ref* 2.52 2.83 3.94 6.03 8.83 7.94		Low to moderate arsenic exposure area Did not analyse males and females separately

F, female; M, male; Ref, reference population

Ahsan et al. (2006) reported the dose–response effect of arsenic on the risk of skin lesions from a cross-sectional study conducted in Araihazar, Bangladesh. From a population exposed to the full dose range of inorganic arsenic (0.1–864 µg/l), 11 746 participants were interviewed and examined individually for skin lesions. Exposure was estimated for each participant based on well water arsenic concentration, cumulative exposure and urinary arsenic concentration. Consistent dose–response effects were observed for all three arsenic exposure measures. Compared with drinking-water containing less than 8.1 µg/l of arsenic, drinking-water containing inorganic arsenic concentrations of 8.1–40.0, 40.1–91.0, 91.1–175.0 and 175.1–864.0 µg/l was associated with adjusted prevalence ORs of skin lesions of 1.91, 3.03, 3.71 and 5.39, respectively. Males were found to be more susceptible than females to inorganic arsenic–related skin lesions.

Xia et al. (2009) recently reported a United States Environmental Protection Agency (USEPA)–supported cross-sectional study in Inner Mongolia, China. Drinking-water seemed to be the only significant source of exposure to inorganic arsenic in this area (Heng et al., 1999). With a low inorganic arsenic concentration in well water (mean 37.9 µg/l; median 21.0 µg/l), 5% of the 12 334 residents surveyed had skin lesions characteristic of arsenic exposure. Skin lesions were strongly associated with well water arsenic level. Compared with the reference population, which consumed drinking-water with an arsenic level below 5 µg/l, there was an elevated prevalence among those with exposures to drinking-water with arsenic concentrations as low as 5–10 µg/l. In this study, the risks in males and females were not analysed separately.

(ii) Developmental effects

Effects of arsenic on fetal development

The information contained in this summary relies heavily on the 2009 EFSA evaluation, as there were no new studies reported in the open literature since the EFSA report (EFSA, 2009).

In spite of the prevalence of inorganic arsenic exposure, there are only a few studies on fetal development in relation to inorganic arsenic exposure reported in the scientific literature. Epidemiological studies suggest that there is an association between pregnant women's exposure to elevated arsenic concentrations in drinking-water and increased risk of spontaneous abortion, stillbirth, preterm birth, neonatal death, birth defects and fetal loss (Hopenhayn-Rich et al., 2000; Ahmad et al., 2001; Milton et al., 2005; Kwok, Kaufmann & Jakariya, 2006; von Ehrenstein et al., 2006; Rahman et al., 2007). Studies in Chile and Taiwan, China, showed that infants born to women who drank water with elevated inorganic arsenic concentrations during pregnancy had significant reduction in birth weights (Hopenhayn et al., 2003; Yang et al., 2003; Huyck et al., 2007).

A recent longitudinal study by Rahman et al. (2009) showed a significant negative association between birth weight or head and chest circumferences and urinary arsenic concentrations in the low exposure range (<100 µg/l in urine), where birth weight decreased by 1.7 g for each microgram per litre of maternal urinary

arsenic concentration. However, in a study carried out in Mongolia for which inorganic arsenic levels in maternal drinking-water of up to 100 µg/l were measured, no adverse birth outcomes or significant increases in neonatal death rate were observed (Myers et al., 2010).

Effects of arsenic on child health and development

Prenatal and post-weaning exposure of infants to inorganic arsenic may affect child health and development and result in infant mortality as a result of its effects on fetal growth and immune function. Cohort studies found that infants born to mothers who consumed drinking-water with arsenic concentrations of 164–275 µg/l during pregnancy had significantly increased mortality during the first year of life; the dose–response relationship indicated that the increased risk of infant mortality started at arsenic concentrations of about 50 µg/l in water. The increase in infant mortality may be attributable to arsenic-related mechanisms, such as growth retardation and impaired immune function (Soto-Pena et al., 2006; Ferrario et al., 2008; Raqib et al., 2009).

Recent cross-sectional studies conducted in Bangladesh (Wasserman et al., 2004, 2007) and India (von Ehrenstein et al., 2007) reported links between inorganic arsenic exposure through drinking-water and neurobehavioural deficits in schoolchildren, although the studies did not include many children and held little information on exposure early in life. In another cross-sectional study, chronic exposure to arsenic and lead was found to be associated with impaired neuropsychological development in children living in the vicinity of a smelter in Mexico, compared with children living in an area with lower, although still elevated, arsenic exposure, but similar lead exposure (Calderon et al., 2001). In a similar study conducted in Taiwan, China, Tsai et al. (2003) observed impaired development of cognitive function among adolescents due to long-term inorganic arsenic exposure.

The evidence for a link between inorganic arsenic and neurobehavioural deficits in schoolchildren, provided by the current studies, and the notion that arsenic is a developmental neurotoxicant is bolstered by the earlier evidence of severe clinical effects caused by inorganic arsenic contamination, at concentrations of 4–7 mg/l, of milk powder used for preparation of infant formula in Japan in 1955. Follow-up of the children exposed to contaminated milk powder revealed neurological diseases, neurobehavioural dysfunction and decreased cognitive skills (Yamashita et al., 1972; Dakeishi, Murata & Grandjean, 2006; Grandjean & Murata, 2007). However, a longitudinal study conducted in Bangladesh reported that problem-solving ability and motor development were not related to prenatal inorganic arsenic exposure (Tofail et al., 2009).

Taken together, these studies provide some evidence for neurobehavioural effects of inorganic arsenic exposure during childhood, at exposure levels occurring in areas with elevated concentrations in drinking-water. More longitudinal studies are warranted to evaluate the most critical windows of exposure, the type of effects and dose–response relationships.

(iii) Cardiovascular disease

The cardiovascular effects following non-therapeutic oral exposure to inorganic arsenic have been investigated in a large number of studies. As reviewed by Navas-Acien et al. (2005) and EFSA (2009), the cardiovascular outcomes from chronic drinking-water exposure include blackfoot disease, increased mortality or prevalence of coronary heart disease, peripheral arterial disease, myocardial infarction and stroke. Recent studies have begun to investigate other cardiovascular end-points, such as blood pressure and the duration of the electrocardiogram QT interval.

Although the association between blackfoot disease and inorganic arsenic exposure has been confirmed by many studies, blackfoot disease is reported only in an area along the south-western coast of Taiwan, China, where arsenic contamination in well water is very high (170–880 µg/l) (NRC, 2001).

Among studies based on disease mortality or prevalence, many were conducted in the blackfoot disease–endemic area in Taiwan, China, and unanimously showed a positive association between cardiovascular end-points and inorganic arsenic exposure. Y. Yuan et al. (2007) reported on a study conducted in Chile, which showed that young adult men aged 30–49 years had the highest risk for acute myocardial infarction. These men were born during the high-exposure (~700 µg/l) period, with probable exposure in utero and in early childhood. From a population-based cohort study conducted in Bangladesh, Sohel et al. (2009) reported that the mortality rate of cardiovascular disease was associated with inorganic arsenic level in drinking-water in a dose-dependent manner ($P < 0.001$). Compared with the reference population, which was exposed to drinking-water with an average inorganic arsenic level below 10 µg/l, those exposed to inorganic arsenic levels of 10–49, 50–149, 150–299 and 300+ µg/l had an adjusted hazard ratio of 1.03 (95% CI 0.82–1.29), 1.16 (95% CI 0.96–1.40), 1.23 (95% CI 1.01–1.51) and 1.37 (95% CI 1.07–1.77), respectively. Although this study provided dose–response information at low-level exposure, the definition of cardiovascular disease was not specified, making it difficult to compare with other studies. Studies conducted in the USA (reviewed by Navas-Acien et al., 2005; EFSA, 2009) and Spain (Medrano et al., 2010) also included populations with low-level arsenic exposures. However, they all reported no or weak associations. Owing to the inconsistent results and unstandardized outcome definitions among different studies, the relationships between inorganic arsenic exposure and cardiovascular prevalence and mortality are not very convincing.

Recently, there have been a number of studies investigating the relationship between arsenic exposure and cardiovascular end-points such as blood pressure. In a cross-sectional study conducted in Bangladesh, baseline blood pressure of 10 910 participants was used to derive an association with the time-weighted well water arsenic concentration. The authors found that inorganic arsenic exposure was positively associated with systolic hypertension and high pulse pressure, and the associations were more pronounced among participants with lower intake levels of folate and the B vitamins. No apparent association was observed between inorganic arsenic exposure and general hypertension (Chen et al., 2007). A dose-dependent

association between inorganic arsenic exposure and systolic blood pressure was also reported in another cross-sectional study with 8790 women of reproductive age in Inner Mongolia, China (Kwok et al., 2007). Compared with the reference population (inorganic arsenic exposure <20 µg/l), the adjusted population mean systolic blood pressure rose 1.88 (95% CI 1.03–2.73) mmHg (0.25 [95% CI 0.14–0.36] kPa), 3.90 (95% CI 2.52–5.29) mmHg (0.52 [95% CI 0.36–0.71] kPa) and 6.83 (95% CI 5.39–8.27) mmHg (0.91 [95% CI 0.72–1.1] kPa) as the drinking-water arsenic concentration increased from 21–50 µg/l to 51–100 µg/l to greater than 100 µg/l, respectively.

There are four population-based studies using the end-point of the duration of corrected QT interval (QT_c) from individual electrocardiograms. They all reported a positive association between high inorganic arsenic exposure and a prolonged QT_c (Ahmad et al., 2006; Mumford et al., 2007; Yildiz et al., 2008; C.H. Wang et al., 2009). However, the data at low levels of exposure are limited for a dose–response evaluation.

(iv) Neurotoxicity

The information contained in this summary relies heavily on the 2009 EFSA evaluation, as there were no new studies reported in the open literature since the EFSA report (EFSA, 2009).

Effects of arsenic on the peripheral nervous system

Exposure to arsenic may affect both the central and peripheral nervous systems, but the most frequent neurological manifestation of inorganic arsenic is peripheral neuropathy. Acute exposure of humans to inorganic arsenic is commonly associated with peripheral neuropathy with both axonopathy and demyelination. Chronic exposure to inorganic arsenic compounds may lead to peripheral and central neurotoxicity. Early events may include paraesthesia followed by muscle weakness. In the periphery, both motor and sensory neurons are affected.

Unlike acute exposure, chronic inorganic arsenic exposure was not found to be consistently associated with peripheral neuropathy. An earlier study indicated that no dose–response relationship existed between daily arsenic ingestion from well water with levels up to 5 mg/l and peripheral neuropathy (Kreiss et al., 1983). However, two more recent studies (Hafeman et al., 2005; Tseng et al., 2006) reported positive associations between cumulative inorganic arsenic exposure from well water and parameters for peripheral neuropathy (nerve conduction velocity, vibrotactile threshold). In its 1999 assessment, the United States National Research Council concluded that there was no consistent evidence of peripheral neuropathy in humans exposed to inorganic arsenic in drinking-water at levels below 1 mg/l (NRC, 1999). However, recent studies indicate that adverse neurosensory effects of chronic arsenic exposure occur at concentrations well below 1 mg/l drinking-water (Hafeman et al., 2005; Otto et al., 2007).

Peripheral neurotoxicity of organic arsenic compounds is not well documented. Apart from the occasional report of peripheral neuropathies in syphilitic and trypanosomiasis patients, resulting from use of arsenic in the forms of

arsphenamine and melarsoprol as therapeutic agents, no overt human peripheral neurotoxicity has been observed from exposure to the dietary organic arsenic compounds, such as AB and AC. Similarly, the neurotoxicity of the various arsenic metabolites (e.g. MMA and DMA) has never been decisively established on a clinical level.

Effects of arsenic on the central nervous system

Several reports indicate that arsenic encephalopathy occurs following acute exposure to inorganic arsenic–containing fumes or after ingestion of inorganic arsenic and that the severity of the symptoms is related to the ingested dose (ATSDR, 2007). However, there are no reports of overt encephalopathy resulting from chronic ingestion of arsenic at low dosages. The central nervous system is more subtly affected on a neurobehavioural level, as evidenced by impairment of cognitive functions, such as learning, memory, hand–eye coordination and attentive processes.

Earlier studies indicated that syphilis patients treated with the organic arsenic compounds arsphenamine and melarsoprol developed acute conditions called arsphenamine encephalitism and severe reactive arsenical encephalopathy, respectively. Beyond these therapeutically used organic arsenic compounds, no overt human central neurotoxicity has been observed as a result of exposure to the dietary organic arsenic compounds, such as AB and AC. Similarly, neurotoxicity of the various arsenic metabolites (e.g. MMA and DMA) has never been decisively established on a clinical level.

Summary

In summary, available epidemiological studies indicate a relationship between high-level oral exposures to inorganic arsenic and sensitive end-points for peripheral and central neurotoxicity. Moreover, exposures of the developing central nervous system and probably the peripheral nervous system, including in utero, may lead to serious health effects later in life. Therefore, longitudinal studies are necessary to better establish the relationship between exposure in a specific time frame during development and neurotoxic effects.

(v) Diabetes

The effect of oral exposure to inorganic arsenic on abnormal glucose metabolism and diabetes was recently reviewed in the EFSA opinion (EFSA, 2009), and no new studies have been published since. In general, studies conducted in Bangladesh and Taiwan, China, indicated an extra risk of diabetes among high-exposure populations. However, many of these studies lacked adjustment for body mass index. In studies of general populations with low to moderate exposures, none of them showed a positive association. Using data from the United States National Health and Nutrition Examination Survey (NHANES), Navas-Acien et al. (2009) reported an increased prevalence of type II diabetes for those with higher (80th percentile) versus those with lower (20th percentile) urinary arsenic levels, adjusted for the organic arsenic species, AB. However, using the same data, Steinmaus et

al. (2009) reported no association when AB was subtracted from total arsenic to reflect the inorganic exposure. In conclusion, the relationship between arsenic exposure and diabetes remains uncertain.

(vi) Other effects

In a small-scale study conducted in India, the immunoresponse to concanavalin A, a potent mitogen, was examined in patients with inorganic arsenic–induced skin lesions and in unexposed controls. T cell proliferation and cytokine levels were significantly lower ($P < 0.001$) in exposed individuals than in the unexposed (Biswas et al., 2008). The same group also reported significantly ($P < 0.001$) impaired macrophage functions, such as loss of cell adhesion capacity and decrease in nitric oxide production and phagocytic capacity, in arsenic-exposed individuals ($n = 70$) compared with the unexposed ($n = 64$) (Banerjee et al., 2009).

3. ANALYTICAL METHODS

3.1 Sample preparation for total arsenic determination

Sample digestion can be achieved by wet or dry mineralization. Wet digestion is the technique most widely used in food, because it requires less time than methods based on dry ashing. The systems most commonly utilized in the laboratory currently are the high-pressure asher, which can attain temperatures of over 320 °C, and the microwave-assisted digestion (MAE) systems, in which the temperatures do not go above 260 °C, because the Teflon material used in most of the systems starts to melt at that temperature (Goessler & Pavkov, 2003). Nitric acid is the oxidant most often used, although combinations of various acids are also common. Generally, the acid is combined with hydrogen peroxide, which enhances the digestion yield as a result of an extra oxidation.

A major problem presented by the MAE system is the complete decomposition of some organoarsenical species. AB, TMAO and tetramethylarsonium ion (TMA^+) are resistant to the attack of oxidizing agents and require high temperatures to break the arsenic–carbon bonds (≥300 °C), which cannot be achieved in MAE systems (Fecher & Ruhnke, 1998). This leads to an underestimation of the total arsenic concentration in samples containing these species when the detection method used after MAE digestion is based on hydride generation (HG): HG–atomic absorption spectrometry (HG-AAS) or HG–atomic fluorescence spectrometry (HG-AFS). Duarte et al. (2009) used a microwave-induced combustion method, generating adequate recoveries in the quantification in seafood by flow injection–HG-AAS.

At present, there are various official methods for the determination of total arsenic in foods based on digestion of samples by wet mineralization (USEPA, 1996; European Committee for Standardization, 2004, 2005b, 2009).

Dry ashing involves oxidation of organic compounds in open systems at elevated temperatures by air oxygen. An advantage of these methods is the possibility of handling relatively large amounts of samples. The main drawback with

regard to wet digestion is that the process requires more time. The use of ashing aid reagents (MgO, $Mg(NO_3)_2$ or mixtures of both) avoids the losses of arsenic due to the formation of volatile compounds and also accelerated mineralization of samples. Dry ashing mineralization is used in several official methods (AOAC, 1990; European Committee for Standardization, 2005a).

3.2 Sample preparation for arsenic species determination

The extractants most commonly used are polar solvents, such as methanol, water and methanol/water mixtures, nitric acid, tetramethylammonium hydroxide, trifluoroacetic acid, phosphoric acid, sodium hydroxide and enzyme mixtures at neutral pH.

Certain considerations must be taken into account when developing extraction methods for arsenic species. First of all, it is necessary to study whether transformations take place in the arsenic species during the extraction and storage of the extract obtained. Also, an extractant may give very different efficiencies for the same type of food, and even optimal results in certified reference materials may not be reproduced in food products purchased from retail outlets (Heitkemper et al., 2001). Application of the method to cooked food may also alter the extraction efficiency. Finally, mass balance calculations should form part of the quality control performed to select the extraction method (Schaeffer et al., 2005). The more polar or ionic organoarsenic species (AB, DMA, MMA, TMAO, TMA^+, arsenosugars) are easily extractable, even with the less aggressive methods, but As^{III} is difficult to extract because covalent bonds are formed with the sulfhydryl groups of proteins. The extraction of arsenolipids requires non-polar solvents such as hexane.

From the toxicological point of view, special attention must be paid to the separation of As^{III} and As^{V} from the other species, as at present they are the main focus of attention in health institutions and regulating organizations. Methods for a selective inorganic arsenic extraction have been described. Muñoz, Vélez & Montoro (1999) developed a quantitative extraction with chloroform followed by back-extraction with hydrochloric acid and determination by dry-ashing HG-AAS or inductively coupled plasma mass spectrometry (ICP-MS). This method has been applied effectively to a wide variety of foods (Muñoz et al., 2000, 2002; Almela et al., 2006; Rose et al., 2007; Jorhem et al., 2008).

Many techniques have been used to assist arsenic species extraction. Mechanical agitation has been widely used in food samples. A recent study by van Elteren et al. (2007) shows the variability in extraction from the certified reference material IAEA-140/TM (*Fucus* sp.) using different methanol/water ratios assisted by a mechanical agitation. None of the conditions assayed achieved quantitative extraction of the arsenic species. Some acids have been used for extraction of arsenic species. Trifluoroacetic acid has been effectively applied at high temperatures for arsenic speciation in rice (Williams et al., 2005), vegetables (Nam et al., 2006) and baby food products (Vela & Heitkemper, 2004). The use of nitric acid (0.3 mol/l) at 80 °C also allows quantitative extraction in *Hizikia fusiforme* (Hamano-Nagaoka et al., 2008).

Bath or focused probe sonication does not always improve the results obtained with simple mechanical agitation (Caruso, Heitkemper & B'Hymer, 2001; Nam et al., 2006; Salgado, Quijano Nieto & Bonilla Simón, 2006). However, ultrasound-assisted extraction with enzymatic solutions achieves satisfactory efficiencies in rice and meat (Sanz, Muñoz-Olivas & Cámara, 2005a,b). Another technique that has been used in recent years is accelerated solvent extraction, with possibilities of working with pressure and with high extraction temperatures, conditions that cannot be attained in sonication. The use of accelerated solvent extraction in fish products (methanol/water, methanol/acetic acid, 100 °C) and carrots (water, 100 °C) allows quantitative extractions in some food matrices (McKiernan et al., 1999; Vela, Heitkemper & Stewart, 2001; Wahlen et al., 2004).

With regard to MAE, many applications of this methodology have been described in recent years. Larsen et al. (2005) applied MAE and an alkaline alcoholic mixture to seafood samples, although the method was unsuitable for fatty fish. MAE has been applied to seaweed, with satisfactory recoveries, using as extractants water, nitric acid (2%) and methanol/water (Tukai et al., 2002; Salgado, Quijano Nieto & Bonilla Simón, 2006; Foster et al., 2007). A suitable extraction of arsenic species from vegetables and cereals using MAE has been achieved in the presence of protein extraction solution (Rahman, Chen & Naide, 2009), enzyme mixture (Guzmán Mar et al., 2009; Rahman, Chen & Naide, 2009) or water (Narukawa et al., 2008). Finally, the use of a sequential extraction procedure in MAE improved the arsenic extraction efficiency in samples in which extraction is difficult (seaweed, plant and animal digestive tissue) (Tukai et al., 2002; Foster et al., 2007).

3.3 Separation of arsenic species

The nature of the food to be analysed determines which arsenic species are present and, consequently, the chromatographic separation selected and its complexity. A number of reviews (Guerin, Astruc & Astruc, 1999; Gong et al., 2002; McSheehy et al., 2003; Francesconi & Kuehnelt, 2004; Niegel & Matysik, 2010) provide an overview of the various chromatographic conditions used for arsenic speciation analysis. High-performance liquid chromatography (HPLC) is the separation technique that has been most commonly used.

The foods in which the largest number of speciation studies has been conducted are vegetables, cereals and aquatic products. In the majority of vegetables and cereals, the major species are As^{III}, As^{V}, MMA and DMA, which can be separated without difficulty in anion exchange columns using isocratic elution over a very variable pH range (generally between 5 and 7) and with various kinds of mobile phase (particularly phosphates or carbonates).

A considerable number of arsenic species coexist in aquatic food products (freshwater fish, marine fish, shellfish and algae). Generally, to avoid overlapping, misidentification and errors in the quantification, it is best to use multidimensional chromatography. Two different chromatographic columns, anion and cation exchange, placed in line (Nischwitz & Pergantis, 2006) or connected by a column switching system (Suñer et al., 2001) permit good resolution of a considerable number of species with one chromatographic run. Other systems have been carried

out off-line, using different columns in two (Kirby et al., 2004) or three chromatographic runs (Schaeffer et al., 2005). An interesting development in HPLC systems is the high-speed separation method using micro-HPLC columns. Only one application of this system for speciation of arsenic in foods has been described (Wangkarn & Pergantis, 2000).

Often the complexity of the arsenic profile and the presence of the unknown species make it necessary to perform a structural identification of the peaks eluted from the HPLC by electrospray ionization mass spectrometry (ESI-MS) or tandem mass spectrometry (ESI-MS/MS) (McSheehy et al., 2001; Sloth, Larsen & Julshamn, 2005; Nischwitz & Pergantis, 2006). In addition, it is necessary to take account of another factor, the non-quantitative elution of arsenic injected in the column (Raab et al., 2003; Soeroes et al., 2005).

3.4 Detection systems

The detection systems most commonly used for the determination of total arsenic and its species in foods are AAS, AFS, inductively coupled plasma atomic emission spectrometry (ICP-AES) and ICP-MS:

- *Atomic absorption spectrometry (AAS):* HG-AAS is one of the techniques most commonly used for the detection of arsenic and its species in foods. HG-AAS allows good preconcentration and chemical separation of the arsenic from potential matrix interferences and has the advantage of being cheaper in terms of both equipment and maintenance. For speciation of the organoarsenic species that do not generate hydrides or that do so with low efficiency, post-column derivatization after their separation is required. This process is performed by means of on-line thermo-oxidation (microwave, heated bath) or photo-oxidation (UV light) using an oxidant, generally potassium persulfate. Although its limits of detection (LODs) are slightly higher than those obtained by ICP-MS, they are suitable for quantification of arsenic species in foods (Koch et al., 2007; Signes et al., 2007).
- *Atomic fluorescence spectrometry (AFS):* As in the case of AAS, combination with HG increases sensitivity and reduces matrix effects. In comparison with detection by AAS, AFS offers advantages in terms of linearity and LODs. In recent years, HG-AFS has been used extensively for detection of arsenic species. For the organoarsenical species, thermo-oxidation or photo-oxidation is necessary prior to HG-AFS. Gómez-Ariza et al. (2000) conducted a comparative study of ICP-MS and AFS for arsenic speciation in which they showed that LODs and linear range were comparable. AFS equipment is less expensive and easier to handle than that for ICP-MS, and therefore it is an excellent alternative for detecting arsenic species.
- *Inductively coupled plasma atomic emission spectrometry (ICP-AES):* This technique has a larger working range, but its instrumental LODs are not good enough for the determination of arsenic in many food samples, and it suffers from various matrix interferences. Research into the determination of arsenic by ICP-AES has basically followed a combination of the technique of HG with ICP. The LODs are at least an order of magnitude lower than those obtained with conventional nebulization. Arsenic speciation studies in seafood products using

HPLC-ICP-AES have shown that this technique is suitable for the determination of major compounds, such as AB in marine organisms.

- *Inductively coupled plasma mass spectrometry (ICP-MS):* With this methodology, sub-nanogram per gram LODs are achieved without the need for preconcentration and derivatization. Another advantage of this technique is the wide linearity range, which can be extended by several orders of magnitude. One of its main disadvantages is the high cost of instrumentation and maintenance and the spectral interferences. The chloride ion present in food samples combines with the plasma gas to form $^{40}Ar^{35}Cl^{+}$, creating polyatomic interferences. Only ICP-MS equipped with a high-resolution mass analyser (sector field system) can improve the separation of these signals, but this equipment is very expensive. One technology introduced to eliminate these polyatomic interferences is the use of collision/reaction cells; however, the gas used in these cells can cause other interferences (Dufailly, Noël & Guérin, 2008). HG prior to ICP-MS was used not only to reduce the interferences but also to improve the LODs.

HPLC-ICP-MS is the hyphenated technique most commonly used for the analysis of arsenic species in foods. LODs below 1 µg/l for the various arsenic species are achieved with single quadrupole instruments. An appropriate choice of chromatographic conditions can also help to avoid this interference, eluting $^{40}Ar^{35}Cl^{+}$ with a retention time different from that of the arsenic species.

Recently, HPLC coupled to MS or MS/MS has been used as the sole technique for identification and quantification of a great variety of arsenic species (Van Hulle et al., 2002; Kato, Nagashima & Shiomi, 2004; Nischwitz & Pergantis, 2005; Ninh, Nagashima & Shiomi, 2006). Near-infrared spectroscopy is also a valuable non-destructive technique that offers low cost and speed of analysis, combining applied spectroscopy and statistics. The potential of near-infrared spectroscopy for screening the inorganic arsenic contents of rice and red crayfish has been assessed (Font et al., 2004, 2005). Other non-destructive methodologies are the X-ray spectroscopic methods, such as X-ray near-edge spectroscopy, X-ray fluorescence and particle-induced X-ray emission. Lombi et al. (2009), using X-ray absorption near-edge spectroscopy, identified and quantified arsenic–glutathione complexes [$As(Glu)_3$], not observable by routine HPLC-ICP-MS analysis because of dissociation of the complex during extraction and analysis.

4. EFFECTS OF PROCESSING

Although there has been little research on the effects of preparation or preservation processes applied to food products on arsenic content, some quantitative and qualitative changes in arsenic have been shown. The effects of processing on arsenic contents and its species in foods have been reviewed in a recent publication (Devesa, Vélez & Montoro, 2008).

Studies on vegetables show that total arsenic contents are much higher in potato and carrot skin (Helgesen & Larsen, 1998; Muñoz et al., 2002; Roychowdhury et al., 2002) than in food that has been peeled. Similar results have

been shown in samples of beetroot and garlic, with concentrations of arsenic in the skin 5 and 70 times greater, respectively, than the levels in the edible part (Muñoz et al., 2002). It has also been described that dehusking/polishing rice reduces total arsenic contents (Signes et al., 2008a). The hepatopancreas of some crustaceans has total arsenic contents equal to or greater than those of muscle (Sekuli, Sapunar & Bažuli, 1993; Devesa et al., 2002), and it also accumulates a greater inorganic arsenic content (Devesa et al., 2002); therefore, its removal may also produce a decrease in the arsenic content.

With regard to washing, studies have concentrated on rice washing, a usual practice in some regions. Various studies (Sengupta et al., 2006; Mihucz et al., 2007; Signes et al., 2008b) show that washing rice with water (5–6 times) and disposing of the water before cooking can eliminate up to 23% of the arsenic. Mihucz et al. (2007) found that As^{III} was the arsenic species with the highest removal.

Among the treatments prior to consumption, cooking is the most studied. Quantitative changes after cooking may be due to an increase in the concentration of arsenic correlating with a decrease in weight or to a decrease in arsenic resulting from solubilization. In fish and shellfish, these changes are significant only for a few of the product/treatment combinations (Dabeka et al., 1993; Devesa et al., 2001a; Ersoy et al., 2006; Perelló et al., 2008). There have been studies confirming the transfer of AB, DMA, As^{V} and arsenosugars into the broth during the process of boiling and steaming crustaceans and bivalves (Devesa et al., 2001a; Lai et al., 2004). For algae, processes such as baking do not alter the arsenic content; however, soaking and boiling can reduce the inorganic arsenic content by up to 82% (Hanaoka et al., 2001a; Laparra et al., 2004; Almela et al., 2005; Ichikawa et al., 2006; Rose et al., 2007). Regarding studies on vegetables and manufactured cereal products, boiling can cause the loss of up to 60% (She & Kheng, 1992; Cubadda et al., 2003). The most detailed studies have been conducted on rice samples, for which boiling produces a loss of arsenic. However, a large volume of water is needed in order to remove substantial quantities of total arsenic (35%) and inorganic arsenic (45%) (Raab et al., 2009).

Studies on qualitative changes after cooking treatments are very sparse. In fish and shellfish, it has been shown that treatments in which the surface of the food reaches temperatures above 150 °C (baking, frying or grilling) can lead to the appearance of TMA^{+} (Devesa et al., 2001b, 2005; Hanaoka et al., 2001b). The elucidation of this phenomenon in standards of arsenic species has confirmed that TMA^{+} can be generated by decarboxylation of AB (van Elteren & Šlejkovec, 1997; Devesa et al., 2001c).

Cooking with water that contains arsenic deserves a separate mention. Most of the studies report changes in the contents of total arsenic and show that cooking with polluted water increases the arsenic concentration to values that depend on the amount of water, the concentration of arsenic in the water and the cooking time (Bae et al., 2002; Del Razo et al., 2002; Roychowdhury et al., 2002; Díaz et al., 2004; Ackerman et al., 2005; Torres-Escribano et al., 2008). Very few studies have attempted to quantify arsenic species in food cooked in these conditions. They all

show that inorganic arsenic is the major species (Díaz et al., 2004; Smith et al., 2006; Torres-Escribano et al., 2008).

There are no studies that evaluate contents of total arsenic or its chemical forms before and after subjecting food to preservation processes similar to those applied in the food industry, in commerce or in the home. Concerning freezing processes, Edmonds & Francesconi (1988) considered the possible decomposition of AB in frozen fish. However, their conclusions that the content of AB in fish decreases upon freezing were not obtained by analysing the product before and after freezing, so the possibility that the difference in AB concentrations in fish before and after freezing was due to the size or source of the raw material cannot be excluded. In contrast, the possibility that the decrease in AB was due to its solubilization during the defrosting process also cannot be ruled out. Among preserved foods, canned seafood products have been studied the most, with reported arsenic concentrations lower than those present in raw products of the same animal species (Vélez & Montoro, 1998; Muñoz et al., 2000; Ikem & Egiebor, 2005). It has been shown that there is a transfer of AB and DMA to the brine (Vélez, Ybáñez & Montoro, 1997).

Kato, Nagashima & Shiomi (2004) conducted a study on fish sauces purchased in retail outlets, prepared by fermentation of raw fish for a long period of time. They compared the arsenic composition of the fish sauces with that of the same fresh fish and observed differences with regard to the predominant species (DMA in sauces and AB in fresh product). The authors suggested that AB was transformed to DMA by bacterial action during manufacturing. A later study (Rodriguez, Raber & Goessler, 2009) on the same type of fermented fish sauces showed that AB was the predominant species; therefore, the authors concluded that there was no transformation during the fermentation. The discrepancies between the two studies might be due to differences in the fermentation process (conditions, inoculum, time).

Van Elteren & Šlejkovec (1997) studied the effect of gamma irradiation (100–10 kGy) on the stability of arsenic species, showing a partial decomposition of AB, DMA and MMA. The gamma ray doses used in commercial foods are lower (5 kGy) than those assayed, so these changes may not occur in the products purchased by consumers.

5. PREVENTION AND CONTROL

Arsenic sources such as mining and some pesticides and wood preservatives may contribute to human exposure and should be controlled in order to prevent environmental contamination. However, the great majority of exposure occurs through naturally contaminated groundwater—through drinking-water, water used in food preparation or water used to irrigate food crops, particularly rice. Paddy rice may also contain relatively high levels of arsenic at low soil arsenic levels due to the high availability of arsenic in flooded soils.

5.1 Strategies for reducing arsenic exposure from water

The ideal solution is to use alternative sources of water that are low in arsenic. However, it is important that this does not result in risk substitution—for example, if the alternative water source, although low in arsenic, increases exposure to waterborne pathogens and results in acute gastrointestinal infections, which are a major source of mortality and morbidity in many parts of the world (Howard, 2003). This is important for most alternative water sources other than water from tube wells. Water safety frameworks should be used during planning, installation and management of all new water points, especially ones based on surface water and very shallow groundwater, to minimize risks from faecal and other non-arsenic contamination. Screening for arsenic and other possible chemical contaminants of concern that can cause problems with health or acceptability, including fluoride, nitrate, iron and manganese, is also important to ensure that new sources are acceptable. Occasional screening may also be required after a source is established to ensure that it remains safe.

Where there are large urban supplies, resources are often available to treat water to remove arsenic or to exploit alternative low-arsenic sources, such as surface water that can be treated to avoid microbiological and other hazards. These low-arsenic sources can be used to blend with higher-arsenic sources to lower the concentration to acceptable levels while still retaining the resource.

Many of the major problems lie in rural areas, where there are many small supplies, sometimes down to the household level. At this level, water availability and financial and technical resources are all limited. There are several available approaches, but there is a basic requirement for education. In particular, there is a need to understand the risks of high arsenic exposure and the sources of arsenic exposure, including the uptake of arsenic by crops from irrigation water and the uptake of arsenic into food from cooking water.

A number of approaches have been successfully used in rural areas, including source substitution and the use of both high- and low-arsenic sources blended together. These sources may be used to provide drinking-water and cooking water or to provide water for irrigation. High-arsenic water can still be used for bathing and clothes washing or other requirements that do not result in contamination of food. However, it is important to remember that there may be other contaminants present as well as arsenic, and so it is important to determine whether other contaminants of concern are present.

Low-cost approaches that have been developed to lower exposure to arsenic where contamination of groundwater is a problem include the following:

- alternative sources, including dug wells that are properly protected to prevent microbiological contamination and rainwater harvesting, which may be possible for at least some months of the year, with steps taken to minimize contamination;

- surface ponds, which require appropriate steps to minimize microbial and chemical contamination and also require treatment to ensure microbial safety before drinking;
- identifying high- and low-arsenic tube wells by painting them different colours and sharing wells (spatial variability in groundwater arsenic contamination in Argentina, Chile and the river deltas of South and South-east Asia is very high, so there are mixtures of arsenic-contaminated and arsenic-uncontaminated wells in most villages);
- sinking new wells into low-arsenic strata. This requires significant technical support to ensure that low arsenic levels are known and can be exploited without other problems arising. Deeper groundwater aquifers can be used to develop community water supplies, which generally succeed where there is community involvement in their establishment and operation;
- removal of arsenic by low-cost village or household treatment systems, usually using absorptive media, such as elemental iron, iron or aluminium oxides and carbon. Shallow groundwater that is anoxic (e.g. in South and South-east Asia) is generally high in dissolved iron, so a pretreatment step involving the formation and precipitation of iron hydroxide, which will then adsorb arsenic, is advantageous. Many household treatment systems in Bangladesh and West Bengal, India, may fail prematurely because of high levels of phosphate, which competes with inorganic arsenic species for adsorption, in the water. Safe disposal of arsenic-contaminated wastes should also be considered.

In areas where there is observable arsenicosis, there is usually no problem in persuading the local population to follow arsenic mitigation measures, even though they often require significant extra effort. Involvement of individuals and communities in the planning, implementation and management of the mitigation strategy is a key factor for successful intervention. Studies in Bangladesh have shown that most rural households prefer sharing of uncontaminated wells or filtration of low-arsenic surface water through sand to treatment of groundwater (Howard, 2003; Johnston, Hanchett & Khan, 2010).

Where arsenic levels are lower and the adverse effects of arsenic exposure are less obvious, there will be a much greater requirement for education in order for mitigation measures to be carried out effectively over an extended time period. More information can be found in sources such as Howard (2003), JICA/AAN (2004) and WHO (in preparation).

5.2 Strategies for reducing arsenic exposure from foods

General strategies for reducing human exposure to arsenic from foods include reducing arsenic uptake into food crops, increasing the proportion of less toxic organic forms relative to inorganic arsenic in food crops and reducing the arsenic content of foods by processing, preparation or cooking methods. These strategies are discussed briefly below.

5.2.1 Reducing arsenic uptake into food crops

(a) Soil amendments

Because arsenic is toxic to plants, various soil amendments aimed at counteracting its toxicity have been investigated, and these should also lower arsenic concentrations in plants. Selection of amendments has been based on our understanding of the factors that regulate arsenic solubility and speciation in soils and plant uptake of arsenic.

(i) Phosphate

As an essential nutrient, phosphate additions to soils are generally needed for crop production purposes. Arsenate, the major arsenic species in aerobic soils, is a close chemical analogue of phosphate, and these two oxyanions exhibit similar chemical behaviour. Plant uptake of arsenate is via the phosphate transport system and is competitive with plant uptake of phosphate (Meharg & MacNair, 1992; Abedin, Feldmann & Meharg, 2002), suggesting that addition of phosphate to soil will reduce arsenic uptake by plants. However, phosphate and arsenate also compete for adsorption sites in soils, especially iron (Fe^{III}) oxides and oxyhydroxides (hereafter termed oxides), and phosphate addition will displace some adsorbed arsenate. Thus, phosphate addition can either decrease or increase plant uptake of arsenate, depending on the effect of its addition on the ratio of phosphate to arsenate in soil solution. Most studies of plant uptake of arsenic from soils show that phosphate additions increase plant tissue arsenic concentration for crops grown in aerobic soils (e.g. Jiang & Singh, 1994; Cao & Ma, 2004) and either have no effect on or increase arsenic concentrations in vegetative tissues and grains of paddy rice (Hossain et al., 2009). Very large additions of phosphate, which have sometimes been used to leach arsenic from surface soil, would likely reduce plant uptake of arsenic. However, this strategy is undesirable, as it just creates a different environmental pollution problem.

(ii) Silicate

This element is required in large amounts by rice, where the straw may contain as much as 15% on a dry weight basis. Uptake of arsenite, the major inorganic arsenic species in flooded or reduced soils, is by the same aquaporin channel and is competitive with uptake of silicate (Ma et al., 2008). Two studies show a reduction in rice grain arsenic with silicate addition (Bogdan & Schenk, 2008; Li et al., 2009), but they appear to have been carried out in soil with marginal to deficient levels of silicate for rice. While silicate addition would be expected to lower arsenite uptake in this circumstance, it may not do so under conditions of silicate sufficiency.

(iii) Iron- or manganese-containing materials

Soil additions of iron or manganese salts and inexpensive iron metal grits that will generate oxides sometimes reduce arsenic concentrations in vegetable crops (Warren et al., 2003; Hartley & Lepp, 2008) and paddy rice (Hossain et al.,

2009; Ultra et al., 2009). There are several challenges to this approach, which suggest that it may not be very viable: 1) these materials also reduce phosphorus availability, requiring increased phosphorus additions, which, in turn, increase arsenic solubility; 2) freshly precipitated oxides are initially amorphous but reorganize to crystalline phases with much lower adsorption capacity; and 3) other soil constituents, including silicate, humic substances and simpler organic acids, also complex with oxide surfaces, reducing their capacity for complexing arsenate. These factors are more problematic for aerobic soils, where iron oxides are not as subject to the dissolution and reprecipitation cycles that occur in a rice paddy.

(b) Inoculation with arbuscular mycorrhizal (AM) fungi

Infection of root systems with AM fungi can increase nutrient acquisition by plants, especially phosphorus, which can lead to downregulation of the high-affinity phosphate uptake transport system and reduce arsenic uptake. This was recently reported for barley (Christophersen, Smith & Smith, 2009). Here, an AM–inorganic phosphorus transport system compensated for reduced direct root uptake of phosphate and did not transport arsenate. Unfortunately, other studies of AM inoculation have shown inconsistent results and have usually not been carried out to crop maturity, so there is often no information on arsenic levels in edible plant parts. AM infection significantly lowered arsenic concentrations in tobacco leaves from 22.1 mg/kg to between 14.3 and 18.0 mg/kg, depending on the AM species, with essentially no effect on phosphorus concentration or biomass production (Hua et al., 2009). In this case, AM infection lowered soil pH and increased arsenic adsorption by crystalline iron oxides, which was thought to be the main explanation for the observed result. In maize, AM infection reversed arsenic toxicity and increased phosphorus uptake, but it increased leaf tissue arsenic concentration with one AM species and reduced it with a second species (Bai et al., 2008). More work is needed to establish whether inoculation with AM fungi can have practical value. It should also be recognized that AM infection is not considered important to phosphorus nutrition of crops in well-fertilized soils and does not apply at all to paddy rice.

(c) Varietal selection

Varietal differences in arsenic uptake have been reported for rice (Norton et al., 2009a). However, emerging information is showing that the environment is more important than genetics as a determinant of rice grain arsenic concentration (Ahmed, 2009; Norton et al., 2009b). In a study in Bangladesh, Ahmed (2009) found that 70–80% of the variability in rice grain arsenic was explained by the environment, 10% by genetics and 10–20% by the interaction between genetics and the environment, in a study across nine environments, two seasons and 38 varieties. A few varieties had consistently high or low grain arsenic concentrations across environments, so that limited recommendations could probably be made to farmers, and low-arsenic varieties can be used in breeding programmes in Bangladesh. As reported by others (Duxbury et al., 2003; Williams et al., 2005), strong seasonal effects on grain arsenic were observed, with the mean arsenic concentration in the monsoon season rice varieties (0.154 mg/kg) being approximately half that of the

dry, winter season varieties (0.288 mg/kg). Highly photoperiod-sensitive aromatic Indica varieties had the lowest grain arsenic concentrations, consistent with a market basket survey in which aromatic rice from India and Pakistan contained significantly lower levels of arsenic than did rice from the USA or Europe (Zavala & Duxbury, 2008).

Other research has shown that rice varieties from different countries vary in their tolerance of toxicity from inorganic arsenic, with tolerance decreasing in the order China > USA > Bangladesh (Ahmed, 2009). Chinese rice varieties are also much more tolerant, compared with USA varieties, of monosodium methane arsonate (Yan et al., 2005), which was formerly used on cotton in some current USA rice production areas. Tolerance of inorganic arsenic was associated with higher grain arsenic concentrations, but increased proportions of DMA^V, in rice grain in a greenhouse study with many Bangladeshi and two USA rice varieties (Ahmed, 2009). Tolerance was also associated with the amount of oxygen diffusing from roots in a greenhouse study with varieties grown in China (Mei, Ye & Wong, 2009). In the latter case, grain and straw arsenic concentrations were also lower with increasing tolerance, but grain arsenic levels for the five most tolerant varieties were all about 1 mg/kg when arsenic was added to soil at the very high rate of 100 mg/kg. These results indicate that exclusion of arsenic from rice may play some role in tolerance of arsenic toxicity, but may not adequately protect against high grain arsenic levels.

(d) Growing rice under less reduced conditions

Growing rice "more aerobically" lowers the solubility of arsenic and can reduce grain arsenic concentrations substantially (Table 7). A challenge to this approach is that yields of rice are generally reduced to unacceptable levels, perhaps averaging about 30% under "aerobic" (or less reducing) conditions. On the positive side, much effort is currently being directed towards "aerobic" rice production methods as water availability is becoming physically and economically limited (Tuong & Bouman, 2003). Partial drainage of fields, either periodically or throughout rice growth (Xie & Huang, 1998), and growing rice on raised beds (Duxbury & Panaullah, 2007) have been used to reduce arsenic toxicity, and these practices also reduce the arsenic content of rice grain and straw (Table 7). Greenhouse studies have shown greater potential for reduction in grain and straw arsenic concentrations than has so far been realized in the field (Table 7). Except for the study by Xu et al. (2008), yield decreases under "aerobic" conditions were found when arsenic was not toxic, but yield increases were found where arsenic toxicity was mitigated.

5.2.2 Increasing the proportion of organic arsenic in food crops

There are only a few reports of arsenic speciation in vegetables, where it is largely inorganic (Muñoz et al., 2002; Signes-Pastor et al., 2008), with variable distribution between arsenite and arsenate (Smith et al., 2009).

Table 7. Effect of growing rice under flooded and less reducing conditions on grain yield and arsenic concentrations in grain and straw

Study	Soil total arsenic concentration (mg/kg)	Grain yield		Arsenic concentration (mg/kg)			
				Grain		Straw	
Duxbury & Panaullah (2007)		(Mg/ha)					
Arsenic gradient in Bangladeshi farmer's field from 20 years of use of irrigation water		Fld.	Aer.	Fld.	Aer.	Fld.	Aer.
Aer. is raised beds	12	8.9	7.8	0.54	0.26	7.3	1.1
	26	8.1	8.2	0.53	0.28	9.7	1.2
	40	6.4	7.0	0.38	0.34	9.9	3.2
	58	3.0	5.2	0.34	0.36	11.5	3.9
Xu et al. (2008)		(g/pot)					
Greenhouse, non-rice soil		Fld.	Aer.	Fld.	Aer.	Fld.	Aer.
As^{III} or As^{V} added							
Aer. is 70% field capacity	Control 15	8.0	9.2	1.0	0.09	13	1
	+ As^{III} 25	6.0	8.0	2.5	0.18	26	3
	+ As^{V} 25	6.2	10.2	2.2	0.15	30	2
Xie & Huang (1998)		(Mg/ha)					
Arsenic-contaminated field site, China		Fld.	Aer.	Fld.	Aer.	Fld.	Aer.
Aer. is maintaining moist conditions after a few days of flooding, Eh ~260	Control 68	5.5	6.6	0.65	0.49	48 (flag leaf)	18 (flag leaf)

Table 7 (contd)

Study	Soil total arsenic concentration (mg/kg)	Grain yield			Arsenic concentration (mg/kg)					
					Grain			Straw		
Y.J. Zavala & J.M. Duxbury (unpublished)		(g/plant)								
Greenhouse, non-rice soil		Fld.	A1	A2	Fld.	A1	A2	Fld.	A1	A2
Aer. is water table at 10 cm (A1) or 20 cm (A2)	7	95	75	45	0.35	0.20	0.01	7.3	2.0	0.1
Arao et al. (2009)		(g/pot)								
Greenhouse, arsenic- and cadmium-contaminated rice soils		Fld.	B1	B2	Fld.	B1	B2	Fld.	B1	B2
Aer. is flooded from transplanting to heading (B1) or 3 weeks before heading (B2)	Soil A 25	36	31	27	0.95	0.30	0.11	28	26	25
	Soil B 48	46	40	36	1.7	0.59	0.17	42	31	38

Aer., “aerobic” or less reducing; Eh, redox potential; Fld., flooded

Arsenic speciation in rice grain varies considerably, with DMA^V comprising from 1% to 90% (Schoof et al., 1999; Heitkemper et al., 2001; Ackerman et al., 2005; Williams et al., 2005; Smith et al., 2008; Zavala et al., 2008). Strong positive relationships between either DMA^V or inorganic arsenic in grain and total grain arsenic have been found (Zavala et al., 2008; Zhu et al., 2008; and when data from Torres-Escribano et al., 2008, were plotted), leading to classification of rice into DMA^V and inorganic arsenic types (Zavala et al., 2008). Differences in speciation in grain also appear to be associated with differences in speciation in other plant parts. In greenhouse-grown rice, where grain was dominated by DMA^V with some arsenite, the major species in stems and leaves was arsenite, with some arsenate and DMA^V, whereas roots contained only arsenite and arsenate (Smith et al., 2008). In contrast, field-grown rice from West Bengal, India, contained predominantly arsenite in grain and arsenate in straw (Sanz et al., 2007). It has recently been shown that DMA^V is preferentially mobilized to rice grain (Carey et al., 2010), explaining why grain can be dominated by DMA^V when there are only low to moderate levels of DMA^V in foliar tissue. What is not yet known is whether DMA^V is synthesized by rice or is taken up as such from soil. In the former case, current varieties with the DMA^V "trait" could be more widely used, and this trait could be incorporated into preferred local varieties and even into other food crops. If DMA^V is coming from soil, strategies to increase DMA^V in soil could be investigated.

5.2.3 Reducing the arsenic content of foods by preparation or cooking methods

Preparation and cooking methods can both increase and decrease arsenic levels in foods. For foods that are cooked by boiling, it is important to use water that has low arsenic levels. Arsenic concentrations in foods may be decreased as much as 60% when the arsenic concentration in water is low and excess water is discarded (Díaz et al., 2004; Devesa, Vélez & Montoro, 2008). However, if cooking water is contaminated with arsenic, adsorption by the food may occur, leading to elevated arsenic levels. Foods that absorb a lot of water, such as dry beans and rice, are especially vulnerable to increases in arsenic concentrations. The traditional method of cooking rice in West Bengal, India—namely, washing rice until the water is clear (5–6 times), followed by cooking in about 6 times the amount of water and discarding excess water—reduced grain arsenic concentration by 56–58% when cooking water contained arsenic at less than 3 µg/l (Sengupta et al., 2006). Approximately equal amounts of arsenic were removed by the washing and cooking steps. However, the same cooking method increased arsenic concentrations in cooked rice by 2- to 4-fold when cooking water had an arsenic concentration twice as high as that in rice over a range of concentrations. Other studies confirm these results (Bae et al., 2002; Roychowdhury et al., 2002; Ackerman et al., 2005; Laparra et al., 2005). Soaking and boiling the seaweed *Hizikia fusiforme* reduced the arsenic concentration up to 82%, but had no effect on other species of red and green seaweed (Laparra et al., 2003; Devesa, Vélez & Montoro, 2008).

The peelings of root vegetables contain 2–7 times higher arsenic concentrations than the peeled root vegetables (Muñoz et al., 2002), but constitute a small fraction of the total weight. Consequently, peeling does not change the arsenic concentration by much. Polishing of brown rice removes arsenic associated

with the bran, so that white rice contains less arsenic than brown rice (Zavala & Duxbury, 2008). In one study, grain arsenic concentration was reduced by an average of 27% (range 18–40%) when 7% of the grain mass was removed, which also selectively removed inorganic arsenic, so further reducing the grain toxicity hazard (Sun et al., 2008). In a village-level study in Bangladesh, parboiling and polishing rice reduced grain arsenic concentration by an average of 19% (range 5–31%) (Duxbury et al., 2003).

6. LEVELS AND PATTERNS OF CONTAMINATION IN FOOD COMMODITIES

6.1 Arsenic content of food

Data on total arsenic contents of foods for evaluation in the present monograph were obtained from the literature and from submissions to the Committee by Brazil, France, Japan and Singapore. The total number of analytical results (single or composite) evaluated was 17 498. Table 8 summarizes the ranges of total arsenic concentrations by food category, based on results with quantified values (minimum to maximum). The highest total arsenic concentrations have been found in seaweed, fish and shellfish, mushrooms and fungi, rice and some meat products. The levels in the remaining food products usually do not exceed 1 mg/kg. In some food groups, the number of non-detectable/non-quantifiable results was important ($n = 9081$) and influences the derivation of mean concentrations. This was the case with milk products (66%), meat and meat products (74%), eggs and egg products (65%), bakery wares (70%), cereals other than rice (80%) and vegetables other than mushrooms (86%).

Table 9 summarizes the ranges of levels of inorganic arsenic obtained from the literature and from data submitted by Japan, France and Singapore (minimum to maximum).

Levels of inorganic arsenic in foods and beverages do not usually exceed 0.1 mg/kg, with mean values generally less than 0.03 mg/kg. However, seaweed, rice and some fish and shellfish products have higher inorganic arsenic levels. Food crops grown in arsenic-contaminated soils can have higher inorganic arsenic levels. In the seaweed *Hizikia fusiforme*, inorganic arsenic is more than 50% of total arsenic, with levels usually ranging from 30 to 130 mg/kg. In other seaweed species, inorganic arsenic is less than 15% of total arsenic, with levels normally below 2 mg/kg. The proportion of inorganic arsenic in rice varies from 17% to 100% of total arsenic and in vegetables from 33% to 74%. For fish and fish products, the proportion of inorganic arsenic usually does not exceed 10% of the total arsenic, but was found to reach 15% in some shellfish samples.

Table 8. Summary of available data on total arsenic concentrations in food products[a]

Food category	*n*	*n* < LOR	Concentration range (mg/kg)
Dairy products and analogues			
Milk and milk powder	284	65	0.001–0.15
Milk products	92	61	0.010–0.35
Fats and oils	39	0	0.003–0.18
Meat and meat products			
Meat	4977	4124	0.004–0.78
Offal	2074	1096	0.009–0.45
Meat products	50	20	0.003–3.25
Eggs and egg products	171	111	0.003–0.04
Confectionery products	186	61	0.002–1.13
Sweeteners	138	21	0.003–0.26
Bakery wares	71	49	0.002–0.25
Beverages			
Alcoholic beverages (except rice distilled spirits)	462	64	0.001–0.05[b]
Rice distilled spirits	8	2	0.050–1.64[b]
Non-alcoholic beverages	120	16	0.001–0.26[b]
Vegetables/fruits/nuts/seaweed			
Fruits	966	800	0.005–2.20
Vegetables (except mushrooms and fungi)	2503	2164	0.001–1.27
Mushrooms and fungi	302	60	0.011–5.79
Nuts and oilseeds	70	15	0.005–0.88
Dried seaweeds	953	3	0.114–236
Cereals and cereal products			
Cereals (except rice)	410	325	0.007–0.43
Rice	1693	0	0.002–1.83
Breakfast cereals	17	10	0.017–0.27
Pasta	19	9	0.003–0.18
Fish and shellfish			
Marine fish	1409	0	0.10–62

Table 8 (contd)

Food category	*n*	*n* < LOR	Concentration range (mg/kg)
Shellfish	171	0	0.090–66
Freshwater fish	238	0	0.060–4.72
Baby food products	75	5	0.001–4.66

LOR, limit of reporting (detection or quantification limit)

[a] Results presented for detected values only (samples in which arsenic was not detected were assigned a concentration of 0).

[b] Data expressed as mg/l.

Sources:

Data submissions: Brazil, Japan, France, Singapore

Literature sources: Bruno, Campos & Curtiust (1994); Cervera, Lopex & Montoro (1994); Pedersen, Mortersen & Larsen (1994); Berti et al. (1998); Sancho et al. (1998); Herce-Pagliai et al. (1999, 2002); Segura, Madrid & Cámara (1999); Viñas, Pardo-Martínez & Hernández-Córdoba (1999); Wangkarn & Pergantis (1999); Bhandari & Amarasiriwardena (2000); Demirözü-Erdinç & Saldamli (2000); López-Alonso et al. (2000, 2007); Moreno et al. (2000); Queirolo et al. (2000); Simsek et al. (2000); Šinigoj-Ganik & Doganoc (2000); Zaidi et al. (2000); Chen et al. (2001); Martínez et al. (2001); Matusiewicz & Mikoajczak (2001); Pardo-Martínez et al. (2001); Wyrzykowska et al. (2001); Carbonell-Barrachina et al. (2002, 2003); D'Ilio et al. (2002); Galani-Nikolakaki, Kallithrakas-Kontos & Katsanos (2002); Karadjova & Venelinov (2002); Kilic, Kenduzler & Acar (2002); Malmauret et al. (2002); Muñoz et al. (2002, 2005); Ronda et al. (2002); Roychowdhury et al. (2002); Vázquez-Moreno et al. (2002); Barbaste, Medina & Perez-Trujillo (2003); Cava-Montesinos et al. (2003, 2004); Coelho et al. (2003); Cubadda et al. (2003); Delgado-Andrade et al. (2003); Jureša & Blanuša (2003); Li et al. (2003); Meharg & Rahman (2003); Miranda et al. (2003); Roychowdhury, Tokunaga & Ando (2003); Viñas et al. (2003a,b); Waheed, Zaidi & Ahmad (2003); Wei et al. (2003); Bordajandi et al. (2004); Castiñeira et al. (2004); Y.-H. Chen et al. (2004); Díaz et al. (2004); Erdogan, Celik & Erdogan (2004); Jos et al. (2004); Julshamn et al. (2004); Laparra et al. (2004); Terrab, Hernanz & Heredia (2004); Zarcinas et al. (2004); Al Rmalli et al. (2005); Dugo et al. (2005); Karadjova et al. (2005); Liu, Probsta & Liao (2005); Patel et al. (2005); Pérez-Carrera & Fernández-Cirelli (2005); Soeroes et al. (2005); Tašev, Karadjova & Traje (2005); Williams et al. (2005, 2007); Almela et al. (2006); Catarino et al. (2006); Falcó et al. (2006); Hirata, Toshimitsu & Aihara (2006); van Overmeire et al. (2006); Weeks et al. (2006); Burger et al. (2007); Chanthai et al. (2007); El-Hadri, Morales-Rubio & de la Guardia (2007); Maduabuchi et al. (2007); Ohno et al. (2007); Pérez et al. (2007); Rose et al. (2007); Signes et al. (2007); Beni, Diana & Marconi (2008); Bronkowska et al. (2008); Cheung, Leung & Wong (2008); Donadini, Spalla & Beone (2008); Fu et al. (2008); Gülda et al. (2008); Hamano-Nagaoka et al. (2008); Jorhem et al. (2008); Lin et al. (2008); Meharg et al. (2008); Pellerano et al. (2008); Pisani, Protano & Riccobono (2008); Torres-Escribano et al. (2008); Zavala et al. (2008); Zhu et al. (2008); Ayar, Sert & Akin (2009); Baeyens et al. (2009); Besada et al. (2009); Caldas et al. (2009); Gonzálvez et al. (2009); Laoharojanaphand et al. (2009); Nardi et al. (2009); Raab et al. (2009); Roberge et al. (2009); Signes-Pastor et al. (2009); Sun et al. (2009); Uluozlu et al. (2009); Waegeneers et al. (2009a,b).

Table 9. Summary of available data on inorganic arsenic concentrations in food products[a]

Food product	*n*	*n* < LOD	Concentration range (mg/kg)
Dried seaweed	539	4	0.1–130
Rice	837	0	0.01–0.51
Fish and fish products	325	1	0.001–1.2
Vegetables	36	1	0.008–0.61

[a] Results presented for detected values only (samples in which arsenic was not detected were assigned a concentration of 0).

Sources:

Data submissions: Japan, France, Singapore

Literature sources: Suñer et al. (1999); Storelli & Marcotrigiano (2001); Almela et al. (2002, 2006); Muñoz et al. (2002); Williams et al. (2005); Rose et al. (2007); Jorhem et al. (2008); Sloth & Julshamn (2008); Torres-Escribano et al. (2008); Zavala et al. (2008); Besada et al. (2009).

Besides inorganic forms, there are a variety of organoarsenic species in foods. In meat, speciation studies are sparse (Zbinden, Andrewy & Blake, 2000; Pizarro et al., 2003; Polatajko & Szpunar, 2004; Sanz, Muñoz-Olivas & Cámara, 2005b; Sánchez-Rodas, Gómez-Ariza & Oliveira, 2006), and they show differences in the profile of arsenic species. DMA has been detected as the major species in many of the samples analysed, and AB and MMA have also been found. The presence of nitarsone, a phenylarsonic acid used as a coccidiostat, has also been reported (Sánchez-Rodas, Gómez-Ariza & Oliveira, 2006).

The greatest variety of arsenic species in vegetables has been detected in mushrooms, food matrices that contain AB, MMA, TMAO, DMA, AC and TMA^+ (Šlejkovec et al., 1997; Larsen, Hansen & Gössler, 1998; Soeroes et al., 2005; Smith, Koch & Reimer, 2007). For other vegetables, MMA has been found in carrot, radish and potato (Signes-Pastor et al., 2008), and MMA and DMA have been found in chard and aubergine (Reyes et al., 2008). Arsenic species found in fish and fish products include AB, arsenosugars, MMA, DMA, AC, TMA^+, TMAO, dimethylarsionylethanol (DMAE), trimethylarsoniopropionate (TMAP), arsenolipids and thioarsenic compounds. AB is the major species (80–90%), except in some kinds of shellfish, where arsenosugars are the major species found. In seaweeds, arsenosugars are the major species, with smaller amounts of DMA, arsenolipids and thioarsenic compounds. The valencies of MMA and DMA in food have not been determined.

6.2 Occurrence of arsenic in water

Arsenic is found widely in the earth's crust in oxidation states of –3, 0, +3 and +5, often as sulfides or metal arsenides or arsenates. In water, it is mostly present as arsenate (+5), but in anaerobic conditions, it is likely to be present as arsenite (+3). It is usually present in natural waters at concentrations below 1–2 µg/l. However, in waters, particularly groundwaters, where there are sulfide mineral deposits and sedimentary deposits derived from volcanic rocks, the concentrations can be significantly elevated. Groundwater environments that are prone to naturally high levels of arsenic (above the World Health Organization [WHO] drinking-water guideline value of 10 µg/l; WHO, 2008) are generally characterized by low rates of flushing and a large volume of young sediments (Smedley & Kinniburgh, 2002). These are mostly associated with shallow aquifers. High arsenic concentrations can be present under both reducing and oxidizing conditions. Amini et al. (2008) modelled the probability that arsenic concentrations would be above 10 µg/l in shallow groundwater for reducing and high pH/oxidizing conditions in many parts of the world. This provides a means of identifying areas potentially at risk of having drinking-water with high arsenic concentrations and prioritizing where investigations should be conducted (Table 10). Often the greatest problems are with supplies to small communities or household wells, where there are only limited resources for finding alternative supplies or for installing and maintaining treatment.

Table 10. Predicted arsenic contamination of groundwater in different countries

Country/region	Condition	% area[a]
Cambodia	Reducing	45.8
Amazon basin[b]	Reducing	37.6
Estonia	Reducing	37.2
Bangladesh	Reducing	35.4
Lithuania	Both	35.0
Finland	Unknown	34.7
Congo	Reducing	30.1
Viet Nam	Reducing	15.8
Russian Federation	Both	14.8
Cameroon	Both	14.0
Myanmar	Both	9.2
Nigeria	Oxidizing	9.0
Poland	Both	8.8
USA	Both	8.3
China, Province of Taiwan	Reducing	8.2

Country/region	Condition	% area[a]
Hungary	Reducing	7.4
Ukraine	Oxidizing	7.0
Zambia	Oxidizing	7.0
India	Both	6.4
Angola	Oxidizing	5.5
Ethiopia	Oxidizing	5.3
Argentina	Oxidizing	4.9
Romania	Reducing	3.5
Belarus	Oxidizing	3.3
Nepal	Reducing	3.2
China	Both	2.5
Kenya	Oxidizing	2.4
Greece	Unknown	0.1

[a] % area in each country with probability of arsenic contamination $P > 0.75$.
[b] Average values for Peru, Brazil and Colombia.
Source: Amini et al. (2008).

Arsenic contamination of groundwater is widespread, and there are a number of regions where arsenic contamination of drinking-water is important. Exposure via water can be very variable, with high and low arsenic sources present in close proximity, and there is also variation with depth of the well. This means that assessment of the probability of wells in an area being contaminated is not easy to judge without data on individual wells. Areas affected include southern Asia (e.g. Bangladesh, India), South-east and East Asia (e.g. China, including Taiwan, Mongolia, Viet Nam), the Americas (e.g. Argentina, Canada, Chile, Mexico, USA) and Europe (e.g. Finland, Hungary, Romania). Concentrations can vary widely, and contaminated water that is used for drinking and food preparation can contain concentrations of inorganic arsenic up to several hundred micrograms per litre, although more normally the concentration would be between 10 and 200 µg/l. Extensive surveys in West Bengal, India (Chakraborti et al., 2009), and Bangladesh (Kinniburgh & Smedley, 2001) reported that 42–50% of household wells contained arsenic concentrations above 10 µg/l and 25% contained concentrations above 50 µg/l. More data on the proportion of samples of shallow groundwater in Asian countries, showing different concentration ranges, are given in Table 11. These data should be considered illustrative, because the sampling was not necessarily systematic.

Table 11. Distribution of arsenic concentrations in shallow groundwater in Asian countries

Country and sampling area	No. of samples	% of samples in concentration range (µg/l)					
		<10	10–50	50–100	100–200	200–300	>300
Bangladesh – national[a]	3 534	58	17	9	7	4	5
India – West Bengal[b]	135 555	50	25	19	8	3	3
Nepal – Terai[c]	12 949	73	22	4	1	<1	<1
Viet Nam/Cambodia – Mekong Delta[d]	352	63	11	4	8	3	11
Viet Nam – Red Delta[e]							
- Private wells, wet season	68	9	35	16	22	6	12
- Private wells, dry season	68	56	13	16	6	4	4
- Hanoi city supply wells, raw water	8	0	25	38	25	13	0
- Hanoi city supply wells, treated water	8	0	50	50	0	0	0

[a] Kinniburgh & Smedley (2001).
[b] Chakraborti et al. (2004, 2009).
[c] Shrestha, Whitney & Shrestha (2004).
[d] Buschmann et al. (2007, 2008).
[e] Berg et al. (2001, 2007).

7. FOOD CONSUMPTION AND DIETARY EXPOSURE ESTIMATES

Dietary exposure estimates for arsenic were reported by the Committee at its twenty-seventh meeting and were not revised at the thirty-third meeting. Only values for total arsenic were given for several European countries, the USA, Canada and the Republic of Korea; these ranged from 10 to 200 µg/day from food (0.17–3.33 µg/kg bw per day, assuming a 60 kg body weight). Estimated dietary exposures to total arsenic from water ranged from 15 to 750 µg/day (0.25–12.5 µg/kg bw per day), the range reflecting normal arsenic concentrations in water (10 µg/l) and elevated arsenic concentrations (500 µg/l), assuming consumption of 1.5 litres of water a day. The Committee at its twenty-seventh meeting noted that water and seafood were the major sources of total arsenic, with other foods making minor contributions.

In the majority of studies on dietary arsenic exposure, available results were reported for total arsenic rather than for inorganic arsenic, which was of more interest for the evaluation. Results available for total arsenic are discussed below, with more focus given to the available estimates of inorganic arsenic dietary exposure. In the earlier studies that report inorganic arsenic dietary exposures, set

conversion factors were used to estimate inorganic arsenic dietary exposures from total arsenic dietary exposures, rather than analytical values.

Many of the early studies, total diet studies in particular, did not include consumption of water, and only one mentioned dietary supplements. They are therefore likely to underestimate total and inorganic arsenic dietary exposures.

At the present meeting, the Committee considered dietary exposure estimates submitted by China, Japan, Australia and New Zealand, published total diet studies and other reports in the literature.

7.1 Arsenic levels used in dietary exposure estimates

The main factors influencing arsenic content in food are the water supply, type of food and food preparation methods.

Reported arsenic levels in water from different countries were given in section 6.2. Arsenic levels in water used in the dietary exposure estimates evaluated are given in Table 12, with the high consumption or maximum amount of water drunk reported.

As noted in section 6, total arsenic levels are higher in fish and seafood commodities than in most other foods, but the arsenic is mainly organic. Levels vary a great deal with species, region and level of water contamination (Cheung, Leung & Wong, 2008). Total arsenic levels tend to be higher in marine fish than in freshwater fish (Larsen & Francesconi, 2003), except for fish from areas with geothermal waters (Whyte et al., 2009). Individual concentrations can be assigned to the correct fish species for the purpose of estimating dietary exposure only if food consumption records are sufficiently detailed. However, more often, a mean concentration is derived for a broad food group such as fish or shellfish, thus losing some accuracy in the dietary exposure estimate.

Rice tends to be a major source of inorganic arsenic in the diet, particularly in Asian countries, where it is a staple food. Speciation of arsenic in rice varies between different regions, with a higher inorganic content in rice grown in Asia compared with the USA (Williams et al., 2005; Meharg et al., 2009). Enhanced arsenic assimilation in rice also results in elevated concentrations compared with other grains (Williams et al., 2007).

The level of inorganic arsenic in the food consumed also depends on food processing and preparation methods. Water can be a major source of inorganic arsenic in food if the food is produced by irrigation with arsenic-contaminated water and/or from food preparation and cooking with contaminated water (see section 6).

The fact that water consumption and water used in cooking are not always included in dietary exposure estimates means that exposure will be underestimated where water has not been included. It also makes direct comparison of reported total and inorganic dietary exposures from different studies difficult.

There are numerous reports of total arsenic levels used in reported dietary exposure estimates, particularly from total diet and duplicate diet studies.

Table 12. Total arsenic levels in water used in dietary exposure estimates

Country or region (references)	Sample number	Mean level (mg/kg)	Median level (mg/kg)	Maximum level (mg/kg)	Water legislation limit (mg/kg)	Maximum amount of water consumed (litres/day)	Comment
Europe (EFSA, 2009)	15 365	0.0013 LB 0.0022 UB	0.000 LB 0.001 UB	0.47	0.01	2.4 95th percentile	Scenarios assume water is 50%, 70% or 100% inorganic
USA (Meliker et al., 2006)	12 520 homes, 1300 communal sources		0.0004 DW 0.0004 cooking	0.099 DW	0.01	4.25 DW 0.97 cooking 2.36 beverages 5.31 coffee	Analytical values used in dietary exposure estimates
USA (Schoof et al., 1999; NRC, 2001; Tsuji et al., 2007)	4	0.0018 total 0.0008 inorganic			0.01	1.9–2.2 DW 95th percentile 1.3–1.7 food processing 95th percentile	Water sampled in one state Two-day averaged water consumption over four regions
Canada (Health Canada, 2006)		0.0018		0.580	0.01		Levels higher in groundwater than in surface water
Bangladesh (Watanabe et al., 2004)				0.001–0.6	0.05	3 mean 6 maximum	Watanabe et al. (2004) study: rural communities using tube wells, 38 participants

Table 12 (contd)

Country or region (references)	Sample number	Mean level (mg/kg)	Median level (mg/kg)	Maximum level (mg/kg)	Water legislation limit (mg/kg)	Maximum amount of water consumed (litres/day)	Comment
Australia (NHMRC, 2004; FSANZ, 2009a)					0.007	2.2 90th percentile	
New Zealand (MOH, 2008; FSANZ, 2009b)					0.01	2.0 90th percentile	

DW, drinking-water; LB, lower bound; UB, upper bound

The 2009 EFSA review used 100 857 results from European countries reported in a 2008 data call to derive total arsenic levels for 15 major food groups, statistically adjusting mean levels for the proportion of individual foods in each food group for use in the dietary exposure assessment (Table 13; EFSA, 2009). These included previously reported values in Europe for total diet studies and from a previous Scientific Cooperation on Questions relating to Food (SCOOP) project (Leblanc et al., 2000; SCOOP, 2004; COT, 2008).

Table 13. Mean adjusted total arsenic content of foods used in the EFSA (2009) dietary exposure estimates[a]

Food group	Total arsenic lower bound mean level (mg/kg)	Total arsenic upper bound mean level (mg/kg)
01. All cereal & cereal products	0.0671	0.0848
01.A Cereal-based dishes	0.0157	0.0283
01.B Cereal & cereal products	0.0825	0.1017
02. Sugar products and chocolate	0.0135	0.0320
03. Fats (vegetable and animal)	0.0063	0.0245
04. All vegetables, nuts, pulses[b]	0.0121	0.0212
04.A Vegetable soups	0.0050	0.0110
04.B Vegetables, nuts, pulses[b]	0.0122	0.0213
05. Starchy roots and tubers	0.0031	0.0142
06. Fruits[b]	0.0051	0.0155
07. Juices, soft drinks and bottled water	0.0030	0.0068
07.A Fruit and vegetable juices[b]	0.0048	0.0129
07.B Soft drinks	0.0044	0.0132
07.C Bottled water	0.0023	0.0041
08. Coffee, tea, cocoa[b]	0.0034	0.0051
09. Alcoholic beverages	0.0055	0.0151
09.A Beer and substitutes	0.0054	0.0161
09.B Wine and substitutes	0.0061	0.0110
09.C Other alcoholic beverages	0.0085	0.0155
10. All meat and meat products, offal	0.0044	0.0138
10.A Meat and meat products	0.0042	0.0137

Table 13 (contd)

Food group	Total arsenic lower bound mean level (mg/kg)	Total arsenic upper bound mean level (mg/kg)
10.B Edible offal and offal products	0.0044	0.0139
10.C Meat-based preparations	0.0121	0.0185
11. All fish and seafood	1.6136	1.6159
11.A Seafood and seafood products	5.5537	5.5545
11.B Fish and fish products	1.4426	1.4549
11.C Fish-based preparations	1.1524	1.1573
12. Eggs	0.0042	0.0117
13. Milk and milk-based products	0.0044	0.0139
13.A Milk and dairy-based drinks	0.0026	0.0104
13.B Dairy-based products	0.0068	0.0184
13.C Cheese	0.0065	0.0188
14. Miscellaneous/special dietary products	0.3993	0.4187
14.A Miscellaneous products	0.2449	0.2658
14.B Foods for special dietary uses	0.4383	0.4573
15. Tap water	0.0013	0.0022

[a] Adjusted mean for whole food category obtained by applying relevant sampling adjustment factor to food subcategories to correct for unbalanced proportion of samples analysed in these subcategories in relation to their actual dietary contribution; non-adjusted means for whole food categories where means not reported for subcategories.

[b] Calculated mean values include conversion to fresh mass by applying various dilution factors.

Source: EFSA (2009).

Total arsenic levels for some foods in other countries reported prior to 2004, mainly from total diet studies, were summarized in the review of arsenic in food by Uneyama et al. (2007) and are not reported here. Arsenic levels from some of the more recent studies on dietary exposure are given in Table 14 for seafood and rice and some other foods, with inorganic arsenic levels given where reported.

Inorganic levels are high in a few rarely consumed foods, such as seaweed, particularly hijiki seaweed, and edible algae; these foods were included in the miscellaneous food group in the European estimates (EFSA, 2009), but dealt with separately in a study in the United Kingdom (FSA, 2004a). In Japan, seaweed is a more important part of the diet and can make a significant contribution to dietary exposures, particularly for people with high consumption of these food items (Uneyama et al., 2007; Ogawa & Kayama, 2009).

Table 14. Mean total and inorganic arsenic content for rice, seafood and some other foods used in dietary exposure estimates other than in the EFSA (2009) report

Country/region (reference)	Food group	Amount consumed (g/day)	Total arsenic mean level (mg/kg)	Inorganic arsenic mean level (mg/kg)
Australia (FSANZ, 2003, 2009a)	White rice (ww)	125 90th percentile (2009 estimate)	0.03–0.06 (LB–UB, 2003 estimate) 0.109 (2009 estimate)	
	Infant mixed cereal (2003 estimate)		0–0.07	
	Seafood (2009 estimate)	180 oysters 206 lobsters 232 fish 90th percentile	0.29 salmon 0.42 fish 0.70 crustaceans 0.335 lobsters 2.40 oysters 11.40 crabs	
Bangladesh (Rahman et al., 2008)	Rice from Bangladesh grown under different soil and water conditions, highest value when soil treated with arsenic at 40 mg/kg	400–650	0.05 (SD 0.02)	
Bangladesh (Williams et al., 2005)			0.13	

Table 14 (contd)

Country/region (reference)	Food group	Amount consumed (g/day)	Total arsenic mean level (mg/kg)	Inorganic arsenic mean level (mg/kg)
Bangladesh, China, Egypt, France, India, Italy, Japan, Spain, Thailand, USA (Meharg et al., 2009)	Rice (India lowest arsenic level; USA, France highest arsenic level)	15 mean France 18 mean USA 218 mean China 445 mean Bangladesh (FAO food balance sheet data)	Mean 0.15 (range 0.05–0.28) Median 0.13 (range 0.04–0.25) Maximum 0.82	Mean range 0.03–0.11 Median range 0.03–0.12 Maximum 0.38
Belgium (Baeyens et al., 2009)	Fish (19 species) Seafood (4 species)	14.7 mean 4.0 mean	12.83 (SD 12.01) 21.57 (SD 20.88)	0.132 (SD 0.075) 0.198 (SD 0.068)
China, Hong Kong Special Administrative Region (Cheung, Leung & Wong, 2008)	Fish – ponds Fish – freshwater Fish – marine	142 high consumer	0.70–3.44 0.24–2.13 0.93–8.11	Assumed 10% inorganic
China, Province of Taiwan (Schoof et al., 1998)	Rice Yam	225 ww 500 ww	0.120–0.150 dw 0.081 dw	0.083–0.110 dw 0.058 dw
China, Province of Taiwan (Lin, Wong & Li, 2004)	Rice	178 ww	0.08 (0.05 polished–0.12 unpolished)	
	Seafood	24	1.66 (0.8–3.17) oysters 1.55 (0.51–3.76) mussels	
China, Province of Taiwan (DOH, 2009)	Rice sampled in 1993, 1997	130–365 per capita (highest 1972, lowest 2007)	0.22 unpolished 0.24 polished	
United Kingdom (Meharg et al., 2008)	Infant rice food (17 samples)	20 g serving, up to 3 servings per day	Median 0.22 (range 0.12–0.47)	Median 0.11 (range 0.06–0.16)

Table 14 (contd)

Country/region (reference)	Food group	Amount consumed (g/day)	Total arsenic mean level (mg/kg)	Inorganic arsenic mean level (mg/kg)
United Kingdom (FSA, 2004a)			*As sold*	*As sold*
	Seaweed – hijiki		110.0	77.0
	Seaweed – others[a]		24.0–50.0	<0.3
			As prepared	*As prepared*
	Seaweed – hijiki		16.0	11.0
	Seaweed – others		3.0–4.0	<0.3
USA (Schoof et al., 1999 [selection of foods with higher levels given only]; Batres-Marquez & Jensen, 2005; Tsuji et al., 2007)	Rice	*Rice*	0.303 (SE 0.061) ww	0.074 (SE 0.0096) ww
	Bread (flour)	11 dw	0.039 (SE 0.006)	0.011 (SE 0.0026)
	Watermelon	USA average	0.042 (SE 0.003)	0.009 (SE 0.0005)
	Saltwater finfish	115 dw	2.369 (SE 1.311)	0.0005 (SE 0.0002)
	Freshwater finfish	Asian, Pacific Islander groups	0.160 (SE 0.132)	0.001 (SE 0.0003)
	Tuna	average in USA	0.512 (SE 0.131)	0.001 (SE 0)
	Shrimp		1.890 (SE 0.566)	0.0019 (SE 0.0003)
	Beef		0.052 (SE 0.110)	0.0004 (SE 0.0002)
	Chicken		0.086 (SE 0.006)	0.0009 (SE 0.0001)
	Pork		0.014 (SE 0.001)	0.0006 (SE 0.0000)
USA (Meliker et al., 2006)	Seaweed	Median 0.0 Maximum 2.2		Median 0.36 Maximum 0.57

dw, dry weight; FAO, Food and Agriculture Organization of the United Nations; LB, lower bound; SD, standard deviation; SE, standard error; UB, upper bound; ww, wet weight

[a] Arame, wakame, kombu, nori.

7.1.1 Conversion factors from total arsenic to inorganic arsenic

In studies in which inorganic arsenic levels are not measured or the LOD is too high to measure inorganic arsenic accurately, conversion factors are often used to estimate inorganic arsenic levels from the total arsenic results for use in estimating dietary exposure. However, the factors used vary from study to study (Table 15). Prior to recent studies that have reported measured inorganic arsenic levels accurately in a wide variety of foods as well as fish and seafood commodities, it was common to assume that inorganic arsenic levels for all foods were up to 10% of total arsenic levels, on the assumption that this was the worst case for conversion for fish and seafood commodities, the major contributors to total arsenic dietary exposures.

7.2 Dietary exposure estimates

7.2.1 Estimates of total arsenic dietary exposure

A comprehensive review of total arsenic content and dietary exposure estimates from total diet studies from the 1970s to 2002 was given in Uneyama et al. (2007), with reported total arsenic dietary exposures across many different countries: for Europe, from 0.001 µg/day for Portugal to 458 µg/day for Spain; for Asia, from 27 µg/day for Japan to 658 µg/day for India; for the USA and Canada, from 6 to 137 µg/day; for New Zealand, 55 µg/day; and for South America, from 7 µg/day for Brazil to 394 µg/day for Mexico.

Estimates of dietary exposure to total arsenic for the whole population derived from individual dietary records for 19 European countries were reported in 2009 and are summarized in Table 16. For Europe, mean total arsenic dietary exposures ranged from 0.45 to 4.58 µg/kg bw per day, and 95th-percentile exposures from 1.75 to 11.22 µg/kg bw per day.

Estimates derived from individual records reported elsewhere in the scientific literature are given in Table 17. Total arsenic dietary exposure estimates (including water) for the USA, Australia and New Zealand were lower than those for Europe, although arsenic levels for individual foods were used in the estimates, rather than for wide food groups, as in the European estimates: USA mean 0.39 µg/kg bw per day (tap water included) (Xue et al., 2010); Australia/New Zealand mean 0.5–0.7 µg/kg bw per day (tap water included), 95th percentile 0.8–1.0 µg/kg bw per day (FSANZ, 2009a,b). Those reported for countries with high-rice diets and/or arsenic-contaminated water were much higher: Japan mean 3.82–4.73 µg/kg bw per day (Tsuda et al., 1995); Bangladesh mean 0.91 µg/kg bw per day in an area with no detected arsenic in the drinking-water (Kile et al., 2007b), 10.30–13.48 µg/kg bw per day in rural areas with contaminated tube well water (Watanabe et al., 2004); Chile mean 2.18–23.3 µg/kg bw per day in an area with high arsenic levels in the river water used as drinking-water (Díaz et al., 2004).

Table 15. Reported conversion factors from total arsenic to inorganic arsenic

Data source	Food	No. of samples	Mean % inorganic	Comments
Uneyama et al. (2007)	Vegetable and cereal	21	84.0 (70–100)	Conversion factors extracted from literature values and applied to total arsenic dietary exposures for a variety of total diet studies
	Fish	133	4.2 (0–50)	
	Crustaceans, other seafood	97	2.2 (0–12)	
	Seaweed (except hijiki)	40	3.3 (0–20)	
	Hijiki	15	61.0 (30–82)	
	Meat, dairy	not sampled	100 (assumed value)	
EFSA (2009)	Fish	170	2.0	Standard ratios for fish and seafood commodities used in EFSA assessment regardless of total arsenic concentration, as proportion inorganic decreases as total arsenic concentration increases (Sirot et al., 2009a) For all other foods, EFSA assessment for inorganic arsenic assumes 50%, 70% or 100% inorganic scenarios
	Seafood products	219	3.5	
			Standard ratio	
	Fish		0.015 or 0.03 mg/kg	
	Seafood products		0.05 or 0.10 mg/kg	
	Rice	200	50–60 (30–90 reported in literature)	
	Cereal products and vegetables		30–100 (Schoof et al., 1999; Muñoz et al., 2002; Díaz et al., 2004)	
	Tea		29–88 (C. Yuan et al., 2007)	
	Edible algae		60	

Table 15 (contd)

Data source	Food	No. of samples	Mean % inorganic	Comments
Yost, Schoof & Aucoin (1998)	Milk and dairy products		26	Single samples, duplicates
	Meat		100	
	Poultry		41	
	Fish saltwater		1	
	Fish freshwater		15	
Leblanc et al. (2000)	All foods		10	Based on worst-case scenario for fish and seafood commodities, assuming most inorganic arsenic will derive from fish and seafood commodities, which were major sources of total arsenic

Table 16. Dietary exposure to total arsenic for Europe, individual dietary records

Country	Number of subjects	Mean[b] (µg/kg bw per day)	95th percentile (µg/kg bw per day)	Comments
Europe (summary over all countries)[a]		0.45 minimum LB 0.94 median LB 4.31 maximum LB 0.65 minimum UB 1.22 median UB 4.58 maximum UB	1.75 minimum LB 3.16 median LB 10.96 maximum LB 1.97 minimum UB 3.38 median UB 11.22 maximum UB	Foods assigned mean analytical values for Europe (100 867 results, sampling adjustment factors used in each food category) Differences in dietary exposure due to different food consumption patterns in each country Includes drinking-water
Austria	2123	0.88–1.14 LB–UB	4.15–4.40 LB–UB	24 h recall, 2005–2006
Belgium	1723	0.91–1.19 LB–UB	2.79–3.11 LB–UB	2 × 24 h recall, 2004–2005
Bulgaria	853	0.86–1.08 LB–UB	3.89–4.16 LB–UB	24 h recall, 2004
Czech Republic	1751	0.87–1.16 LB–UB	2.74–3.01 LB–UB	2 × 24 h recall, 2003–2004
Denmark	3159	0.94–1.22 LB–UB	2.10–2.44 LB–UB	7-day pre-coded diary with open fields, 2000–2004
Estonia	2010	0.86–1.10 LB–UB	3.97–4.20 LB–UB	24 h recall, 1997
Finland	2007	0.98–1.21 LB–UB	3.16–3.38 LB–UB	2 × 24 h recall (one interview, consecutive days), 2007
France	1195	1.61–1.88 LB–UB	3.97–4.25 LB–UB	7-day dietary record, 2006–2007
Germany	3550	1.05–1.36 LB–UB	2.41–2.78 LB–UB	2 × 24 h recall, 2005–2007
Great Britain	1724	1.07–1.07 LB–UB	2.89–3.18 LB–UB	7-day dietary record, 2000–2001

Table 16 (contd)

Country	Number of subjects	Mean[b] (µg/kg bw per day)	95th percentile (µg/kg bw per day)	Comments
Hungary	927	0.60–0.84 LB–UB	1.75–1.97 LB–UB	3-day dietary record, 2003–2004
Iceland	1075	1.46–1.75 LB–UB	4.75–5.17 LB–UB	Not available
Ireland	1372	0.98–1.27 LB–UB	2.25–2.65 LB–UB	7-day dietary record, 1997–1999
Italy	1544	2.11–2.37 LB–UB	6.54–6.74 LB–UB	3-day dietary record, 2005–2006
Netherlands	4285	0.79–1.07 LB–UB	2.42–2.69 LB–UB	2 × 24 h recall, 2003
Norway	2321	4.31–4.58 LB–UB	10.96–11.22 LB–UB	Not available
Poland	2692	0.93–1.25 LB–UB	3.58–3.91 LB–UB	24 h recall, 2000
Slovakia	2208	0.45–0.65 LB–UB	2.15–2.48 LB–UB	24 h recall, 2006
Sweden	1088	2.53–2.82 LB–UB	6.46–6.80 LB–UB	7-day dietary record, 1997–1998

LB, lower bound; UB, upper bound

[a] EFSA Concise European Food Consumption Database, 19 countries; individual dietary records, body weights, whole population; arsenic levels reported in 2008.

[b] Except where otherwise indicated.

Source: EFSA (2009)

Table 17. Other estimates of dietary exposure to total arsenic, individual records

Country (reference)	Data source	Mean (µg/kg bw per day)	90th/95th percentile (µg/kg bw per day)	Comments
Australia (FSANZ, 2009a)	Food consumption from 1995 NNS, consumers were whole population 2+ years (all were arsenic consumers) Arsenic levels from TDS and other sources	*Tap water not included* 0.5–0.6 mean 0.1–0.2 median *Tap water included* 0.6–0.7 mean 0.3–0.3 median	*Tap water not included* 0.6–0.7 90th percentile *Tap water included* 0.8–1.0 90th percentile	Range lower to upper bound Assumed inorganic arsenic level at Australian drinking-water guideline, maximum of 7 µg/l, 100% inorganic
Bangladesh (Watanabe et al., 2004)	24 h recall, FFQ and interviews, 38 participants for water consumption study, 230 samples for food recall/FFQ	13.48 males 10.30 females		Assumed 50 kg bw to convert to per kg bw Tube well water, assumed arsenic level of 0.10 mg/l
Bangladesh (Kile et al., 2007b)	Duplicate diets × 6 days, 47 women from longitudinal study on arsenic and biomarker response	0.91 mean background (arsenic not detected in drinking-water)	0.96 median food only 1.36 median food + water	Average 50 kg bw Inorganic fraction of duplicate diets was 81 ± 13.9% Rice main contributor from diet, little seafood consumed, as inland community Tube well water arsenic level 0.0016 mg/l median, range from 0 to 0.45 mg/l
Chile (Díaz et al., 2004)	24 h recall in 1999, 50 participants, foods prepared as consumed prior to analysis (results in Muñoz et al., 2002)	0.63–1.1 mean food 2.18–23.3 mean food + water		Assumed 60 kg bw to convert to per kg bw Range of values due to different analysed water content at two sampling periods (0.041–0.572 mg/l)

Table 17 (contd)

Country (reference)	Data source	Mean (µg/kg bw per day)	90th/95th percentile (µg/kg bw per day)	Comments
France (Leblanc et al., 2000)	Duplicate diet method, only breakfast and lunch meals provided out of home analysed	1.82		Total dietary exposure derived by assuming total = breakfast + 2 × lunch values, drinking-water not included Assumed 60 kg bw
France (Sirot et al., 2009a)	996 participants, FFQ for fish and seafood commodities plus mean consumption from French national survey for other foods, fish and seafood commodities analysed	11.04 males 13.53 females	25.14 males 95th percentile 33.00 females 95th percentile	Frequent fish and seafood commodity consumers (>2 meals per week) from coastal area Includes drinking-water
Japan (Tsuda et al., 1995)	3 × duplicate diets, 79 women, Shiga Prefecture	4.73 (SD 3.46) 1991 3.82 (SD 2.00) 1992	na	Assumed 55 kg bw, drinking-water not included
Japan (Ogawa & Kayama, 2009)	2007 diet history of women and 10-year-old children in fishing and rice-growing communities Analysed composite values for rice, vegetables, seaweed, fish	24.10 fishermen's wives 23.57 rice farmers' wives 19.71 10-year-old children (TDS for whole population 4.91–4.96 reported in 2008)	*95th percentile* 78.00 fishermen's wives 68.86 rice farmers' wives 68.86 10-year-old children *Maximum* 159.57 fishermen's wives 148.86 rice farmers' wives 114.00 10-year-old children	Assumed 50 kg bw Range lower to upper bound Lower-bound non-detects = 0, upper-bound non-detects = ½ LOQ Dietary exposure for high consumers of edible algae, seaweed, fish and shellfish

Table 17 (contd)

Country (reference)	Data source	Mean (µg/kg bw per day)	90th/95th percentile (µg/kg bw per day)	Comments
New Zealand (FSANZ, 2009b)	Food consumption from 1997 NNS adults (all were arsenic consumers) Arsenic levels from TDS and other sources	*Tap water not included* 0.4–0.5 mean 0.1–0.2 median *Tap water included* 0.5–0.6 mean 0.2–0.3 median	*Tap water not included* 0.6–0.7 90th percentile *Tap water included* 0.8–0.9 90th percentile	Range lower to upper bound Assumed inorganic arsenic level at WHO drinking-water guideline, maximum of 10 µg/l
USA (Xue et al., 2010)	USA TDS analytical values for total arsenic used from 1991–2004, with conversion factors from Schoof et al. (1999) NHANES 2003–2004 1-day data used for food consumption	0.36 food 0.03 water	1.40 food 95th percentile 0.11 water 95th percentile	SHEDS – Dietary model, probabilistic model, included food and water consumption (recipes used to convert food to raw agricultural commodities)

FFQ, food frequency questionnaire; LOQ, limit of quantification; na, not available; NNS, National Nutrition Survey; TDS, total diet study

For communities where seafood is a major component of the diet, higher total arsenic dietary exposure estimates were reported. For a coastal community in France, a mean exposure of 11.04–13.53 µg/kg bw per day and a 95th-percentile exposure of 25.14–33.00 µg/kg bw per day were reported (Sirot et al., 2009a). For Japanese fishermen's or rice farmers' wives who were known to be high consumers of fish and edible algae and seaweed, mean dietary exposures were 23.57–24.10 µg/kg bw per day, and 95th-percentile dietary exposures were 68.86–78.00 µg/kg bw per day. For 10-year-old children in these communities, mean dietary exposure was 19.71 µg/kg bw per day, and 95th-percentile dietary exposure was 68.86 µg/kg bw per day (Ogawa & Kayama, 2009).

Dietary exposures to total arsenic from total diet studies or other model diets reported since 2003 are presented in Table 18.

A wider range of total dietary exposure was reported in total diet studies or model diet estimates (mean total arsenic dietary exposures from 0.250 to 4.75 µg/kg bw per day for adults), compared with those calculated using individual dietary records, for Europe, Australia and New Zealand, which had estimates using both approaches. Estimates for China, Hong Kong Special Administrative Region, Chile and Bangladesh were within this range. Estimates for children indicate higher total arsenic dietary exposure than for adults from the same country, except for young infants, who rarely consume seafood (FSANZ, 2003; NZFSA, 2006).

7.2.2 Contributions to total arsenic dietary exposure

Most studies reported that fish and seafood commodities were the major contributors to total arsenic dietary exposure, although consumption of these foods usually constitutes only a small part of the diet. For infants, rice and rice cereals also make a major contribution (Tao & Bolger, 1999; Uneyama et al., 2007; FSANZ, 2009a,b).

For the USA, in a summary of total diet studies from 1991 to 1996, Tao & Bolger (1999) reported that for the population 2 years of age and over, the highest proportion of total arsenic dietary exposures was from fish and seafood commodities (76–96%), with infants having a greater contribution from rice and rice cereals (42% fish and seafood commodities, 31% rice cereals). In Uneyama et al. (2007), major contributors to total arsenic dietary exposure were reported to differ by country: for the United Kingdom, fish was the major contributor (93.9%), followed by bread and cereals (3.1%); for Canada, fish was the major contributor (52.2%), followed by bakery goods and cereals (17%), meat and poultry (9.8%) and beverages (5.5%); for Spain, fish was the major contributor (97.7); for Japan, seafood was the major contributor (49.5%), followed by vegetables and seaweed (28.1%) and rice (17.2%).

Table 18. Estimates of dietary exposure to total arsenic using model diets or total diet approach, available since 2003

Country (reference)	Data source	Mean dietary exposure (µg/kg bw per day)	95th-percentile dietary exposure (µg/kg bw per day)	Comments
Australia (FSANZ, 2003)	Total diet study 2000–1001	0.56–0.88 M 25–34 years 0.49–0.78 F 25–34 years 0.50–0.83 boy 12 years 0.28–0.54 girl 12 years 0.55–1.30 child 2 years 0.37–1.40 infant 9 months		Range lower to upper bound (ND = 0 or LOD)
Bangladesh (Rahman et al., 2008)	Mean arsenic from rice grown under experimental conditions, water at maximum limit of 0.05 mg/l	0.20–0.35		Arsenic intake from mean rice consumption of 400–650 g/day plus 4 litres of water
Belgium (Baeyens et al., 2009)	Model diet Arsenic data for seafood collected in 1997–1998, 300 samples, other values from Leblanc et al. (2005)	4.75 whole population	10.82 whole population (90th percentile)	Assumed 60 kg bw for whole population, model diet included consumption of fish, seafood commodities, fruit and soft drinks only
Chile (Muñoz et al., 2005)	Market basket study, food prepared as consumed for analysis	1.13		24 h recall food consumption, mean body weight of 68 kg
China (China, 2010)	Total diet study 2007, 12 provinces for 18–45 males	0.99 overall mean 0.24–3.35 across provinces		63 kg bw for adult males

Table 18 (contd)

Country (reference)	Data source	Mean dietary exposure (µg/kg bw per day)	95th-percentile dietary exposure (µg/kg bw per day)	Comments
China, Hong Kong Special Administrative Region (Cheung, Leung & Wong, 2008)	Fish analysis, assumed 10% inorganic, high consumption amount for seafood only	0.23 freshwater fish 0.66 marine fish		Average body weight 55 kg, 142 g of fish per day
France (Leblanc et al., 2005)	Total diet study 2001–2002	1.04 adult 1.42 child 3–14 years	2.72 adult 3.43 child 3–14 years	Drinking-water included, assumed 60 kg bw adult, 30 kg child 3–14 years
New Zealand (NZFSA, 2006)	Total diet study 2003–2004	1.39–1.44 M 25 years 1.06–1.44 F 25 years 1.27–1.34 boy 11–14 years 0.69–0.74 girl 11–14 years 1.40–1.50 child 5–6 years 1.60–1.77 child 1–3 years 1.45–1.63 infant 6–12 months		Range lower to upper bound (ND = 0 or LOD), presented per week, converted to per day
Slovakia (Pavlovičová & Šalgovičová, 2008)	1) Household budget survey, model diets (diet based on nutrient requirements) 2) Arsenic data collected from 1994 to 2005 68 513 samples	1) 0.25–0.60 adults 2) 0.32–1.16 adults 0.57–1.16 4–6 years 0.42–0.82 7–11 years 0.36–0.70 12–15 years	1) 1.44–3.59 adults 2) 0.92–3.06 adults	2) Dietary exposure ranges are for lowest to highest results reported for individual years from 1994 to 2005, drinking-water not included Average body weight 70 kg adult, 18 kg 4–6 years, 33.5 kg 7–11 years, 47 kg 12–15 years

Table 18 (contd)

Country (reference)	Data source	Mean dietary exposure (μg/kg bw per day)	95th-percentile dietary exposure (μg/kg bw per day)	Comments
Spain (Martí-Cid et al., 2008)	Total diet study	3.19–3.73		Catalonia population, average body weight 70 kg male adult Fish and cereal group main contributors to dietary exposure Arsenic data collected in 2000 survey, ND = ½ LOD; arsenic data collected in 2006 survey, LB (ND = 0) estimate for 50 foods reported

F, female; LB, lower bound; M, male; ND, non-detects

In Australia, fish contributed 13–16% to total arsenic levels when water was excluded and 11–12% when it was included; other seafood commodities contributed 46–55% when water was excluded and 39–43% when it was included (FSANZ, 2009a). Similar results were reported for New Zealand, where fish contributed 26–32% to total arsenic levels when water was excluded and 21–24% when it was included; other seafood commodities contributed 27–38% when water was excluded and 24–29% when water was included (FSANZ, 2009b). Milk and rice were the only other food groups to contribute to total arsenic dietary exposures in Australia (10–13% when water was excluded, 8–11% when water was included) and in New Zealand (13–16% when water was excluded, 9–12% when water was included); rice contributed more than 1% but less than 5% in both countries. Water was reported to contribute 18–21% to total arsenic exposures in Australia and 22–25% in New Zealand (FSANZ, 2009a,b).

7.2.3 Estimates of inorganic arsenic dietary exposure

Estimates of inorganic arsenic dietary exposure either were derived by applying conversion factors to total arsenic levels for different foods or food groups prior to estimating exposure, such as in the 2009 EFSA report (EFSA, 2009), or have used measured inorganic arsenic levels.

Estimates of inorganic arsenic dietary exposure for 19 European countries are presented in Table 19. For Europe, mean inorganic arsenic dietary exposure estimates for different scenarios ranged from 0.21 µg/kg bw per day (lower-bound estimate, 0.05 mg/kg seafood, 50% inorganic arsenic in other foods) to 0.61 µg/kg bw per day (upper-bound estimate, 0.1 mg/kg seafood, 100% inorganic arsenic in other foods) (EFSA, 2009). Other estimates for the United Kingdom were within these ranges (FSA, 2004b, 2009). Estimates for people with high water consumption (mean 0.66 µg/kg bw per day) and high consumption of edible algae (4.03 µg/kg bw per day) were given in the EFSA report (EFSA, 2009).

Reported inorganic arsenic dietary exposures for some non-European countries are given in Table 20. Mean inorganic arsenic dietary exposures for the four studies in the USA were in the same range for the general population, from 0.0 to 0.10 µg/kg bw per day (Meacher et al., 2002; Meliker et al., 2006; Tsuji et al., 2007; Xue et al., 2010), the highest being from the probabilistic estimate by Tsuji et al. (2007), noting that the ranges given for each study relate to the inclusion of different sources of inorganic arsenic. Xue et al. (2010) reported 95th-percentile inorganic arsenic dietary exposures of 0.19 µg/kg bw per day for the general population; Meliker et al. (2006) reported higher 95th-percentile exposures of 0.34 µg/kg bw per day, with a maximum reported exposure of 1.80 µg/kg bw per day, from an area in south-east Michigan where the drinking-water was contaminated.

For France, there was a contrast between a duplicate diet study and estimates for coastal communities, with the latter estimated exposures being much higher than those for the general population (mean duplicate diet 0.18 µg/kg bw per day, assuming 10% total arsenic is inorganic; coastal communities mean 0.43–0.48 µg/kg bw per day, 95th percentile 0.95–0.98 µg/kg bw per day, using measured seafood analysis and other reported values).

Table 19. Predicted dietary exposure to inorganic arsenic, individual records for whole population, median of country means and 95th-percentile estimates for 19 European countries (EFSA, 2009)

Data source	Mean dietary exposure (µg/kg bw per day)	95th-percentile dietary exposure (µg/kg bw per day)	Scenario assumptions
19 European countries (EFSA, 2009), food consumption amounts based on EFSA Concise European Food Consumption Database - General diet Individual dietary records, body weights, whole population Arsenic levels reported in 2008	0.41–0.61 LB–UB	0.72–0.99 LB–UB	Scenario 1: Actual data for fish and seafood commodities, 100% total arsenic in other food is inorganic
	0.29–0.43 LB–UB	0.51–0.69 LB–UB	Scenario 2: Actual data for fish and seafood commodities, 70% total arsenic in other food is inorganic
	0.21–0.31 LB–UB	0.36–0.51 LB–UB	Scenario 3: Actual data for fish and seafood commodities, 50% total arsenic in other food is inorganic
	0.42–0.61 LB–UB	0.73–0.99 LB–UB	Scenario 4: 0.03 mg/kg fish, 0.1 mg/kg seafood commodities, 100% total arsenic in other food is inorganic
	0.30–0.43 LB–UB	0.51–0.69 LB–UB	Scenario 5: 0.03 mg/kg fish, 0.1 mg/kg seafood commodities, 70% total arsenic in other food is inorganic
	0.22–0.31 LB–UB	0.37–0.52 LB–UB	Scenario 6: 0.03 mg/kg fish, 0.1 mg/kg seafood commodities, 50% total arsenic in other food is inorganic
	0.41–0.60 LB–UB	0.72–0.97 LB–UB	Scenario 7: 0.015 mg/kg fish, 0.05 mg/kg seafood commodities, 100% total arsenic in other food is inorganic
	0.29–0.42 LB–UB	0.50–0.68 LB–UB	Scenario 8: 0.015 mg/kg fish, 0.05 mg/kg seafood commodities, 70% total arsenic in other food is inorganic
	0.21–0.30 LB–UB	0.36–0.49 LB–UB	Scenario 9: 0.015 mg/kg fish, 0.05 mg/kg seafood commodities, 50% total arsenic in other food is inorganic

Table 19 (contd)

Data source	Mean dietary exposure (µg/kg bw per day)	95th-percentile dietary exposure (µg/kg bw per day)	Scenario assumptions
- Special diets			
Consumers of algae as food	4.03		Specific diet (10 g algae) added to inorganic exposure from scenario 5
Consumers of bran and germ	0.48		Specific diet (2 g bran and germ) added to UB inorganic exposure from scenario 5
Consumers of fish and seafood commodities	0.47 fish 0.52 seafood		Specific diet (600 g fish or 400 g seafood commodities) added to UB inorganic exposure from scenario 5
Consumers of rice-based diets	1.02 ethnic 0.45 European		Specific diet (300 g raw rice ethnic diet, 9 g European diet plus water) added to UB inorganic exposure from scenario 5
Vegetarians	0.27–0.40		Scenario 5: 0.03 mg/kg fish, 0.1 mg/kg seafood commodities, 70% total arsenic in other food is inorganic; LB and UB values
People with high consumption of water	0.66		Specific diet (2 litres water at 0.002 mg/l [maximum concentration reported in EFSA data set]) added to UB inorganic exposure from scenario 5
France (Leblanc et al., 2000)	0.18		Duplicate diet method, only breakfast and lunch meals provided out of home analysed Total dietary exposure derived by assuming total = breakfast + 2 × lunch values, assumed 10% total arsenic is inorganic, drinking-water not included Assumed 60 kg bw

Table 19 (contd)

Data source	Mean dietary exposure (µg/kg bw per day)	95th-percentile dietary exposure (µg/kg bw per day)	Scenario assumptions
France (Sirot et al., 2009b)	0.43 males 0.48 females	0.98 males 0.95 females	996 participants, FFQ for fish and seafood commodities plus mean consumption from French national survey for other foods, fish and seafood commodities analysed Frequent consumers of fish and seafood commodities (>2 meals per week) from coastal area Includes drinking-water
United Kingdom (FSA, 2004b)	0.02–0.08 adults 0.03–0.1 children (4–18 years) 0.02–0.07 vegetarians	*97.5th percentile* 0.05–0.1 adults 0.08–0.2 children 0.05–0.1 vegetarians	Inorganic arsenic analysed when total arsenic high enough for measurement in 1999 total diet study, food consumption from NDNS (range LB–UB values given) Results for elderly "free living" and elderly "institutional" similar to adults
United Kingdom (FSA, 2009)	0.03–0.09 adults 0.06–0.16 children (4–18 years) 0.04–0.10 vegetarians	*97.5th percentile* 0.07–0.17 adults 0.13–0.29 children 0.08–0.16 vegetarians	Inorganic arsenic analysed when total arsenic high enough for measurement in 2006 total diet study, food consumption from NDNS (range LB–UB values given) Results for elderly "free living" and elderly "institutional" similar to adults

FFQ, food frequency questionnaire; LB, lower bound; NDNS, National Diet and Nutrition Survey; UB, upper bound

Table 20. Other estimates of dietary exposure to inorganic arsenic, individual records

Country (references)	Data source	Mean dietary exposure (µg/kg bw per day)	High-percentile dietary exposure (µg/kg bw per day)	Comments
Chile (Díaz et al., 2004)	24 h recall in 1999, 50 participants in area known to have contaminated water, foods prepared as consumed prior to analysis (results in Muñoz et al., 2002)	0.52–0.92 mean food 2.08–21.48 mean food + water		Assumed 60 kg bw to convert to per kg bw Range of values due to different analysed water content at two sampling periods (0.041–0.572 mg/l), water source is river water
Japan (Ogawa & Kayama, 2009)	2007 diet history of middle-aged fishermen's wives (n = 201), rice farmers' wives (n = 125) and 10-year-old children (n = 231)	0.39 fishermen's wives 0.36 rice farmers' wives 0.46 10-year-old children	*95th percentile* 1.29 fishermen's wives 0.83 rice farmers' wives 0.83 10-year-old children *Maximum* 2.87 fishermen's wives 1.63 rice farmers' wives 2.27 10-year-old children	Individual body weight Range LB–UB LB non-detects = 0, UB non-detects = ½ LOQ, conversion factors as in Uneyama et al. (2007), assumed 50% loss of inorganic arsenic from washing nori (algae), 10% from cooking seaweed High dietary exposure for consumers of edible algae, seaweed

Table 20 (contd)

Country (references)	Data source	Mean dietary exposure (µg/kg bw per day)	High-percentile dietary exposure (µg/kg bw per day)	Comments
USA (Schoof et al., 1999; Meacher et al., 2002)	1999 market basket survey, focused on foods likely to contain inorganic arsenic Adults 16–59 years, individual dietary records, CSFII 1989–1991	*Females* 0.04 food 0.046 water 0.08 all sources *Males* 0.05 food 0.04 water 0.09 all sources		Monte Carlo analysis, different source of inorganic arsenic (food, drinking-water, soil, inhalation) Assumed 70 kg bw to convert reported values to per kg bw values
USA (Meliker et al., 2006)	Individual dietary records 2003–2004 Case–control study, 440 elderly adults from south-east Michigan (87% males in study population) Water analysed for each household, other inorganic arsenic levels from literature	0.0–0.06 median	0.16–0.34 95th percentile 0.06–0.23 90th percentile 0.73–1.80 maximum	Monte Carlo analysis of individual dietary records, range for eight metrics taking inorganic arsenic from water from different sources, food and cigarettes into account 8% population with water levels >10 µg/l Estimates given per week, converted to per day

Table 20 (contd)

Country (references)	Data source	Mean dietary exposure (µg/kg bw per day)	High-percentile dietary exposure (µg/kg bw per day)	Comments
USA (Tsuji et al., 2007)	Inorganic arsenic levels from USEPA water data (Schoof et al., 1999) Whole population, children 1–6 years, individual dietary records, CSFII 1994–1996, 1998 Supplemental Children's Survey	0.20–0.21 1–6 years 0.09–0.10 all From food and water	*90th percentile* 0.34–0.36 1–6 years 0.18–0.19 all From food and water	Probabilistic analysis (FARE program) of inorganic arsenic exposure from food, water, soil, treated wood 2-day water consumption calculated from drinking-water plus food processing water Assume 60 kg bw for whole population, 17.3 kg for 1- to 6-year-old children Range LB (water distribution truncated at 0.01 µg/l) to UB (not truncated)
USA (Xue et al., 2010)	TDS analytical values for total arsenic used from 1991–2004, with conversion factors from Schoof et al. (1999) NHANES 2003–2004 1-day data used for food consumption	0.05 food 0.03 water	*95th percentile* 0.19 food 0.11 water	SHEDS – Dietary model, probabilistic model, included food and water consumption (recipes used to convert food to raw agricultural commodities) Assumed all arsenic in water inorganic

CSFII, Continuing Survey of Food Intakes by Individuals; FFQ, food frequency questionnaire; LB, lower bound; UB, upper bound

The European and USA values were much lower than those reported from studies in Chile and Japan. In Chile, where the drinking-water was known to be contaminated, mean inorganic arsenic dietary exposure from food and water ranged from 2.08 to 21.48 µg/kg bw per day. In Japan, in two rural fishing and rice-growing communities known to have high consumption of either fish, algae and seaweed or rice, respectively, mean inorganic arsenic dietary exposures for middle-aged women ranged from 0.36 to 0.39 µg/kg bw per day, with 95th-percentile exposures ranging from 0.83 to 1.29 µg/kg bw per day. For 10-year-old children, mean inorganic arsenic dietary exposure was 0.46 µg/kg bw per day, with a 95th-percentile exposure of 0.83 µg/kg bw per day.

For infants (Table 21), assuming single food consumption, the highest inorganic arsenic dietary exposure would be from 90 g of rice-based cereal (1.63 µg/kg bw per day), followed by 600 ml of water (0.69 µg/kg bw per day), then 800 ml of formula (0.10 µg/kg bw per day) or breast milk (0.03 µg/kg bw per day).

For young children, estimated mean inorganic arsenic dietary exposure from individual dietary records for the United Kingdom, Italy and the USA were in a similar range: Italy, 0.39–0.54 µg/kg bw per day for 0.5- to 7-year-old children (Meharg et al., 2008); United Kingdom, 0.05–0.30 µg/kg bw per day for 1.5- to 4.5-year-old children (FSA, 2004b, 2009); USA, 0.18 µg/kg bw per day for 1- to 6-year-old children (Yost et al., 2004) or from 0.08 µg/kg bw per day for 3- to 5-year-old children to 0.23 µg/kg bw per day for children less than 1 year of age (Xue et al., 2010). For European children aged 1–3 years, predicted median dietary exposures to inorganic arsenic were estimated to be slightly higher, ranging from 0.74 to 1.39 µg/kg bw per day for 1- to 3-year-old children, depending on the scenario used.

For infants and young children with high consumption, 95th-percentile estimates were as follows: Italy, 0.61–1.63 µg/kg bw per day for 0.5- to 7-year-old children (Meharg et al., 2008, as quoted in EFSA, 2009); Europe, 1.47–2.66 µg/kg bw per day for 1- to 3-year-old children (EFSA, 2009); and USA, 0.36 µg/kg bw per day for 1- to 6-year-old children (Yost et al., 2004) or from 0.21 µg/kg bw per day for 3- to 5-year-old children to 0.53 µg/kg bw per day for children less than 1 year of age (Xue et al., 2010).

Inorganic arsenic dietary exposures estimated from total diet studies or model diets are given in Table 22. Uneyama et al. (2007) used set inorganic to total arsenic ratios (see Table 15) to convert reported total arsenic dietary exposures from total diet studies to inorganic arsenic dietary exposures for the United Kingdom, Canada, Spain and Japan, as presented in Table 22. Dietary exposures have been given per kilogram body weight, assuming a 60 kg body weight for adults in all countries. Mean or median inorganic arsenic dietary exposure estimates ranged from 0.02 to 0.909 µg/kg bw per day, with the highest values reported for China (up to 0.76 µg/kg bw per day in one province), Japan (0.56 µg/kg bw per day), Bangladesh (median 0.60 µg/kg bw per day) and China, Province of Taiwan (mean 0.909 µg/kg bw per day, maximum 3.836 µg/kg bw per day), and lower values for Europe, the USA and India.

Inorganic arsenic dietary exposures for infants and young children reported from total diet studies (Table 23) were similar to those from individual dietary records for Europe and the USA.

Table 21. Estimated dietary exposure to inorganic arsenic for infants below 6 months of age and young children

Data source	Food consumption (g/day)	Mean/median dietary exposure (µg/kg bw per day)	95th-percentile exposure (µg/kg bw per day)	Assumptions
Europe				
Infants Arsenic values reported in Sternowsky, Moser & Szadkowsky (2002), Meharg et al. (2008)	*Infants* 800 breast milk 800 formula 90 rice-based food 600 water	 0.03 mean 0.10 mean 1.63 mean 0.69 mean		Mean body weight 6.1 kg for infants below 6 months of age Arsenic in breast milk all inorganic arsenic Maximum level for arsenic in water permitted in legislation (0.01 mg/l) Lower and upper bound values
Children Arsenic levels reported in 2008, food consumption from INRAN or EFSA	*Children* 0.5–3 years, Italy 4–7 years, Italy 8–12 years, Italy	 0.39–0.62 mean 0.38–0.54 mean 0.31–0.45 mean	 0.61–1.24 1.50–1.63 1.32 –1.51	
Concise European Food Consumption Database (EFSA, 2009)	1–3 years, Europe	0.74–1.39 median	1.47–2.66	Range of median values across different countries
United Kingdom (FSA, 2004b)	1999 TDS	0.05–0.20 mean (1.5–4.5 years)	0.08–0.25 97.5th percentile (1.5–4.5 years)	Composite samples for 20 TDS food groups, individual diet records from NDNS Range lower bound–upper bound
United Kingdom (FSA, 2009)	2006 TDS	0.10–0.30 mean (1.5–4.5 years)	0.17–0.40 97.5th percentile (1.5–4.5 years)	Composite samples for 20 TDS food groups, individual diet records from NDNS Range lower bound–upper bound

Table 21 (contd)

Data source	Food consumption (g/day)	Mean/median dietary exposure (µg/kg bw per day)	95th-percentile exposure (µg/kg bw per day)	Assumptions
USA (Yost et al., 2004)	Inorganic arsenic levels from Schoof et al. (1999) 2 days of records, 1994–1998 CSFII, 1998 Supplemental Children's Survey	0.18 1–6 years	0.36 1–6 years 97.5th percentile	FARE program probabilistic model, foods consumed converted to raw commodity level, includes water used in cooking (0.8 mg/kg) but not drinking-water Assumes 17.3 kg child
USA (Xue et al., 2010)	TDS analytical values for total arsenic used from 1991–2004, with conversion factors from Schoof et al. (1999) Food consumption NHANES 2003–2004 1-day data	0.23 food <1 year 0.014 water 0.10 food 1–2 years 0.03 water 0.08 food 3–5 years 0.04 water	*95th percentile* 0.53 food <1 year 0.05 water 0.29 food 1–2 years 0.15 water 0.21 food 3–5 years 0.15 water	SHEDS – Dietary model, probabilistic model, included food and water consumption (recipes used to convert food to raw agricultural commodities) Assumed all arsenic in water inorganic

CSFII, Continuing Survey of Food Intakes by Individuals; INRAN, National Research Institute for Food and Nutrition, Italy; NDNS, National Diet and Nutrition Survey; TDS, total diet study

Table 22. Estimated dietary exposure to inorganic arsenic using model diets or total diet approach

Country (reference)	Data source	Mean[a] dietary exposure (µg/kg bw per day)	90th-percentile dietary exposure (µg/kg bw per day)	Comments
Belgium (Baeyens et al., 2009)	Arsenic data for seafood collected in 1997–1998, 300 samples, other values from Leblanc et al. (2005)	0.10 whole population	0.16 whole population	Assumed 60 kg bw for whole population, consumption of fish and seafood commodities, fruit and soft drinks only Measured inorganic arsenic values used for seafood, assumed 50% total arsenic was inorganic for fruit and soft drinks
China (China, 2010)	Total diet study 2007, 12 provinces, diets for males 18–45 years of age	0.43 overall mean 0.24–0.76 across provinces		63 kg bw reported for adult males
China, Province of Taiwan (Schoof et al., 1998)		0.909 mean 0.273 minimum 3.836 maximum		Includes consumption of rice and yams only (225 g wet weight of rice per day, 500 g wet weight of yams per day) Assumed 55 kg body weight to convert to per kg bw
United Kingdom (COT, 2008)	Total diet study	0.02 LB 0.12 UB		Assumed 60 kg bw Tap water not included
USA/Canada (Yost, Schoof & Aucoin, 1998)	Total diet study USA Total diet study Canada	0.2 adults 0.116 F 20–39 years 0.181 M 20–39 years		Conversion factors applied to food groups (Table 15) Assumed 70 kg bw to convert to per kg bw

Table 22 (contd)

Country (reference)	Data source	Mean[a] dietary exposure (μg/kg bw per day)	90th-percentile dietary exposure (μg/kg bw per day)	Comments
Various (Uneyama et al., 2007; Matsuda & Watanabe, 2008)	Total diet study, conversion ratios applied (Table 15)	0.12 United Kingdom 0.29 Canada 0.26 Spain 0.56 Japan		Assumed 60 kg bw for all populations
Various (Meharg et al., 2009)	Per capita rice consumption from FAO production and processing data for 2004 for 10 countries, rice analysis	0.60 median Bangladesh 0.40 median China 0.19 median India 0.02 median Italy 0.035 median USA		Assumed 60 kg bw Inorganic arsenic content derived by regression equation from median total arsenic content, assuming polished rice is 66.7% by weight husked rice (not calculated for all countries)

F, female; FAO, Food and Agriculture Organization of the United Nations; LB, lower bound; M, male; UB, upper bound

[a] Unless otherwise stated.

Table 23. Estimates of dietary exposure to inorganic arsenic for infants up to 1 month of age and young children using model diets or total diet approach

Country (reference)	Data source	Mean dietary exposure (µg/kg bw per day)	95th-percentile dietary exposure (µg/kg bw per day)	Comments
United Kingdom (Meharg et al., 2008)	Analysis of infant rice	0.45 median inorganic arsenic level, 1 serving 0.21 median intake from water	0.74 inorganic arsenic level for 1 serving	Assumed 9.25 kg bw for 1-year-old, 1 serving of infant rice = 20 g Assumed 1 litre of water consumed
USA/Canada (Yost, Schoof & Aucoin, 1998)	Total diet study USA Total diet study Canada	1.19 infant 0.94 toddler 0.32 child 1–4 years		Conversion factors applied to food groups (Table 15) Body weights of 7 kg, 10 kg, 15 kg for infant, toddler, 1- to 4-year-old child, respectively (Egan, Bolger & Carrington, 2007)

7.2.4 Contributions to inorganic arsenic dietary exposure

Uneyama et al. (2007) estimated inorganic arsenic dietary exposure from total arsenic exposures reported from total diet studies for four countries. Major contributors to inorganic dietary exposure were reported, assuming the conversion ratios given in Table 15 and dietary exposures in Table 19. The major food groups contributing to inorganic arsenic dietary exposure differed by country: for the United Kingdom, fish was the major contributor (36.7%), followed by beverages (14.3%), bread (12%) and other cereals (12%); for Canada, bakery goods and cereals were the major contributor (31.3%), followed by meat and poultry (21.6%), beverages (12.2%) and milk and dairy products (9.7%); for Spain, fish was the major contributor (66.1%), followed by milk (6.3%), meat (6.3%), bread (5.3%), potatoes (5.3%), vegetables (5.3%) and fruits (5.3%); for Japan, rice was the major contributor (47.4%), followed by vegetables and seaweed (29.9%), potatoes and cereals (9.3%) and seafood (8.1%).

In a case–control study of bladder cancer of 440 elderly individuals in an area in the USA where 8% of the population was exposed to arsenic in water at levels above 10 µg/l (south-east Michigan), the major contributors to inorganic arsenic dietary exposure were determined by a Monte Carlo analysis of eight metrics that included potential exposure from water, food and cigarettes (Meliker et al., 2006). Results indicated that arsenic in home drinking-water accounted for 55.1% variance and food 37.3% variance, with rice being the largest contributor. In the upper decile

of inorganic arsenic exposure, consumption of plain water and beverages made with water at home and ingestion of arsenic in water at work also contributed to exposure estimates, although water used for cooking and arsenic exposure from cigarettes only minimally altered the inorganic arsenic exposure estimates (95th percentile of inorganic arsenic exposure ranged from 11 to 24 µg/day or from 0.16 to 0.34 µg/kg bw per day, assuming an average body weight of 70 kg). The influence of inorganic arsenic levels in the drinking-water was shown by further analysis of the western area of the USA with arsenic levels in water higher than those in Michigan, where 71% variance was attributed to home drinking-water; and the north-eastern area of the USA, with arsenic levels in water lower than those in Michigan, where 30% variance was attributed to home drinking-water and 57% from food, mainly rice (Meliker et al., 2006).

In a more recent probabilistic analysis for the population of the USA, Xue et al. (2010) reported major food contributors to inorganic arsenic dietary exposure to be vegetables (24%), fruit and fruit juices (18%), rice (17%), beer and wine (12%) and flour, corn and wheat (11%); although water was included, it was not a major contibutor (Xue et al., 2010).

In a duplicate diet study in a rural area of Bangladesh, 90% variance in total arsenic dietary exposures was explained by the inorganic fraction of the diet, with tube well drinking-water concentrations contributing most to the variance; 60% of tube wells contained water with arsenic concentrations below the WHO guideline value of 0.01 mg/l, and 70% were below the Bangladesh water standard of 0.05 mg/l (Kile et al., 2007b).

8. DOSE–RESPONSE ANALYSIS AND ESTIMATION OF CARCINOGENIC RISK

8.1 Identification of key data for risk assessment

8.1.1 Pivotal data from biochemical and toxicological studies

Most studies in experimental animals have not shown increased tumour incidences following chronic oral exposure to inorganic arsenic, and it is considered that experimental animals do not provide a good model for the carcinogenicity of arsenic. Maternal oral exposure to arsenite has resulted in tumours in the offspring. The studies in which increased tumour incidence has been reported were generally designed for mechanistic research. In contrast, studies conducted according to standardized protocols for bioassays used for regulatory purposes were frequently negative. Taking into account the lack of a good animal model for carcinogenicity of arsenic compounds and the large number of data available from epidemiological studies, the Committee did not consider the data from experimental animals appropriate for the dose–response analysis.

Inorganic arsenic compounds have also shown reproductive and neuro-behavioural effects and lesser evidence of immunotoxicity, nephrotoxicity and cardiovascular effects. Although these data provide support for the plausibility of

associations reported in epidemiological studies, they are not pivotal for the dose–response analysis.

8.1.2 Pivotal data from human clinical/epidemiological studies

The main adverse effects reported to be associated with long-term ingestion of inorganic arsenic in humans are cancer, skin lesions, developmental effects, cardiovascular disease, neurotoxicity and diabetes. Of these, the greatest strength of evidence for a causal association is for cancers of the skin, urinary tract and lung and for skin lesions (hyperkeratosis, hyperpigmentation and hypopigmentation) observed in studies in which inorganic arsenic exposure was relatively high due to high levels of inorganic arsenic in drinking-water (e.g. ≥100 µg/l). The nutritional status of exposed populations has been observed to influence cancer risk. Thus, compromised nutrition (e.g. low protein intake) is also likely to be associated with significantly higher risk in these populations (USEPA, 2007; EFSA, 2009). For this report, studies were preferred that included documentation of relatively high concentrations of inorganic arsenic in drinking-water (e.g. >300 µg/l) and also relatively low concentrations (e.g. <100 µg/l) in order to avoid extrapolation below the observed range in the dose–response modelling. Pivotal studies were identified from epidemiological studies reporting a positive association with inorganic arsenic exposure and those adverse effects with the greatest strength of evidence for a causal association, as described in the following section. The relevant populations were located in Bangladesh and north-eastern Taiwan, China (see section 8.2.1).

For bladder cancer, at low levels of exposure to arsenic in drinking-water, smoking appears to be a consistent effect modifier: for those studies in which smoking behaviour was documented, smokers were observed to have a significantly increased risk of bladder cancer compared with non-smokers at a similar level of exposure.

A prospective cohort study in north-eastern Taiwan, China, was selected as a pivotal study for urinary cancer (Chen et al., 2010a). In total, 8086 subjects aged 40 years and older were recruited into the study, with “12 years” of follow-up. Arsenic concentrations in drinking-water were available for 6888 of these subjects. An advantage of the prospective cohort study design is that the cohort is classified in relation to exposure before disease develops, thereby reducing the likelihood of exposure misclassification. Standardized incidence ratios can also be estimated from this study design, unlike for the case–control design, which yields only OR estimates.

Cited bladder cancer case–control studies in which “never smoked” and “ever smoked” subjects were analysed separately are Bates, Smith & Cantor (1995), Kurttio et al. (1999) and Karagas et al. (2004) (Table 4). Karagas et al. (2004) found that among smokers, an elevated OR for bladder cancer was observed for toenail arsenic levels above 0.330 µg/g compared with below 0.06 µg/g. Among never smokers, there was no association between toenail arsenic and bladder cancer risk. A maximum likelihood estimate change point of 0.326 µg/g (95% CI 0.121–0.466) toenail arsenic was observed, which equates to approximately 50 µg/l in drinking-water. Owing to uncertainties regarding the precise relationship between toenail

arsenic and arsenic exposure in food and water, as described below, these studies were not selected for dose–response modelling.

A recent prospective cohort study of lung cancer involving 6888 participants 40 years of age and older with measured arsenic concentrations in drinking-water and "11 years" of follow-up in north-eastern Taiwan, China, was selected as a pivotal study (Chen et al., 2010b). A significant dose–response trend of lung cancer risk was associated with increasing arsenic drinking-water concentration. Advantages of this prospective cohort study design are described above. Smoking 25 pack-years or more and consuming well water with an arsenic level of 100 µg/l or higher yielded an RR of 6.97 (95% CI 3.4–14.3) for lung cancer, with no significant interaction between smoking and arsenic concentration. There was no significant association in non-smokers. The Committee noted that the papers of Chen et al. (2010a,b) related to the same cohort with the same follow-up time, which was an average of 11.5 years.

For the skin cancer end-point, studies with arsenic concentrations of 100 µg/l and below in drinking-water are shown in Table 3. Three studies that reported significant increases in skin cancer related to low-level arsenic exposure in drinking-water used toenail arsenic as a biomarker of exposure (Karagas et al., 2001b; Karagas, Stukel & Tosteson, 2002; Beane Freeman et al., 2004). Although toenail arsenic is deemed qualitatively useful to assess total arsenic exposure over the previous few months, uncertainty in relating this quantitatively to total exposure to inorganic arsenic in food and drinking-water precludes using the results from these studies in the evaluation of dietary exposure to arsenic. A large cohort study in Denmark that examined skin cancer effects found no significant effects with drinking-water arsenic concentrations up to 25 µg/l and thus was not useful for dose–response modelling.

The studies of Rahman et al. (2006) and Ahsan et al. (2006) were selected for dose–response modelling of skin lesions (hyperkeratosis, hyperpigmentation, hypopigmentation) characteristic of arsenic exposure. The foregoing studies established uniform diagnostic criteria to define arsenic-related skin lesions. A recent study conducted in Inner Mongolia, China, reported a significant increase in arsenic-induced skin lesions (Xia et al., 2009); however, concise diagnostic criteria for identification of skin lesions were not described, and therefore this study was not preferred for dose–response modelling.

The concentration of inorganic arsenic in drinking-water was used as the exposure metric in these studies; total dietary exposure to inorganic arsenic was not assessed. This approach does not allow for exposure to inorganic arsenic present in food, which in some populations exceeded that consumed in drinking-water, particularly where the concentration of inorganic arsenic in drinking-water was low. In order to provide an opinion on the risks to health related to the presence of inorganic arsenic in foodstuffs, it was necessary to make assumptions about the total dietary exposure to inorganic arsenic for the populations in which the respective health end-points were studied. Underestimating the total dietary exposure in the study populations will lead to an overestimation of the risk at an estimated exposure in other populations. Similarly, overestimating the total dietary exposure in the study

populations will lead to an underestimation of the risk at an estimated exposure in other populations.

Estimates of the total dietary exposure to inorganic arsenic for the different regions considered varied and are subject to a number of limitations that differ between studies—for example, small numbers of individuals surveyed for consumption habits, analysis of selected foods only and measurement of total arsenic rather than inorganic arsenic. In addition, foods that absorb water during cooking can absorb considerable amounts of arsenic if cooked in water containing relatively high amounts of arsenic (see section 6). This is particularly important for rice and therefore also needs to be taken into account in considering total dietary exposure to inorganic arsenic if foods have been analysed dry rather than as consumed.

The USEPA Science Advisory Board recommended that a range of values from at least 50 µg/day to as high as 200 µg/day should be used in a sensitivity analysis for the Asian study populations (USEPA, 2007). From the available information on occurrence and exposure, the Committee agreed that the lower end of this range was appropriate for the study populations in Bangladesh and north-eastern Taiwan, China, but noted that even higher arsenic exposure from rice should be considered for Bangladesh. To assess an appropriate upper level for modelling for the Bangladeshi population, information on food consumption and known total arsenic levels were used to estimate potential dietary exposures. Reported rice consumption for an adult male in South Asia varies from 400 to 650 g dry weight per day in Bangladesh (Watanabe et al., 2004; Williams et al., 2005; Rahman et al., 2008). Zavala & Duxbury (2008) analysed data sets (total of 887 samples) for total arsenic in rice produced in Bangladesh. The 75th-percentile arsenic concentrations for the two Bangladeshi data sets for a geographically structured national survey and data from five upazilas or subdistricts (~100 samples each from four areas and 40 from a fifth, where four of the five were known to have arsenic-contaminated irrigation water) were 0.3 mg/kg and 0.41 mg/kg, respectively. The highest reported total arsenic level was 1.08 mg/kg. A person consuming 500 g rice daily containing a total arsenic concentration of 1 mg/kg would ingest 500 µg of total arsenic per day. Inorganic arsenic has been found to range from 68% to 85% of total arsenic content, with the lower proportion of inorganic arsenic found at higher total arsenic levels—85% at 0.1 mg/kg and 68% at 1 mg/kg; the remaining arsenic in rice is predominantly DMA^V (Zavala et al., 2008). If inorganic arsenic is assumed to be 70% of the total arsenic concentration, the inorganic arsenic exposure from consuming 500 g rice containing total arsenic at 1 mg/kg would be 350 µg/day. This is an upper estimate compared with that reported by Watanabe et al. (2004) of 52 µg/day and 90 µg/day from rice for females and males, respectively. Inorganic arsenic is found in foods other than rice (Watanabe et al., 2004). Watanabe et al. (2004) also reported total arsenic dietary exposure from other foods for adult females and males in Bangladesh of, respectively, 46 and 63 µg/day from wheat, 22 and 60 µg/day from fish and <1 µg/day from potato. Taking into account that the arsenic in fish is predominantly organic, but there would be a higher proportion of inorganic arsenic in wheat, 400 µg of inorganic arsenic per day was taken as a reasonable estimate for high dietary exposure to inorganic arsenic from

rice and vegetables for adult males in the Bangladeshi study populations before considering the influence of cooking water. Thus, for Bangladesh, a range of 50–400 µg of inorganic arsenic per day from food was selected for modelling purposes.

For north-eastern Taiwan, China, the Committee considered that the range of 50–200 µg of inorganic arsenic per day from food for modelling purposes was consistent with the data available.

For modelling purposes, it is important to estimate the range of water consumed as drinking-water and via food due to use in food preparation. As discussed in EFSA (2009), estimated values for daily water intake were 1.7–3.5 litres direct consumption and 1 or 1.6 litres indirect consumption through use in cooking (e.g. preparation of food such as rice, sweet potato, yam, bread) (NRC, 1999, 2001; Watanabe et al., 2004; Kile et al., 2007b; Signes-Pastor et al., 2008; Pal et al., 2009). This provides a combined range of about 3–5 litres of water per day and was considered appropriate for the Bangladeshi study populations. For north-eastern Taiwan, China, rice forms a lesser proportion of the diet, and hence less water is likely to be used in cooking rice. It was noted that some would be used in the preparation of yams. Therefore, the Committtee identified a lower range of 2–4 litres of water consumption per day for this population.

From within the above ranges, the Committee identified average exposures of 140 and 75 µg of inorganic arsenic per day from food, respectively, for Bangladesh and north-eastern Taiwan, China, together with 4 and 3 litres direct plus indirect water consumption, respectively. These values were used in extrapolating from concentration in water to total dietary exposure to inorganic arsenic in the dose–response modelling. From the available data, an average body weight of 55 kg was identified for the epidemiological study populations.

8.2 General modelling considerations

8.2.1 Dose–response modelling and BMD calculations

In the dose–response analysis using the USEPA benchmark dose (BMD) software (BMDS version 2.1.1), the nine different dichotomous models were fitted to the adjusted data. Those resulting in acceptable fits based on statistical considerations were selected to derive BMD and lower limit on the BMD (BMDL) values for a benchmark response (BMR) at the low end of the observed range of the data. Doses in units of milligrams per person per day were initially used for deriving BMDs. These were then converted to milligrams per kilogram body weight per day by dividing by a body weight of 55 kg per person.

8.2.2 Selection of data

For modelling of urinary and lung cancer dose–response, the studies of Chen et al. (2010a,b) were preferred, respectively, as these are prospective studies with a reasonable follow-up time of an average of 11.5 years that have documented exposure categories below 100 µg/l and assessed smoking behaviour and water use history.

In order to utilize the adjustment made for other variables (e.g. smoking) in the original analyses in the studies of lung cancer (Chen et al., 2010b) and urinary tract cancer (Chen et al., 2010a) in north-eastern Taiwan, China, and the Ahsan et al. (2006) study of skin lesions in Bangladesh, adjusted cases were calculated for each exposure group (i.e. other than the referent group) from RRs. This two-step process involved calculating case frequency by multiplying the rate in the referent group by the relative risk and then estimating the number of adjusted cases by multiplying the number of subjects by the case frequency. For the two Chen et al. (2010a,b) studies, the resulting adjustment was small relative to the reported cases (see Tables 24 and 25).

The study of urinary tract cancer (Chen et al., 2010a) showed a significantly increased RR trend with increasing arsenic concentration in water when adjusted for sex, age and smoking; for exposures above 100 µg/l, RRs were more than 5, whereas the risk was elevated but not significant for exposures below 100 µg/l. Table 24 shows the data used in dose–response modelling.

Table 24. Association of urinary cancer in relation to person-years of observation with arsenic exposure in an arseniasis-endemic area in north-eastern Taiwan, China

Inorganic arsenic in water		Inorganic arsenic total dietary exposure[b]		Cohort incidence[c]	RR	*N*	Adjusted cases[d]
Category range (µg/l)	Central estimate[a] (µg/l)	µg/person per day	µg/kg bw per day				
<10	5	90	1.6	0.002 2	1	2288	5
10–49.9	30	165	3.0	0.003 6	1.66	2093	8
50–99.9	75	300	5.5	0.005 3	2.42	907	5
100–299.9	200	675	12.3	0.009 05	4.13	909	8
≥300	450	1425	25.9	0.017 0	7.8	691	12

[a] Point estimate of the range of inorganic arsenic in drinking-water.
[b] Central estimate, assuming consumption of 3 litres of water per day, including that used in cooking, and 75 µg of inorganic arsenic in food per day and body weight of 55 kg.
[c] Referent group (<10 µg/l) is actual case rate per person; other rates are calculated from RRs.
[d] Referent group is actual cases. Other case estimates are obtained by multiplying group size by incidence.
Source: Chen et al. (2010a).

The study of lung cancer (Chen et al., 2010b) found a significant dose–response trend ($P = 0.001$) of lung cancer risk associated with increasing arsenic concentration. Increase in RR was nonsignificant below 100 µg/l, but a significant increase in RR was shown for exposures above 100 µg/l. Table 25 shows the data used in dose–response modelling.

Table 25. Association of lung cancer cases in relation to total population studied with arsenic exposure in an arseniasis-endemic area in north-eastern Taiwan, China

Inorganic arsenic in water		Inorganic arsenic total dietary exposure[b]		Cohort incidence[c]	RR	*N*	Adjusted cases[d]
Category range (µg/l)	Central estimate[a] (µg/l)	µg/person per day	µg/kg bw per day				
<10	5	90	1.6	0.021	1	2288	48
10–49.9	30	165	3.0	0.023	1.1	2093	48
50–99.9	75	300	5.5	0.021	0.99	907	19
100–299.9	200	675	12.3	0.032	1.54	909	29
≥300	450	1425	25.9	0.047	2.25	691	33

[a] Point estimate of the range of inorganic arsenic in drinking-water.
[b] Central estimate, assuming consumption of 3 litres of water per day, including that used in cooking, and 75 µg of inorganic arsenic in food per day and body weight of 55 kg.
[c] Referent group (<10 µg/l) is actual case rate per person; other rates are calculated from RRs.
[d] Referent group is actual cases. Other case estimates are obtained by multiplying group size by incidence.
Source: Chen et al. (2010b).

For skin lesions, data from two pivotal studies in Bangladesh were modelled. In a cross-sectional study reported by Ahsan et al. (2006), dose-dependent effects were observed with increased inorganic arsenic exposure. Adjusted cases were calculated from adjusted relative risks in the same manner as for the two Chen et al. (2010a,b) studies. However, as the total number of cases in the Ahsan et al. (2006) study was increased by about 15% by this adjustment, the case estimates were further adjusted by normalizing relative to the total number of reported cases so that the overall case frequency in the cohort was the same (i.e. the number of cases in the referent group was adjusted as well). The data set from this study is listed in Table 26.

Rahman et al. (2006) reported a case–control study also conducted in Bangladesh. In this study, the referents were randomly selected in the study areas. The OR for skin lesions, in both males and females, increased along with arsenic exposure. The results from a case–control study cannot be used for dose–response modelling because the ratios are not based on population rates. However, information was provided in the paper that allowed estimation of relative prevalence rates for the area from which the study cohort was drawn. Two assumptions played a role in this estimation. First, it was assumed that the distribution of arsenic exposures in the rest of the population was proportional (n = 164 000) to those in the study cohort (n = 2334). Second, it was assumed that there were additional cases in the rest of the population, with a prevalence determined by the number of cases detected in the individuals who were originally selected for the control group (6 of 1830).

Table 26. Association of skin lesion cases and controls with arsenic exposure in Bangladesh

Inorganic arsenic in water		Inorganic arsenic total dietary exposure[b]		Prevalence[c]	RR	*N*	Actual cases	Adjusted cases[d]
Category range (µg/l)	Category average estimate [a] (µg/l)	µg/person per day	µg/kg bw per day					
0.1–8	1.8	147	2.7	0.025	1	2259	57	48
8.1–40	23	232	4.2	0.048	1.91	2122	90	86
40.1–91	62	388	7.1	0.076	3.03	2202	144	141
91.1–175	125	640	11.6	0.094	3.71	2185	162	171
175.1–864	255	1160	21.1	0.136	5.39	2183	242	249

[a] Median of the concentration range of inorganic arsenic in drinking-water.
[b] Central estimate, assuming consumption of 4 litres of water per day, including that used in cooking, and 140 µg of inorganic arsenic in food per day and body weight of 55 kg.
[c] Referent group (<0.1–8 µg/l) is actual case rate per person; other rates are calculated from RRs.
[d] Adjusted cases for groups other than the referent group were estimated by first multiplying group size by prevalence and then normalizing all groups (including the referent group) to the total unadjusted cohort prevalence.
Source: Ahsan et al. (2006).

The data and estimated cases from this study are listed in Table 27.

8.3 Benchmark dose estimates

8.3.1 Chen et al. (2010b), lung cancer

The data (see Table 25) were fit with all nine dichotomous models provided by the BMDS modelling software. The log-probit model was fit in both constrained (*c* parameter > 1) and unconstrained forms. In the former case, the log-probit model provided a relatively poor fit and relatively high BMD and BMDL values (see Table 28 and Figure 2). Although the fit was improved by removing the constraint, the BMDL values were over 100-fold lower than for any of the other models and were outside the dose range from the study (see Table 28 and Figure 3). The Committee therefore found it preferable to exclude both forms of the log-probit model. The BMR selected at the low end of the observed data range was 0.5% increased incidence. The lowest $BMDL_{0.5}$ of 3 µg/kg bw per day was generated by the quantal-linear model (along with several other equivalent models).

Plots for the probit model, which provided the best fit, and the quantal-linear model, which provided the lowest BMDL (along with several other equivalent models), are shown in Figures 4 and 5, respectively.

Table 27. Association of skin lesion cases and controls with arsenic exposure in Bangladesh

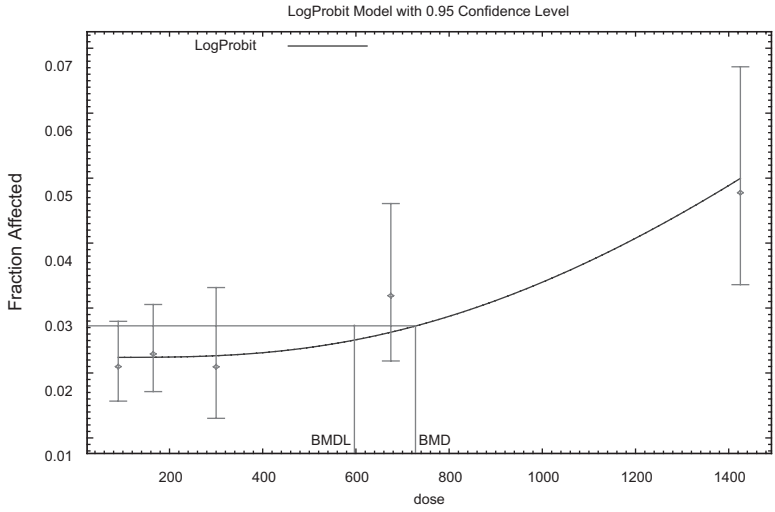
Inorganic arsenic in water		Inorganic arsenic total dietary exposure scenario[b]		Original cohort		Estimates for total population	
Category range (µg/l)	Average estimate[a] (µg/l)	µg/person per day	µg/kg bw per day	Cases	Controls	Cases[c]	Estimated group size[c]
<10	5	160	2.9	25	230	52	20 902
10–49	30	260	4.7	53	261	110	23 770
50–149	100	540	9.8	124	551	256	50 205
150–299	225	1040	18.9	194	551	401	50 350
≥300	450	1940	35.3	108	237	223	21 708

[a] Midpoint except for the highest category.
[b] Central estimate, assuming consumption of 4 litres of water per day, including that used in cooking, and 140 µg of inorganic arsenic in food per day and body weight of 55 kg.
[c] Number of additional cases and subjects estimated for the population from which the cohort was drawn; see text for additional explanation.
Source: Rahman et al. (2006).

Table 28. $BMD_{0.5}$ for lung cancer based on Chen et al. (2010b)

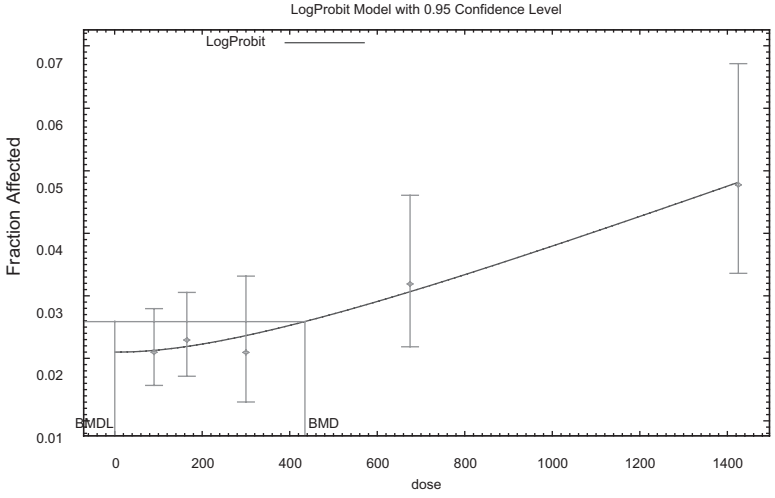
Model name	*P*-value	$BMD_{0.5}$ (µg/person per day)	$BMDL_{0.5}$ (µg/person per day)	$BMD_{0.5}$ (µg/kg bw per day)	$BMDL_{0.5}$ (µg/kg bw per day)
Gamma	0.79	402	167	7.3	3.0
Logistic	0.92	351	273	6.4	5.0
Log-logistic	0.79	400	165	7.3	3.0
Log-probit (constrained)	0.67	728	597	13.2	10.8
Log-probit (unconstrained)	0.80	435	0.4	7.9	0.006
Multistage	0.78	357	167	6.5	3.0
Multistage cancer	0.89	250	165	4.5	3.0
Probit	0.92	336	257	6.1	4.7
Weibull	0.79	399	167	7.2	3.0
Quantal-linear	0.89	250	165	4.5	3.0

Figure 2. Log-probit model with constraint



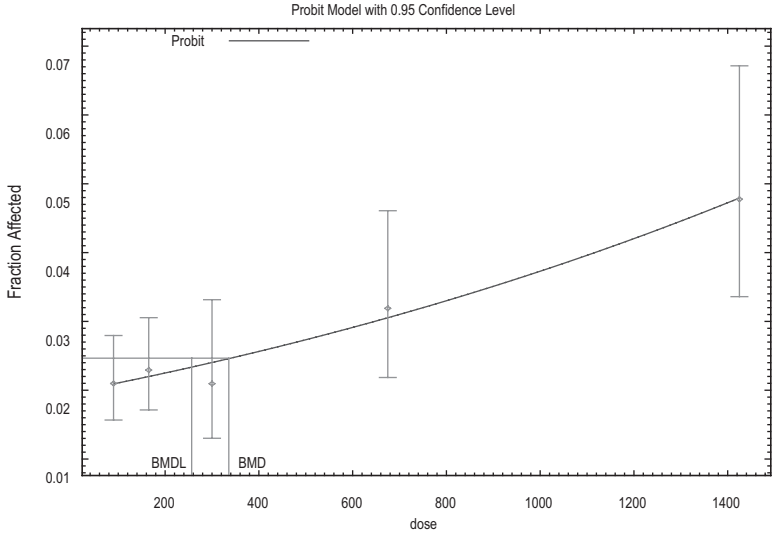
Notes: x-axis: exposure in µg/person per day; y-axis: cohort incidence. The line is the central estimate resulting from the fit of the model to the data. The vertical bars are the confidence intervals around the data.

Figure 3. Log-probit model without constraint



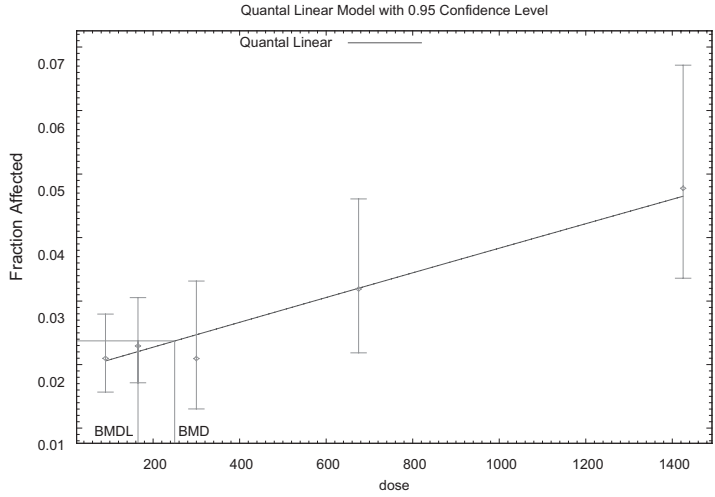
Notes: x-axis: exposure in µg/person per day; y-axis: cohort incidence. The line is the central estimate resulting from the fit of the model to the data. The vertical bars are the confidence intervals around the data.

Figure 4. Probit model for lung cancer based on Chen et al. (2010b)



Notes: x-axis: exposure in µg/person per day; y-axis: cohort incidence. The line is the central estimate resulting from the fit of the model to the data. The vertical bars are the confidence intervals around the data.

Figure 5. Quantal-linear model for lung cancer based on Chen et al. (2010b)



Notes: x-axis: exposure in µg/person per day; y-axis: cohort incidence. The line is the central estimate resulting from the fit of the model to the data. The vertical bars are the confidence intervals around the data.

8.3.2 Chen et al. (2010b), lung cancer, sensitivity analyses

Two sensitivity analyses using four different models (probit, logistic, log-logistic and quantal-linear) were performed to evaluate the impact of some of the dosimetry assumptions on the $BMD_{0.5}$ calculation. For this analysis, four different models were examined. The first analysis examined the impact of the estimates of dietary arsenic concentrations and water intakes. The second examined the impact of assuming that the risk is driven entirely by water intake. The latter analysis would be more accurate if the dietary exposure is nearly proportional to arsenic well water concentrations and allows calculation of a benchmark concentration (BMC). $BMD_{0.5}$ estimates are presented in Table 29 and Table 30.

Table 29. $BMD_{0.5}$ for lung cancer based on Chen et al. (2010b) with varying dietary arsenic exposures and water consumptions

Model name	Arsenic exposure in diet (µg/day)	Water consumption (litres/day)	*P*-value	$BMD_{0.5}$ (µg/person per day)	$BMDL_{0.5}$ (µg/person per day)	$BMD_{0.5}$ (µg/kg bw per day)	$BMDL_{0.5}$ (µg/kg bw per day)
Quantal-linear	200	4	0.89	333	220	6.1	4.0
Logistic	200	4	0.92	489	385	8.9	7.0
Probit	200	4	0.92	466	361	8.5	6.6
Log-logistic	200	4	0.79	580	219	10.5	4.0
Quantal-linear	50	4	0.89	333	220	6.1	4.0
Logistic	50	4	0.92	459	354	8.3	6.4
Probit	50	4	0.92	439	333	8.0	6.1
Log-logistic	50	4	0.79	510	220	9.3	4.0
Quantal-linear	200	2	0.89	167	110	3.0	2.0
Logistic	200	2	0.92	266	215	4.8	3.9
Probit	200	2	0.92	252	201	4.6	3.6
Log-logistic	200	2	0.78	337	109	6.1	2.0
Quantal-linear	50	2	0.89	167	110	3.0	2.0
Logistic	50	2	0.92	234	182	4.3	3.3
Probit	50	2	0.92	224	171	4.1	3.1
Log-logistic	50	2	0.79	267	110	4.9	2.0

The scenarios with respect to exposure to inorganic arsenic from food and volume of drinking-water consumed resulted in a range of 2.0–7.0 µg/kg bw per day for the $BMDL_{0.5}$, with the volume of drinking-water having the larger impact.

Table 30. $BMD_{0.5}$ and $BMC_{0.5}$ for lung cancer based on Chen et al. (2010b) and drinking-water alone

Model name	Arsenic exposure in diet (µg/day)	Water consumption (litres/day)	*P*-value	$BMD_{0.5}$ (µg/person per day)	$BMDL_{0.5}$ (µg/person per day)	$BMC_{0.5}$ (µg/l)	$BMCL_{0.5}$ (µg/l)
Quantal-linear	0	3	0.89	250	165	83	55
Logistic	0	3	0.92	337	258	112	86
Probit	0	3	0.92	323	243	108	81
Log-logistic	0	3	0.79	363	165	121	55

While changes in drinking-water assumptions produced proportional changes in the $BMD_{0.5}$ estimates, the impacts of assumed dietary exposure were more variable and model dependent. For the quantal-linear model (and the other related models), the dietary exposure estimate had virtually no effect. For the other models, changing dietary exposure altered the $BMD_{0.5}$ and $BMDL_{0.5}$ estimates somewhat (20–25%), but the difference was considerably less than the proportional change in the assumed dietary exposures (a factor of 4). Focusing on drinking-water only, as reported in the epidemiological studies, resulted in a $BMCL_{0.5}$ of 55 µg/l.

8.3.3 Chen et al. (2010a), urinary tract cancer

The data (see Table 24) were fit with all nine dichotomous models provided by the BMDS modelling software. The log-probit model provided a relatively poor fit and relatively high BMD and BMDL values (see Table 31). The log-probit model was therefore also excluded from the analysis of the Chen et al. (2010a) urinary tract data set. The BMR selected at the low end of the observed data range was 0.5% increased incidence. The lowest $BMDL_{0.5}$ of 5.2 µg/kg bw per day was again generated by the quantal-linear model (and other equivalent models).

A plot for the log-logistic model, which provided the best fit together with the lowest BMDL, is shown in Figure 6.

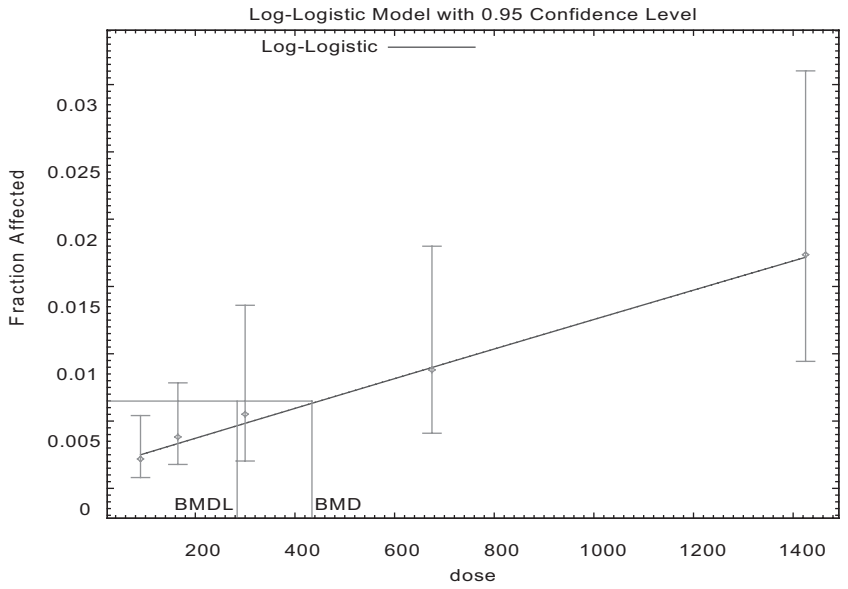
8.3.4 Ahsan et al. (2006), skin lesions

The data (see Table 26) were fit with all nine dichotomous models provided by the BMDS modelling software. None of the models provided a good fit, as judged by the *P*-value. Because it provided a much better fit than any of the other models, the unconstrained log-probit model was included (see Table 32). The BMR selected at the low end of the observed data range was 5% increased prevalence. The log-probit model, which also provided the lowest $BMDL_5$ (5.4 µg/kg bw per day), is illustrated in Figure 7. Most of the other models, with the possible exception of the log-logistic, are excluded on the basis of fit.

Table 31. $BMD_{0.5}$ for urinary cancer based on Chen et al. (2010a)

Model name	*P*-value	$BMD_{0.5}$ (µg/person per day)	$BMDL_{0.5}$ (µg/person per day)	$BMD_{0.5}$ (µg/kg bw per day)	$BMDL_{0.5}$ (µg/kg bw per day)
Gamma	0.96	436	286	7.9	5.2
Logistic	0.65	763	625	13.9	11.4
Log-logistic	0.96	434	284	7.9	5.2
Log-probit	0.26	923	753	16.8	13.7
Multistage	0.96	436	286	7.9	5.2
Multistage cancer	0.96	436	286	7.9	5.2
Probit	0.70	727	587	13.2	10.7
Weibull	0.96	436	286	7.9	5.2
Quantal-linear	0.96	436	286	7.9	5.2

Figure 6. Log-logistic model for urinary tract cancer based on Chen et al. (2010a)

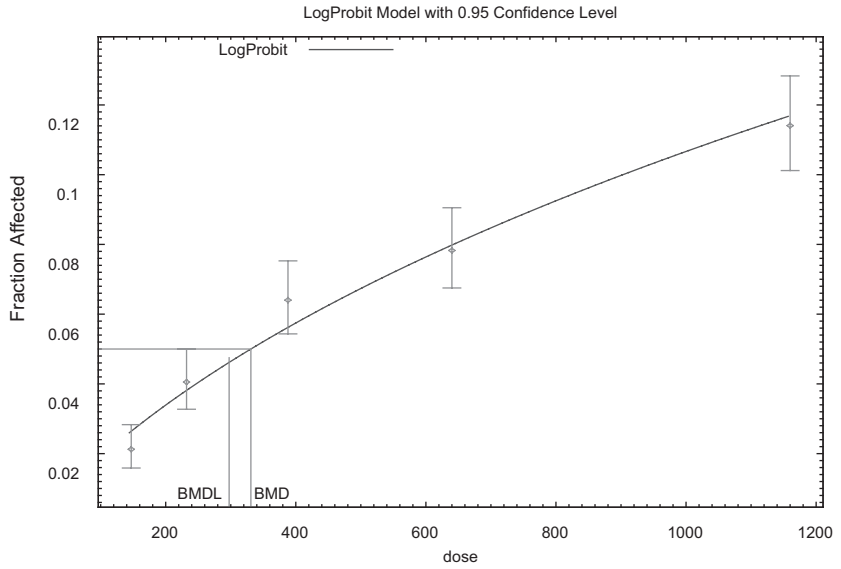


Notes: x-axis: exposure in µg/person per day; y-axis: cohort incidence. The line is the central estimate resulting from the fit of the model to the data. The vertical bars are the confidence intervals around the data.

Table 32. BMD_5 for skin lesions based on Ahsan et al. (2006)

Model name	*P*-value	BMD_5 (µg/person per day)	$BMDL_5$ (µg/person per day)	BMD_5 (µg/kg bw per day)	$BMDL_5$ (µg/kg bw per day)
Gamma	0.004	507	447	9.2	8.1
Logistic	0.000	779	729	14.2	13.3
Log-logistic	0.007	485	424	8.8	7.7
Log-probit	0.148	331	297	6.0	5.4
Multistage	0.004	507	447	9.2	8.1
Multistage cancer	0.004	507	447	9.2	8.1
Probit	0.000	746	695	13.6	12.6
Weibull	0.004	507	447	9.2	8.1
Quantal-linear	0.004	507	447	9.2	8.1

Figure 7. Log-probit model for Ahsan et al. (2006) skin lesion study



Notes: x-axis: exposure in µg/person per day; y-axis: prevalence. The line is the central estimate resulting from the fit of the model to the data. The vertical bars are the confidence intervals around the data.

8.3.5 *Ahsan et al. (2006), skin lesions, sensitivity analyses*

Two sensitivity analyses using three different models (log-probit, log-logistic and multistage cancer) were performed to evaluate the impact of some of the dosimetry assumptions on the BMD calculation. The first analysis examined the impact of the estimates of dietary arsenic exposures and water intakes. The second examined the impact of assuming that the risk is driven entirely by water intake. The latter analysis would be more accurate if the dietary exposure is nearly proportional to arsenic well water concentrations and allows calculation of a BMC. BMD_5 estimates are presented in Table 33 and Table 34.

The scenarios with respect to exposure to inorganic arsenic from food and volume of drinking-water consumed resulted in a range of 2.8–11.5 µg/kg bw per day for the $BMDL_5$, with the assumption for food having the larger impact. However, most of the models did not provide a good fit.

Focusing on drinking-water only, as reported in the epidemiological studies, the log-probit model gave the best fit and also the lowest $BMCL_5$, which was 47 µg/l.

Table 33. BMD_5 for skin lesions based on Ahsan et al. (2006) with varying dietary arsenic exposures and water consumptions

Model name	Arsenic exposure in diet (µg/day)	Water consumption (litres/day)	*P*-value	BMD_5 (µg/person per day)	$BMDL_5$ (µg/person per day)	BMD_5 (µg/kg bw per day)	$BMDL_5$ (µg/kg bw per day)
Log-probit	400	5	0.012	665	616	12.1	11.2
Log-logistic	400	5	0.004	671	622	12.2	11.3
Multistage cancer	400	5	0.009	671	631	12.2	11.5
Log-probit	50	5	0.773	248	213	4.5	3.9
Log-logistic	50	5	0.007	612	536	11.1	9.8
Multistage cancer	50	5	0.004	633	558	11.5	10.1
Log-probit	400	3	0.002	567	535	10.3	9.7
Log-logistic	400	3	0.001	571	536	10.4	9.7
Multistage cancer	400	3	0.000	528	496	9.6	9.0
Log-probit	50	3	0.568	178	156	3.2	2.8
Log-logistic	50	3	0.007	366	321	6.7	5.8
Multistage cancer	50	3	0.004	380	335	6.9	6.1

Table 34. BMD_5 and BMC_5 for skin lesions based on Ahsan et al. (2006) and drinking-water alone

Model name	Arsenic exposure in diet (µg/day)	Water consumption (litres/day)	*P*-value	BMD_5 (µg/person per day)	$BMDL_5$ (µg/person per day)	BMC_5 (µg/l)	$BMCL_5$ (µg/l)
Log-probit	0	4	0.6189	310	189	78	47
Log-logistic	0	4	0.0066	492	431	123	108
Multistage cancer	0	4	0.0038	507	447	127	112

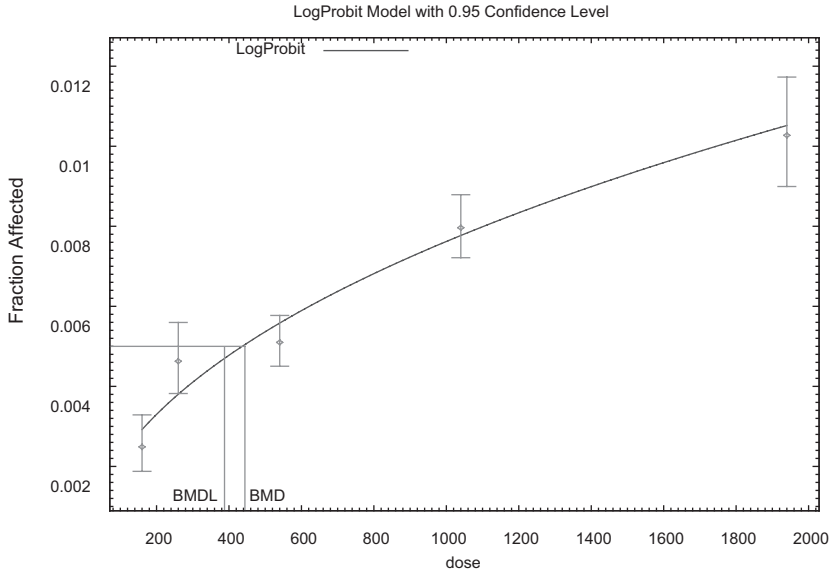
8.3.6 Rahman et al. (2006), skin lesions

The data (see Table 27) were fit with all nine dichotomous models provided by the BMDS modelling software. None of the models provided a good fit, as judged by the *P*-value. Because it provided a much better fit than any of the other models, the unconstrained log-probit model was included (see Table 35). The BMR selected at the low end of the observed data range was 0.5% increased prevalence. As it provided both the best fit and the lowest $BMDL_{0.5}$ (5.4 µg/kg bw per day), the log-probit model is illustrated in Figure 8. Most of the other models, with the possible exception of the log-logistic model, are excluded on the basis of fit.

Table 35. $BMD_{0.5}$ for skin lesions based on Rahman et al. (2006)

Model name	*P*-value	$BMD_{0.5}$ (µg/person per day)	$BMDL_{0.5}$ (µg/person per day)	$BMD_{0.5}$ (µg/kg bw per day)	$BMDL_{0.5}$ (µg/kg bw per day)
Gamma	0.0034	507	447	9.2	8.1
Logistic	0	779	729	14.2	13.3
Log-logistic	0.0035	485	424	8.8	7.7
Log-probit (unconstrained)	0.0442	331	297	6.0	5.4
Multistage	0.0034	507	447	9.2	8.1
Multistage cancer	0.0034	507	447	9.2	8.1
Probit	0	746	695	13.6	12.6
Weibull	0.0034	507	447	9.2	8.1
Quantal-linear	0.0034	507	447	9.2	8.1

Figure 8. Log-probit model for Rahman et al. (2006) skin lesion study



Notes: x-axis: exposure in µg/person per day; y-axis: prevalence. The line is the central estimate resulting from the fit of the model to the data. The vertical bars are the confidence intervals around the data.

9. COMMENTS

9.1 Absorption, distribution, metabolism and excretion

Absorption of arsenic depends on the chemical species and its solubility as well as the matrix in which it is present. Soluble arsenicals in water are highly bioavailable. Inorganic arsenic is rapidly cleared from blood both in humans and in most experimental animal species that have been tested; an exception is rats, in which arsenic binds to erythrocytes, delaying clearance. Inorganic arsenic is metabolized primarily by stepwise reduction of pentavalent arsenic (arsenate) to trivalent arsenic (arsenite) followed by oxidative addition of methyl groups, although alternative pathways have also been proposed that include methylated arsenical glutathione metabolites. Most ingested arsenic species are excreted via the kidney within a few days. Ingested inorganic arsenic is excreted as inorganic arsenate and arsenite and as the pentavalent methylated metabolites MMA^{V} and DMA^{V}, with lesser amounts of the trivalent methylated metabolites, MMA^{III}, DMA^{III} and thioarsenical metabolites. Whereas it has previously been assumed that methylation of inorganic arsenic was a detoxification route, it is not entirely clear whether or not this is correct, because, based on limited in vitro and in vivo data, MMA^{III} and DMA^{III} appear to be more toxic than inorganic arsenic and have high affinity for thiols and cellular proteins.

Major organic arsenicals present in fish when ingested undergo very little biotransformation and are excreted almost entirely unchanged. However, some organoarsenicals, such as arsenolipids present in cod liver and arsenosugars in mussels and algae, can be metabolized to DMA^{V} when ingested.

9.2 Toxicological data

Arsenic toxicity depends on the chemical form and its solubility and varies among animal species and with route of administration. Generally, trivalent arsenic is more toxic than the pentavalent forms. Oral administration of inorganic arsenicals to laboratory animals has a number of effects, including effects on the cardiovascular, respiratory, gastrointestinal, haematological, immune, reproductive and nervous systems. MMA^{V} administration to experimental animals has been shown to have effects on the gastrointestinal tract, kidney, thyroid and reproductive system, with the effect seen at the lowest doses being diarrhoea. DMA^{V} has effects on the urinary bladder, kidneys, thyroid and fetal development.

Studies in experimental animals conducted according to standard protocols have generally not shown increased tumour incidences following chronic oral exposure to inorganic arsenic. However, evidence of tumour promotion and co-carcinogenicity has been reported. In addition, studies involving administration of arsenite to pregnant mice in their drinking-water have shown evidence of transplacental carcinogenesis.

MMA^{V} has not shown evidence of carcinogenicity in 2-year cancer bioassays with doses equivalent to up to 100 mg/kg bw per day. DMA^{V} (administered in drinking-water at 50 mg/l) was carcinogenic in the urinary bladder of rats, but not mice. DMA^{V} is not genotoxic, and its carcinogenic mode of action is considered to involve cytotoxicity to the bladder epithelium and sustained increased cell proliferation; the rat is considered to be particularly sensitive to DMA^{V} because of slower elimination and possibly a greater potential for metabolism to DMA^{III} compared with other species. The NOAEL was equivalent to 0.73 mg/kg bw per day.

In its most recent evaluation, IARC concluded that there is sufficient evidence for carcinogenicity of inorganic arsenic compounds in experimental animals and sufficient evidence for carcinogenicity of DMA^{V} in experimental animals. Evidence from a wide range of studies has led to the conclusion that arsenic compounds do not react directly with DNA. There are a number of proposed mechanisms of carcinogenicity of inorganic arsenic, including oxidative damage, epigenetic effects and interference with DNA damage repair.

Because of a general lack of data on both exposure to and toxicity of organic arsenicals, the Committee further considered only inorganic arsenic for this report.

Taking into account the lack of a good animal model for carcinogenicity of inorganic arsenic compounds and the large number of data available from epidemiological studies, the Committee did not consider the data from experimental animals appropriate for the dose–response analysis.

9.3 Observations in humans

The main adverse effects reported to be associated with long-term ingestion of inorganic arsenic by humans are cancer, skin lesions, developmental effects, cardiovascular disease, neurotoxicity and diabetes.

The classification of arsenic as a carcinogen was originally based on evidence of skin cancers. Studies in Taiwan, China, and other regions where high exposures to arsenic in drinking-water occurred have confirmed the relationship. Significant associations between exposure to high levels of ingested arsenic in drinking-water and bladder cancer have been observed in ecological studies from Chile, Argentina and Taiwan, China, and cohort studies in Taiwan, China. Some of the studies showed an association only in smokers. In studies from Chile, Argentina and Taiwan, China, exposure to arsenic at high concentrations in drinking-water has been shown to be associated with lung cancer. Again, when smokers and non-smokers were compared, the associations were stronger in the smokers. Nutritional status of exposed populations has been observed to influence cancer risk. Thus, compromised nutrition (e.g. low protein intake) is likely to be associated with significantly higher risk. The evidence for an association with cancers at other sites, including prostate, liver and kidney, is less conclusive.

Epidemiological studies in different regions of the world have consistently demonstrated a strong association between long-term inorganic arsenic ingestion and skin lesions, typically in the form of hyperkeratosis, hyperpigmentation or hypopigmentation. Observations of skin lesions following low chronic exposure have suggested that these characteristic dermal changes are sensitive indications of the toxic effects of inorganic arsenic.

Available epidemiological studies indicate a positive relationship between high concentrations of inorganic arsenic in drinking-water and sensitive end-points for peripheral and central neurotoxicity. There is some evidence that exposure of children to inorganic arsenic in areas with elevated arsenic concentrations (>50 µg/l) in drinking-water produces effects on cognitive performance, but so far this is not conclusive.

The cardiovascular outcomes that have been associated with chronic exposure to arsenic through drinking-water include blackfoot disease, increased mortality or prevalence of coronary heart disease, peripheral arterial disease, myocardial infarction and stroke, and other cardiovascular end-points, such as increased blood pressure and prolonged QT interval of the electrocardiogram. The association between blackfoot disease and inorganic arsenic exposure has been confirmed by many studies, but blackfoot disease has been reported primarily in an area along the south-western coast of Taiwan, China, where arsenic contamination in well water is very high (170–880 µg/l). Except for blackfoot disease, the reported associations between inorganic arsenic exposure and cardiovascular disease prevalence/mortality and other cardiovascular end-points currently do not provide sufficient evidence of causality and are not considered pivotal for the assessment.

Studies conducted in Bangladesh and Taiwan, China, indicated an extra risk of diabetes among high-exposure populations. In addition, recent findings suggest

that in utero arsenic exposure impaired child thymic development and that enhanced morbidity and immunosuppression might occur. However, as a result of limitations in the studies, the relationship between arsenic exposure and these outcomes remains uncertain.

The Committee concluded that the greatest strength of evidence for a causal association between inorganic arsenic and adverse effects in humans is for cancers of the skin, urinary bladder and lung and skin lesions (hyperkeratosis, hyperpigmentation and hypopigmentation) observed in studies in which levels of arsenic in drinking-water were relatively high (e.g. ≥100 µg/l). For this evaluation, studies were preferred that included documentation of exposure from drinking-water both at higher concentrations (e.g. ≥300 µg/l) and also at relatively lower concentrations (e.g. <100 µg/l). This was in order to assess effects across a broad gradient of exposure and to avoid extrapolation below the observed range in the dose–response modelling. For skin cancer, three of the four most recent studies of low-level exposure utilized toenail arsenic as a biomarker of exposure; however, the relationship between toenail arsenic and total dietary exposure to inorganic arsenic remains uncertain. Further, as arsenic-related skin lesions may be a possible precursor to skin cancer and have been reported at lower concentrations of arsenic in drinking-water compared with skin cancer, the Committee considered the data for skin lesions to be a more sensitive adverse effect than skin cancer. Thus, pivotal data were identified from epidemiological studies reporting a positive association with arsenic exposure and these effects (i.e. cancers of the lung and urinary tract and skin lesions).

9.4 Analytical methods

The most common detection techniques for arsenic are ICP-MS, ICP-AES, HG-AAS and HG-AFS. ICP-AES is generally adequate for determination of total arsenic in foods, and its sensitivity can be improved by coupling to HG. ICP-MS has the highest sensitivity without derivatization. HG-AAS and HG-AFS have LODs in the microgram per kilogram range, which is adequate for all foods. For speciation with HG-based detection systems, some organoarsenic species require oxidation to species that form volatile arsines prior to their detection.

Samples prepared for total arsenic determination are mineralized by either wet or dry methods. Microwave is the most common closed system used in wet mineralization, although temperatures higher than those that can be achieved by microwave are needed for the complete degradation of some organoarsenic species. This leads to an underestimation of total arsenic in some foods when HG-based detection systems are used. Recent developments, such as microwave-induced combustion methods, are solving this problem. In dry mineralization, addition of ashing aids is necessary to avoid arsenic losses by volatilization.

Methodological research in the last decade has been targeted to arsenic speciation. Quantitative extraction of arsenic species from food matrices is one of the main methodological problems, and efficiencies vary widely, depending on the nature of the matrix and the method used. Polar solvents assisted by ultrasound, accelerated solvent extraction or microwave are commonly used. Extraction of

arsenite is especially difficult to achieve, because of binding to thiol groups in proteins. Separation of arsenic species is most commonly achieved by HPLC. Multidimensional chromatography (different columns and conditions) may be needed for samples with a large number of arsenic species; up to 23 species have been found in seaweed and seafood, for example. Further difficulties are that the elution may not be quantitative under certain conditions, and the eluent may change the arsenic oxidation state.

Most of the current work on arsenic speciation has been targeted to characterization of arsenic species profiles in food products, without special attention to inorganic arsenic. There is a current need for validated and horizontal methods for selective extraction and determination of inorganic arsenic and for certified reference materials for inorganic arsenic in foods. Further, it would be more appropriate to report total inorganic arsenic than arsenite and arsenate, because various extraction/analytical procedures may change the oxidation state.

9.5 Effects of processing

Peeling of vegetables and polishing of rice reduce the content of total arsenic. Washing or soaking rice and seaweed and discarding the water before cooking reduce arsenic levels, especially inorganic forms. Decreases in arsenic levels with boiling have been described for rice, pasta, seaweed and seafood products, except where the water used is contaminated with arsenic, when levels may increase. The main arsenic species solubilized are AB, DMA and arsenosugars for seafood products and inorganic arsenic for cereals and seaweed. Limited studies in which seafood was heated at temperatures above 150 °C have reported that up to 11% of AB is transformed to TMAO and TMA^+.

9.6 Prevention and control

Commercial-scale water treatment processes to remove arsenic in water are available. Simple arsenic removal systems for household wells have also been developed. Low-cost systems in arsenic-endemic areas generally utilize elemental iron, iron or aluminium oxides and carbon as adsorbents for arsenic. Many household treatment systems fail prematurely because of high levels of phosphate in water, and maintenance and disposal of arsenic-contaminated wastes are difficult. Studies in Bangladesh have shown that most rural households prefer sharing uncontaminated wells or filtering low-arsenic surface water through sand to treating groundwater. Sand filtration gives mixed results with respect to removal of biological pathogens. Spatial variability in groundwater arsenic contamination in Argentina, Chile and the river deltas of South and South-east Asia is very high, so villages usually have a mixture of contaminated and uncontaminated wells. Deeper groundwater aquifers often have low arsenic levels that can be used to develop community water supplies.

Apart from processing possibilities, practical prevention and control approaches for arsenic in foods are limited. Attempts to reduce arsenic uptake into food crops by additions of phosphate fertilizer and iron oxides have given equivocal and unconvincing results with several vegetable and cereal crops. Silicate additions

to soil have been shown to reduce arsenic levels in rice grain where soils are low in silicate. Growing rice under less reducing soil conditions can dramatically reduce grain arsenic levels. However, the challenge is to do this without substantial loss of yields in uncontaminated soils. Very limited identification of "low" and "high" arsenic rice varieties has been reported, and more data are needed before recommendations can be made to farmers and consumers.

9.7 Levels and patterns of contamination in food commodities

Data on total arsenic contents of foods for evaluation at the present meeting were obtained from the literature and from data submitted to the Committee by Australia, Brazil, France, Japan, New Zealand and Singapore. The total number of analytical results (single or composite) evaluated at the present meeting was 17 498. Table 8 in section 6.1 summarizes the ranges of total arsenic concentrations by food category, based on results with quantified values (minimum to maximum). The highest total arsenic concentrations have been found in seaweed, fish and shellfish, mushrooms and fungi, rice and rice products and some meat products. The levels in the remaining food products usually do not exceed 1 mg/kg. In some food groups, the number of non-detectable/non-quantifiable results was important (n = 9081) and influences the derivation of mean concentrations; this was the case with milk products (66%), meat and meat products (74%), eggs and egg products (65%), bakery wares (70%), cereals other than rice (80%) and vegetables other than mushrooms (86%).

Table 9 in section 6.1 summarizes the ranges of levels of inorganic arsenic obtained from the literature and from data submitted by Japan, France and Singapore (minimum to maximum). The total number of analytical (single or composites) results evaluated at the present meeting was 1737.

Levels of inorganic arsenic in foods and beverages usually do not exceed 0.1 mg/kg, with mean values generally less than 0.03 mg/kg. However, seaweed, rice and some fish and seafood commodities have higher inorganic arsenic levels, as do food crops grown in arsenic-contaminated soils.

In the seaweed *Hizikia fusiforme*, inorganic arsenic is more than 50% of total arsenic, with levels usually ranging from 30 to 130 mg/kg. In other seaweed species, inorganic arsenic is less than 15% of total arsenic, with levels normally below 2 mg/kg. The proportion of inorganic arsenic in rice varies from 17% to 100% of total arsenic and in vegetables from 33% to 74%, with maximum concentrations of 0.5 and 0.6 mg/kg, respectively. The proportion of inorganic arsenic usually does not exceed 10% of the total arsenic in fish and fish products, but it was found to reach 15% in shellfish from areas with some degree of arsenic contamination.

There are a variety of organoarsenic species in foods. For MMA and DMA, no information was available on their oxidation state in food products. In meat, DMA is the major species found in most studies, together with AB and minor amounts of MMA. In poultry meat, the presence of nitarsone, a phenylarsonic acid used as a coccidiostat, has also been reported. The greatest variety of arsenic species in vegetables has been detected in seaweeds, where arsenosugars are the major species, with smaller amounts of DMA, arsenolipids and thioarsenic compounds.

Mushrooms also contain many arsenic species, including AB, MMA, TMAO, DMA, AC and TMA^+. For other vegetables, MMA has been found in carrot, radish and potatoes, and MMA and DMA in chard and aubergines. Arsenic species found in fish and fish products include AB, arsenosugars, MMA, DMA, AC, TMA^+, TMAO, DMAE, TMAP, arsenolipids and thioarsenic compounds. AB is the major species (80–90%), except in some kinds of shellfish, where arsenosugars are the major species found.

9.8 Food consumption and dietary exposure assessment

Dietary exposure estimates for arsenic were reported by the Committee at the twenty-seventh meeting and were not revised at the thirty-third meeting. Only values for total arsenic were given for several European countries, the USA, Canada and the Republic of Korea; these ranged from 10 to 200 µg/day from food (0.17–3.33 µg/kg bw per day, assuming a 60 kg bw). Estimated dietary exposures to total arsenic from water ranged from 15 to 750 µg/day (0.25–12.5 µg/kg bw per day), reflecting arsenic concentrations in water of 10 µg/l and 500 µg/l and assuming a consumption of 1.5 litres of water a day. The Committee at the twenty-seventh meeting noted that water and seafood were the major sources of total arsenic, with other foods making minor contributions.

The focus of the Committee at the present meeting was on dietary exposure to inorganic arsenic; however, the majority of dietary exposure estimates submitted for evaluation were for total arsenic. The main factors influencing dietary exposure to inorganic arsenic are the water supply, type of food consumed and food preparation methods.

Where water is contaminated with arsenic, it is one of the most significant sources of inorganic arsenic exposure. It is also a major source of inorganic arsenic in food produced by irrigation with arsenic-contaminated water and from food preparation and cooking. Rice takes up high amounts of arsenic, but speciation of arsenic in rice varies between different regions, with a higher inorganic content in rice grown in Asia compared with the USA. Rice tends to be a major source of inorganic arsenic from food, particularly in Asia and other countries where it is a staple food. The level of inorganic arsenic in the rice consumed also varies, depending on food processing and preparation methods.

Arsenic contamination of groundwater is widespread, and there are a number of regions where arsenic contamination of drinking-water is important. Areas affected include southern Asia (e.g. Bangladesh, India), South-east and East Asia (e.g. China, including Taiwan, Mongolia, Viet Nam), the Americas (e.g. Argentina, Canada, Chile, Mexico, USA) and Europe (e.g. Finland, Hungary, Romania). Exposure to inorganic arsenic from water can be very variable, with high and low arsenic sources present in close proximity. Contaminated water that is used for drinking and food preparation would normally contain arsenic at concentrations between 10 and 200 µg/l. However, concentrations above 200 µg/l have been reported in some areas. The amount of water consumed also varies according to the region, temperature, physical activity and type of food, with soups and rice being examples of foods that will either contain high quantities of water or take up large

quantities of water. This can result in a total water consumption of between 1.5 and 5 litres per day.

The fact that water consumption and water used in cooking are not always included in dietary exposure estimates also makes direct comparison of reported total and inorganic arsenic dietary exposures found in different studies difficult, as exposure will be underestimated where water has not been included. In estimating dietary exposure to inorganic arsenic, variations in the different species of arsenic within a food category and between food categories need to be considered.

A summary of reported national inorganic arsenic estimates is given in Table 36, with ranges taken from various studies for some countries. It is particularly difficult to predict dietary exposures to arsenic at a regional level due to the complex factors discussed above that influence exposure at a local level. International estimates using the 13 Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diets were not generated, as the Committee considered that this level of generalization was not appropriate for estimating dietary exposures to inorganic arsenic.

Table 36. Summary of inorganic arsenic dietary exposure estimates

Country/region	Mean exposure (µg/kg bw per day)	Upper-percentile exposure (µg/kg bw per day)
Europe		
Europe[a] (EFSA)	0.21–0.61 adult 0.31–1.39 child 1–8 years 0.03–1.63 infant <12 months	0.36–0.99 adult (95th) 0.61–2.66 child 1–8 years (95th) —
Belgium[b]	0.10 all	0.16 all (90th)
France TDS[c]	0.10 adult 0.14 child 3–14 years	0.27 adult (95th) 0.34 child 3–14 years (95th)
United Kingdom TDS[c]	0.02–0.12 adult 0.03–0.20 child 1–18 years 0.45 infant <12 months	0.05–0.16 adult (97.5th) 0.08–0.40 child 1–18 years (97.5th) 0.74 infant (95th)
North America		
Canada TDS[c]	0.29 all	
USA TDS, other studies[d]	0.08–0.20 adult 0.12–0.32 child 1–6 years 0.24–1.19 infant <12 months	0.16–0.34 adult (95th) — —
South America		
Chile[e]	2.08–21.48 adult	

Table 36 (contd)

Country/region	Mean exposure (µg/kg bw per day)	Upper-percentile exposure (µg/kg bw per day)
Asia		
Bangladesh[f]	1.68–3.00 adult	
China TDS[c]	0.24–0.76 adult	
China, Province of Taiwan[g]	0.91 adult	
Japan TDS, other study[h]	0.36–0.46 adult	0.83–1.29 adult (95th)

TDS, total diet study

[a] Individual dietary records for 19 European countries, different scenarios using conversion factors, drinking-water included.

[b] Individual dietary records for Belgium, analysed inorganic values for fish and seafood commodities only, drinking-water not included.

[c] Total diet studies; France 2001–2002 TDS, 10% total arsenic assumed to be inorganic, drinking-water included; Canada 1985–1988 TDS, conversion factors from Uneyama et al. (2007) applied to total arsenic, drinking-water not included; China 2007 TDS analysed inorganic arsenic, drinking-water included; United Kingdom 2006 TDS analysed inorganic arsenic, drinking-water included, previous TDSs did not.

[d] Various studies based on individual dietary records for USA from 1986–1987 Nationwide Food Consumption Survey or 1994–1996, 1998 supplement Continuing Survey on Food Intakes by Individuals, inorganic arsenic levels from Schoof et al. (1998), drinking-water included in some studies.

[e] Small community in Chile, drinking-water included, seasonal contamination of river water used as drinking-water source.

[f] Small community in Bangladesh, total arsenic reported, assumed 70% total arsenic is inorganic, drinking-water not included.

[g] Small community in Taiwan, China, only rice and yams with analysed inorganic arsenic values included, drinking-water not included.

[h] Two studies; Japan 2000 TDS, drinking-water included, conversion factors from Uneyama et al. (2007) applied to total arsenic; other study of women in fishing and rice-farming communities, analysed inorganic arsenic for fish, shellfish, seaweed and edible algae, Japan TDS values for other foods, drinking-water not included.

In most circumstances, it would be expected that estimates of dietary exposure to inorganic arsenic using individual dietary records would be more accurate than those obtained using population food consumption figures, such as normally used in total diet studies or model diets. However, it is not possible to assume this is the case; for example, the EFSA estimates for European countries used individual records but assigned inorganic arsenic values derived from conversion factors applied to total arsenic levels for broad food groups, introducing uncertainties in the estimates and tending to overestimate dietary exposure compared with individual country studies in the region.

In general, the ranges of dietary exposure to inorganic arsenic for North America and Europe were similar but were lower than those reported for countries

in Asia. An exception was Bangladesh, for which mean dietary exposure to inorganic arsenic was estimated to be up to 3 times that in other Asian countries. Mean dietary exposure to inorganic arsenic for adults in a community in Chile was 7 times higher at the upper end of the reported range than that reported for adults elsewhere.

For infants and children, a limited amount of information was available for Europe and the USA; in general, estimates of dietary exposure to inorganic arsenic for children were higher than those for adults from the same population when expressed per kilogram of body weight.

With the exception of dietary exposure estimates for inorganic arsenic for Bangladesh and Chile, mean reported dietary exposures for adults or whole populations were less than 1 µg/kg bw per day, and upper-percentile dietary exposures were less than 1.5 µg/kg bw per day. For infants and children, mean dietary exposure estimates for inorganic arsenic were less than 2 µg/kg bw per day, and upper-percentile estimates were less than 3 µg/kg bw per day. The mean dietary exposures of up to 3 µg/kg bw per day for Bangladesh were for a small community known to have contaminated water; the results from the study in Chile would need to be confirmed.

For countries where rice is the staple food, rice and water were the major contributors to total inorganic arsenic dietary exposures, with wheat and vegetables being minor contributors. In Europe and North America, where wheat-based products and potatoes are staple foods, these were major contributors to inorganic arsenic dietary exposure, as well as other vegetables, milk and meat and their products. Water can contribute up to 50% of total dietary exposure in areas in these regions where the water is not contaminated. Although total arsenic levels are higher in fish and shellfish than in other foods, consumption of fish and shellfish does not have a major influence on dietary exposure to inorganic arsenic, as the majority of arsenic in fish and in the edible portion of shellfish is organic. The exception to this is for populations (e.g. Japan) or individuals in other populations who consume high levels of seaweed and other edible algae, some species of which are very high in inorganic arsenic and consumption of which can make a significant contribution to inorganic arsenic dietary exposure. No studies included dietary supplements, although some of these may contain appreciable amounts of inorganic arsenic, which may also mean that dietary exposures to inorganic arsenic are underestimated for individuals taking these supplements on a regular basis.

9.9 Dose–response analysis

The following studies were selected for dose–response modelling of the respective end-points. For lung cancer, data were from a recent prospective study in north-eastern Taiwan, China, of 6888 residents for whom arsenic concentrations in drinking-water had been ascertained, with an average 11.5 years of follow-up. Residents 40 years of age and older at study initiation with 178 incident lung cancer cases identified (Chen et al., 2010b) were used for modelling. An earlier case–control study of lung cancer (Ferreccio et al., 2000) was not preferred for modelling due to potential selection bias in hospital-based controls. For urinary cancer (Chen et al., 2010a), data from the same prospective study in north-eastern Taiwan, China, with 45 incident cases of urinary cancer were used for dose–response modelling. Three arsenic-related skin lesion case–control studies were considered: two

conducted in Bangladesh (Ahsan et al., 2006; Rahman et al., 2006) and one conducted in Inner Mongolia, China (Xia et al., 2009). Substantial differences exist among the studies in factors such as case definition, exposure assessment methods and assessment of possible confounders, including smoking and sun exposure. Considering these differences, these studies were not used for the evaluation.

The exposure metric in these studies was concentration of arsenic in drinking-water; total dietary exposure to inorganic arsenic from food and water was not assessed. In order to provide an opinion on the risks to health related to the presence of inorganic arsenic in foodstuffs, it was necessary to convert from the arsenic concentrations in drinking-water to total dietary exposure to inorganic arsenic. This conversion required assumptions about the arsenic exposure from food before cooking and the volumes of drinking-water consumed directly and in cooking for the populations in which the respective health end-points were studied. Because of the uncertainty about actual exposure, the Committee used average estimates of exposure from food and volumes of water consumed to extrapolate from concentrations in drinking-water to total dietary exposure to inorganic arsenic from food and water. A range of low to high values for exposure from food and volume of water consumed was identified to be used in a sensitivity analysis, taking into account the dietary habits and levels of arsenic in food in the relevant region (north-eastern Taiwan, China). The identified ranges were 50–200 µg/day from food excluding water and volumes of 2–4 litres of water consumed directly and used in cooking per day. The average estimates were 75 µg/day from food and 3 litres of water per day. From the available data, an average body weight of 55 kg was assumed for this population.

In order to utilize the adjustment made for other variables (e.g. smoking) in the original analyses in the studies in north-eastern Taiwan, China, of cancers of the lung (Chen et al., 2010b) and urinary tract (Chen et al., 2010a), adjusted cases were calculated based on the RRs. This two-step process involved calculating case frequency by multiplying the rate in the referent group by the RR and then estimating the number of adjusted cases by multiplying the number of subjects by the case frequency. The resulting adjustment was small relative to the reported number of cases.

In the dose–response analysis using the USEPA BMD software (BMDS version 2.1.1), the nine different dichotomous models were fitted to the adjusted data. Those resulting in acceptable fits based on statistical considerations were selected to derive BMD and BMDL values for a BMR at the low end of the observed range of the data (Table 37). All nine models resulted in an acceptable fit for the lung and urinary tract data. In modelling the epidemiological data, the BMD and BMDL estimated by the log-probit model differed from those of other models, with higher values when the model was constrained within the BMDS and very much lower values when unconstrained. In consequence, the Committee decided that the outputs of the log-probit model should be excluded from the assessment.

The lowest calculated BMDL was 3.0 µg/kg bw per day for a 0.5% increased incidence of lung cancer above background over the average 11.5 years of follow-up, based on average estimates of the exposure. A sensitivity analysis to investigate the impact of uncertainty in the exposure estimate in this study indicated that this $BMDL_{0.5}$ could be in the range of 2.0–7.0 µg/kg bw per day, with the assumption

made with respect to volume of drinking-water consumed and used in cooking having a greater impact than the assumption regarding inorganic arsenic in food.

Table 37. Ranges of $BMD_{0.5}$ and $BMDL_{0.5}$ values for lung and urinary cancer associated with dietary exposure to inorganic arsenic, based on average estimates of exposure

	$BMD_{0.5}$ (μg/kg bw per day)	$BMDL_{0.5}$ (μg/kg bw per day)
Lung cancer (Chen et al., 2010b)	4.5–7.3	3.0–5.0
Urinary cancer (Chen et al., 2010a)	7.9–13.9	5.2–11.4

$BMD_{0.5}$, benchmark dose for 0.5% increased incidence of cancer over background in north-eastern Taiwan, China, with average 11.5 years of follow-up; $BMDL_{0.5}$, lower 95% confidence limit for the benchmark dose for 0.5% increased incidence of cancer over background.

10. EVALUATION

From epidemiological studies measuring arsenic levels in drinking-water, inorganic arsenic has been identified as a human carcinogen. It is present naturally in food and water because of geochemical conditions, and consequently exposure varies significantly in different regions and even within regions, primarily through the presence or absence of arsenic in groundwater sources for drinking-water.

The approach to quantitative assessment of cancer risk from inorganic arsenic is limited, inter alia, by the lack of information on total exposure in the available epidemiological studies. The inorganic arsenic BMDL for a 0.5% increased incidence of lung cancer was determined by using a range of assumptions to estimate exposure from drinking-water and food with differing concentrations of inorganic arsenic. The $BMDL_{0.5}$ was computed to be 3.0 μg/kg bw per day (2.0–7.0 μg/kg bw per day based on the range of estimated total dietary exposure). The uncertainties in this $BMDL_{0.5}$ relate to the assumptions regarding total exposure and to extrapolation of the $BMDL_{0.5}$ to other populations due to the influence of nutritional status, such as low protein intake, and other lifestyle factors on the effects observed in the studied population. The Committee noted that the PTWI of 15 μg/kg bw (2.1 μg/kg bw per day) is in the region of the $BMDL_{0.5}$ and therefore was no longer appropriate, and the Committee withdrew the previous PTWI.

The Committee noted that more accurate information on the inorganic arsenic content of foods as they are consumed is needed to improve assessments of dietary exposures to inorganic arsenic species. Analytical constraints to achieving this goal include the lack of validated methods for selective determination of inorganic arsenic species in food matrices and the lack of certified reference materials for inorganic arsenic in foods. The proportion of inorganic arsenic in some foods was found to vary widely, indicating that dietary exposures to inorganic arsenic should be based on actual data rather than using generalized conversion factors from total arsenic measurements.

Reported mean dietary exposure to inorganic arsenic in the USA and various European and Asian countries ranged from 0.1 to 3.0 µg/kg bw per day. Drinking-water was a major contributor to total inorganic arsenic dietary exposures and, depending on the concentration, can also be an important source of arsenic in food through food preparation and possibly irrigation of crops, particularly rice. The proportion of total exposure to inorganic arsenic arising from food relative to the proportion from water increases as the concentration of inorganic arsenic in the water decreases. At the lower end of the exposure range, food can also be a major contributor to total inorganic arsenic exposure.

For certain regions of the world where concentrations of inorganic arsenic in drinking-water exceed 50–100 µg/l, some epidemiological studies provide evidence of adverse effects. There are other areas where arsenic concentrations in water are elevated (e.g. above the WHO guideline value of 10 µg/l) but are less than 50 µg/l. In these circumstances, there is a possibility that adverse effects could occur as a result of exposure to inorganic arsenic from water and food, but these would be at a low incidence that would be difficult to detect in epidemiological studies.

10.1 Recommendations

There is a need for validated methods for selective extraction and determination of inorganic arsenic in food matrices and for certified reference materials for inorganic arsenic.

There is a need for improved data on occurrence of different species of arsenic in, and their bioavailability from, different foods as consumed in order to improve the estimates of dietary and systemic exposure. Further information on the toxicity of arsenic species found in food is also required.

The Committee recommended that future epidemiological studies of the health impacts of arsenic should incorporate appropriate measures of total exposure to inorganic arsenic, including from food and from water used in cooking and processing of food.

Further, it is recommended that epidemiological studies not only focus on relative risks, but also analyse and report the data such that they are suitable for estimating exposure levels associated with additional (lifetime) risks, so as to make their results usable for quantitative risk assessment.

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DEOXYNIVALENOL (addendum)

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1. EXPLANATION

Deoxynivalenol (12,13-epoxy-3,4,15-trihydroxy-trichothec-9-en-8-one; DON, also known as vomitoxin; Chemical Abstracts Service [CAS] No. 51481-10-8) is a type B trichothecene mycotoxin produced mainly in cereals by various *Fusarium* species. In addition to DON, 3-acetyl-deoxynivalenol (3-Ac-DON; CAS No. 50722-38-8) and 15-acetyl-deoxynivalenol (15-Ac-DON; CAS No. 88337-96-6) are also naturally occurring fungal secondary metabolites, whereas DON-3β-glucopyranoside (DON-3-glucoside) is a naturally occurring conjugate of DON formed in plants.

DON was previously evaluated by the fifty-sixth meeting of the Committee (Annex 1, reference *152*). The Committee established a provisional maximum tolerable daily intake (PMTDI) of 1 µg/kg body weight (bw) on the basis of the no-observed-effect level (NOEL)[1] of 100 µg/kg bw per day for decreased body weight gain reported in a 2-year feeding study in mice and application of a safety factor of 100. The Committee concluded that intake at this level would not result in effects of DON on the immune system, growth or reproduction. The Committee noted that the available data did not suggest that DON presents a carcinogenic hazard.

DON was on the agenda of the present meeting at the request of the Second Session of the Codex Committee on Contaminants in Food (FAO/WHO, 2008), which asked the Committee to assess exposure on a more global basis, taking new data into account; to review the toxicological data and consider the need for an acute reference dose (ARfD), taking into account data in finished products, but also in raw wheat and other commodities as they are traded internationally, and consideration of processing factors; and to assess the toxicity of 3-Ac-DON and 15-Ac-DON.

The Committee reviewed several new studies on metabolism and toxicokinetics, acute toxicity, genotoxicity, mechanisms of toxicity and developmental toxicity of DON and/or its acetyl derivatives. The Committee also took note of the data previously evaluated at the fifty-sixth meeting. Emphasis was given to studies in which pure DON or acetylated DON was added to defined diets in mammalian species, because naturally contaminated feed commonly contains multiple mycotoxin contaminants. New information on occurrence, processing, prevention and control, and dietary exposure was also considered.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Mice

The effects of oral (gavage) and intranasal exposure to DON (5 mg/kg bw, purity unknown) on tissue distribution and proinflammatory cytokine induction in

[1] At its sixty-eighth meeting (Annex 1, reference *187*), the Committee decided to differentiate between no-observed-adverse-effect level (NOAEL) and no-observed-effect level (NOEL). This NOEL would now be considered a NOAEL.

adult female B6C3F1 mice (five or more per group) were investigated. Competitive direct enzyme-linked immunosorbent assay (ELISA) revealed that, regardless of exposure route, DON concentrations in plasma, spleen, liver, lung and kidney were maximal within 15–30 min and declined by 75–90% after 120 min. After oral exposure, peak concentrations were approximately 1 µg/ml in plasma, 0.77 µg/g in spleen, 1.1 µg/g in liver, 0.9 µg/g in lung and 1.8 µg/g in kidney. Plasma and tissue DON concentrations were 1.5–3 times higher following intranasal exposure compared with oral exposure. The inductions of interleukin-1 beta (IL-1β), IL-6 and tumour necrosis factor-alpha (TNF-α) messenger ribonucleic acids (mRNAs) were measured in spleen, liver and lung of nasally exposed mice, and these were 2–10, 2–5 and 2–4 times greater, respectively, than the mRNA inductions in the tissues of orally exposed mice (Amuzie, Harkema & Pestka, 2008).

Female B6C3F1 mice (4 weeks old) were fed on a diet containing purified DON (purity unknown) at 20 mg/kg for 2–8 weeks. The capacity of mice to accumulate DON in plasma was measured at weeks 2, 4 and 8 of the feeding period. The body weight of animals fed on a control diet increased from 18 to 26 g over the 8-week period, whereas in DON-treated animals, weight increased from 18 g to only 20 g over the same period. DON was detectable in plasma (48 ng/ml) within 2 weeks of initiating the treatment. The mice maintained a near-steady-state concentration of DON in plasma at weeks 4 (63 ng/ml) and 8 (44 ng/ml). Mice fed control diet (without DON) contained no detectable DON in their plasma. These findings indicate that impaired growth in the mice exposed subchronically to DON was associated with detectable levels of the toxin in circulation (Amuzie & Pestka, 2010).

(b) Rats

The metabolism of [^{14}C]DON (5 mg/kg bw, radiochemical purity 93%) was investigated in male Sprague-Dawley rats. The animals (n = 15) received the radiolabelled compound, dissolved in water containing 15% ethanol, by gavage, and the distribution of DON in body fluids was investigated over 72 h. DON and its metabolites were detectable in the plasma of rats, with the highest levels at 8 h, at which time approximately 9% was bound to plasma protein. After 72 h, a total of 37% of the radiolabel was excreted in the urine, and DON-glucuronide was implicated as the major urinary metabolite based on reversed-phase high-performance liquid chromatographic (HPLC) analysis (Meky et al., 2003).

(c) Pigs

Castrated male pigs (n = 11, body weight 88.1 ± 3.9 kg) received a *Fusarium*-contaminated diet (restricted to 2.2 kg/day) containing DON at 4.2 mg/kg diet over a period of 7 days. The pigs were slaughtered at 1, 2, 3, 4, 5, 6, 8, 15, 18 and 24 h after feeding on day 7, with the exception of one pig, which was slaughtered unfed. DON and de-epoxy-DON were analysed in serum and digesta from consecutive segments of the digestive tract (stomach, small intestine divided into three parts of a similar length, caecum, colon, rectum). DON was rapidly and nearly completely absorbed while passing through the stomach and the proximal small intestine. The maximum serum concentration appeared 4.1 h after the DON-containing meal had been ingested, and half of the systemically absorbed

DON was eliminated after 5.8 h. De-epoxy-DON appeared in increasing proportions in the distal small intestine and reached approximately 80% of the sum of DON plus de-epoxy-DON in faeces collected from the rectum. The study authors concluded that de-epoxidation of DON, which occurs primarily in the hindgut, probably does not contribute much to detoxification in the pig (Dänicke et al., 2004).

The toxicokinetics of DON was investigated in castrated male pigs (5–6 per group, body weight 41.5 ± 2.0 kg). Pigs were fed naturally contaminated wheat containing DON at 5.7 mg/kg for at least 4 weeks (subchronic) or on a single day (acute). In addition, a group of pigs received an intravenous injection of DON ("pure", but percentage unknown) at a dose of 53 µg/kg bw. After intravenous DON application, serum DON concentrations decreased biphasically, with terminal elimination half-lives of between 4.2 and 33.6 h. DON was rapidly absorbed following oral exposure and reached maximal plasma concentrations of 21.79 and 15.21 ng/ml serum after 88.4 and 99.1 min in the subchronically and acutely fed groups, respectively. Thereafter, serum DON levels declined slowly, with elimination half-lives of 6.28 h and 5.32 h for the subchronic and acute groups, respectively. The mean bioavailability of DON was 89% for the subchronic group and 54% for the acute group. DON was highly distributed in all groups, with an apparent volume of distribution higher than the total body water volume. Glucuronide conjugation of DON was found in serum samples after oral exposure, but not after intravenous application. Dietary DON caused a significant increase in DON concentrations in urine and faeces, whereas the metabolite de-epoxy-DON was found only in the trials with 4 or more weeks of treatment. The total recovery was about 66.6% and 54.0% for the control and the subchronic DON groups, respectively, with urine being the main excretory route. Twenty-four hours following oral dosing, DON could not be detected in the serum, except in one subchronically fed pig, in which it was detected at the limit of detection (LOD). The study indicates that in pigs orally administered DON, more than 50% of the DON is quickly absorbed, highly distributed and only poorly metabolized (Goyarts & Danicke, 2006).

A dynamic laboratory model simulating the gastrointestinal tract of healthy pigs (TNO-Intestinal Model of the stomach and small intestine) was used to evaluate the small-intestinal absorption of DON and nivalenol (NIV), another type B trichothecene, and the efficacy of activated carbon in reducing the relevant absorption. The in vitro intestinal absorptions of DON and NIV were 51% and 21%, respectively, following the ingestion of 170 µg DON and 230 µg NIV, respectively, through contaminated (spiked) wheat. Most absorption occurred in the jejunal compartment for both mycotoxins. The inclusion of activated carbon produced a significant reduction in the intestinal mycotoxin absorption. At a 2% inclusion level, the absorption with respect to the intake was lowered from 51% to 28% for DON and from 21% to 12% for NIV. The Committee noted that this mechanistic study was not relevant for the evaluation (Avantaggiato, Havenaar & Visconti, 2004).

2.1.2 Biotransformation

Five castrated male pigs (body weight 29 kg), in which the gastrointestinal microflora lacked the ability to transform 3-Ac-DON and NIV to their corresponding de-epoxidated metabolites, were equipped with post-valve T-caecum cannulas for

collection of ileal digesta and were fed on control diet for 2 weeks, followed by a diet naturally contaminated with DON at 0.8 mg/kg for 3 weeks and subsequently a diet naturally contaminated with DON at 1.2 mg/kg for 4 weeks. The gastrointestinal microorganisms did not acquire the de-epoxidation ability during the 7-week-long exposure period. At the end of the exposure period, faeces from pigs with a known de-epoxidation ability were spread out in the pens and left for 24 h. One week after the faeces had been spread out in the pens, the de-epoxidation ability was found in faecal incubations from four out of five experimental pigs. This change in the intestinal de-epoxidation ability was not accompanied by any detectable changes in the deoxyribonucleic acid (DNA) profiles of the bacterial community. The results show that the intestinal de-epoxidation ability is common at pig farms in the Uppsala area in Sweden and that the ability may be transferred between pigs in a stock (Eriksen et al., 2002).

2.1.3 Absorption, distribution and excretion of 3-Ac-DON

The absorption, metabolism and excretion of 3-Ac-DON (purity >95%) in pigs were studied. Pigs with a faecal microflora known to be able to de-epoxidate trichothecenes were used in the experiment. The pigs were fed a commercial diet with 3-Ac-DON added to provide a concentration of 2.5 mg/kg feed for 2.5 days. No traces of 3-Ac-DON or its de-epoxide metabolite were found in plasma, urine or faeces. DON was detected in plasma as soon as 20 min after the start of feeding. The maximum concentration of DON in plasma was reached after 3 h and decreased rapidly thereafter. Only low concentrations close to the LOD were found in plasma 8 h after the start of feeding. A significant part of the DON in plasma was in a glucuronide-conjugated form (42% ± 7%). No accumulation of DON occurred in plasma during the 60 h of exposure. The excretion of DON was mainly in urine (45% ± 26% of the toxin ingested by the pigs), and only low amounts of metabolites of 3-Ac-DON (2% ± 0.4%) were recovered in faeces. De-epoxy-DON constituted 52% ± 15% of the total amount of 3-Ac-DON metabolites detected in faeces. The remaining part in faeces was DON. DON was still present in the urine and faeces at the end of the sampling period 48 h after the last exposure. The results show that no de-epoxides are found in plasma or urine in pigs after trichothecene exposure, even in pigs having a faecal microflora with a de-epoxidation activity. The acetylated form of the toxin is deacetylated in vivo. Furthermore, the experiment shows that the main part of DON is rapidly excreted and does not accumulate in plasma, but a minor part of the toxin is retained and slowly excreted from the pigs. It has to be noted that about half of the administered dose was not accounted for. This study indicates that there is substantial conversion of 3-Ac-DON to DON in vivo in pigs (Eriksen, Pettersson & Lindberg, 2003).

2.2 Toxicological studies

Since the last evaluation, a large number of toxicity studies of DON have been published. Many of those were excluded from this addendum, based on the following criteria:

- studies using naturally contaminated feed, which, although relevant for dealing with DON in practice, are not useful for derivation of a no-observed-adverse-effect level (NOAEL) for pure DON;
- studies dosing DON in combination with other mycotoxins, as no NOAEL for pure DON can be derived;
- in vitro studies aimed at elucidating mechanistic effects of DON toxicity, as no in vivo NOAEL for DON can be derived;
- studies using chickens, ducks or turkeys as test species, as these species are not considered representative for toxicity in humans.

2.2.1 Acute toxicity

Results of acute studies on lethality (median lethal dose [LD_{50}]) and emesis in animals treated with DON were presented in the previous monograph (Annex 1, reference *153*). Since then, a number of acute studies have been performed, mostly on immunological parameters; these are summarized in section 2.2.6. One acute study on 3-Ac-DON has been summarized in the section on metabolites (section 2.2.7). Those studies on the emetic effects of DON in the diet of pigs from the 2001 monograph that had clear dosing regimes are described in more detail below for purposes of derivation of the ARfD.

(a) Pigs

Groups of four young pigs (average weight 8 kg) received feed containing contaminated corn at a concentration of 0% or 36% for 10 days, 0%, 6%, 12%, 18% or 24% for 4 days, 0%, 1.5%, 3%, 4.5% or 6% for 11 days or 0%, 0.3%, 0.6%, 0.9%, 1.2%, 1.5%, 1.8% or 2.1% for 21 days in four trials. The DON and the *Fusarium* mycotoxin zearalenone (ZEA) contents of the diets were analysed. A pilot study was perfomed, but the starting weights of the pigs were not given, and feed refusal occurred. Trial 2 was terminated after 4 days because the pigs that received the diets containing mouldy corn were consuming very little feed. In the third trial, pigs were vomiting at day 1 from mouldy corn concentrations of 3% (determined analytically to contain DON at a concentration of 19.7 mg/kg diet). In the 3%, 4.5% and 6% groups, there were indications that at least one pig vomited. Feed intake was reduced to 45% of that of controls in the 1.5% group and to 12% and less in higher dose groups. There were no pigs vomiting in trial 4, but the inclusion of mouldy corn in the diet resulted in a linear reduction in rate of body weight gain and a linear and quadratic reduction in feed consumption and body weight gain per kilogram of feed. The DON content of the diet used in trial 4 was not given. For this evaluation, it was assumed that the ZEA content did not contribute to the emetic effect. The lowest dose at which no emesis was seen was 9 mg/kg diet. The authors reported that this was equal to a dose of 0.15 mg/kg bw per day, but data on food intake from the first day were not given (Young et al., 1983).

Pollman et al. (1985) exposed groups of eight starter pigs (average body weight 7.7 kg) in a first trial to DON through contaminated wheat in the diet at DON concentrations of 0, 0.9, 2.0 and 2.8 mg/kg diet (analysed values) for 3 weeks. No emesis was seen in any dose group, but feed intake was reduced at 2.0 mg/kg diet, equal to 0.17 mg/kg bw per day (using starting weights). In a second trial, groups

of four pigs (average body weight 8.3 kg) were exposed to DON at 1.3, 1.4, 2.3 or 2.7 mg/kg feed through contaminated wheat in the diet (analysed concentrations, no control group) for 2 weeks. No emesis was seen; feed intake was reduced at 1.4 mg/kg diet, but not at higher doses. This dose is equal to 0.1 mg/kg bw per day based on measured feed intake. A third trial was done with grower-finishing pigs of average body weight 60.8 kg, which were exposed to 0, 0.9, 2.2, 2.8 or 4.2 mg/kg diet (analysed concentrations) for 6 weeks. Evidence of emesis was seen only once in the 2.2 mg/kg diet group, but not at higher doses. Reduced feed intake was seen at 2.2 mg/kg feed. The two highest dose groups were taken off the feed after 2 weeks because of very poor performance. The lowest dose that did not induce emesis in this study was 2.8 mg/kg feed, the highest dose tested, equal to 0.24 mg/kg bw per day based on starting weight and measured feed intake.

Groups of four nursery pigs of mixed breed (Polish White Large × Polish White Ear-pendent) with an average body weight of 35 kg were given a single dose of DON at 0, 0.2 or 0.4 mg/kg bw in the feed. The animals were euthanized on day 5, and, based on macroscopic examination, segments of duodenum, jejunum, ileum, liver and mesenteric lymph nodes were sampled and assigned for histopathological examination. Histopathological examination indicated that the regressive lesions were expressed more in the experimental group treated with the higher concentration of DON (Zielonka et al., 2009).

2.2.2 Short-term studies of toxicity

(a) Rats

The effects of DON (purity not reported) on blood biochemical parameters in growing Wistar rats were studied. Male rats (10 per group) were treated subcutaneously with DON at 1 mg/kg bw per day for 3 days. After 3 days, significant increases in blood insulin, glucose and free fatty acids were observed in the DON-treated animals in comparison with the control group. DON treatment caused an increment in glycogen depots and a reduction in the triglyceride content of the muscle (Szkudelska, Szkudelski & Nogowski, 2002).

(b) Pigs

Groups of 6–10 pigs (sex unknown, body weights 15–20 kg at the start of the study) were fed on a diet containing DON at 0 or 2.85 mg/kg for 5 weeks (equivalent to 0 or 0.11 mg/kg bw per day). In intestinal tissues of pigs treated with DON, an increased intestinal barrier permeability and a reduction in the expression of claudins (a component of tight junctions) were observed. In vitro studies demonstrated that in intestinal epithelial cell lines from porcine (IPEC-1) or human (Caco-2) origin, DON decreased trans-epithelial electrical resistance and increased, in a time- and dose-dependent manner, the paracellular permeability to 4-kilodalton dextran and to pathogenic *Escherichia coli* across intestinal cell monolayers. The data suggested that porcine epithelial cells were more susceptible than human cells to the effects of DON. As only one dose was tested in vivo, a NOAEL could not be established, but would be below 0.11 mg/kg bw per day (Pinton et al., 2009).

In a study in which pigs (five of each sex per dose, 5 weeks old) were fed corn–soya bean diets containing 0, 0.5 or 1.5 mg DON (purity and source unknown) per kilogram (equivalent to 0, 0.02 and 0.06 mg/kg bw per day; conversion from Bohm & Razzazi, 2003) for 15 days, sera samples were collected at day 35 of treatment for biochemical analysis. DON treatment at 0.5 and 1.5 mg/kg diet increased serum urea by 43% and 51%, respectively. Gamma glutamyl transferase (GGT) activity was increased about 2.5-fold at 1.5 mg/kg diet. DON treatment did not affect serum protein levels or aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. As the source and purity of DON could not be determined, the Committee did not consider this study suitable for the evaluation (Dinischiotu et al., 2007).

Female weaned piglets (nine per group; mean body weight 9.8 kg at the start of the study) were given 0, 0.3, 0.6 or 1.2 mg/kg of isolated, pure DON in the diet (equivalent to 0, 0.012, 0.024 and 0.48 mg/kg bw per day) for 8 weeks. Pigs were fed restrictively to allow a complete feed intake by all animals. Body weight, feed intake, parameters of liver integrity, haematological data and blood concentrations of some selected components of energy and protein metabolism were examined weekly. Body weight gain, feed intake and feed conversion rate were not affected by DON treatment. No toxicologically significant effects of DON treatment on plasma levels of AST, ALT, GGT, glutamate dehydrogenase, sorbitol dehydrogenase, haemoglobin, urea, albumin or glucose were observed (Drochner et al., 2006).

The effects of DON on weaned piglets (average body weight 8 kg) were investigated. Two feeding trials were conducted with wheat naturally contaminated with DON. In the first trial, as a preliminary study, weaned piglets (9–13 per age group) in age groups of 3, 4 or 5 weeks were fed for 1 week with DON at 7.7 mg/kg diet (reported to be equal to 0.35 mg/kg bw per day). The intake of DON-contaminated diets was not associated with any obvious negative health effects. In the main feeding trial, in which piglets (13–15 per group) were treated with control feed or feed containing DON at 3 mg/kg diet, no vomiting or other negative clinical symptoms were observed. At the end of the 8-week treatment period, body weights in the control and treatment groups were 49.8 kg and 45.7 kg, respectively. The weekly feed intake was decreased by 4–19% in the DON-fed group compared with the control group, but the feed conversion rates were slightly improved in the DON-fed group (Bohm & Razzazi, 2003).

2.2.3 Long-term studies of toxicity and carcinogenicity

No new long-term studies of toxicity or carcinogenicity were identified.

2.2.4 Genotoxicity

For the present addendum, no new studies into the genotoxic potential of DON conducted according to Organisation for Economic Co-operation and Development (OECD) guidelines were available. In a review by Ma & Guo (2008), it is reported that DON was positive in an unscheduled DNA synthesis (UDS) test and a replicative DNA synthesis (RDS) test in rat primary hepatocytes and in a number of comet assays. However, these studies were not available for the present

evaluation, as indicated in Table 1. In a study by Sakai et al. (2007), in which the effects of various mycotoxins on initiation and promotion of v-Ha-ras-transfected BALB/3T3 cell transformation was studied, DON (and NIV) was negative (see Table 1). In an in vivo study in which chickens were treated for 17 days with DON at 10 mg/kg bw per day, DON induced a slight, but statistically significant, increase in DNA damage in spleen leukocytes, as measured by the comet assay (Frankic et al., 2006).

Table 1. Results of assays for the genotoxicity of DON

End-point	Test object	Concentration	Results	Reference[a]
In vitro				
UDS	Male SD rat primary hepatocytes	0, 0.003, 0.03, 0.3 µg/ml	Positive	Guo & Xu (1997)*
RDS	Male SD rat primary hepatocytes	0, 0.01, 0.1, 1 µg/ml (incubation time 3 h)	Positive	Li & Guo (2000)*
Comet assay (DNA breaks)	Male SD rat primary hepatocytes	0, 0.01, 0.1, 1 µg/ml (incubation time 2 h)	Positive	Li & Guo (2001)*
Comet assay (DNA breaks)	Vero cells	0, 1, 5, 10 µmol (incubation time 4 and 16 h)	Positive	Li & Sun (2004)*
Comet assay (DNA breaks)	Vero cells	0, 10 µmol (incubation time 4 and 16 h, reincubate for 15, 30, 60 and 120 min) 0, 1, 5, 10 µmol (incubation time 4 and 16 h)	Positive	Li & Sun (2004)*
Comet assay (DNA breaks)	Human Caco-2 cells	0.01–0.5 µmol/l (incubation time 24 and 72 h)	Positive	Bony et al. (2006)*
Cell transformation (tumour initiation and promotion)	BALB/c3T3 cells	0.01–0.2 µg/ml	Negative	Sakai et al. (2007)
In vivo				
Comet assay (DNA breaks)	Chicken spleen leukocytes	10 mg/kg bw per day by gavage for 17 weeks in diet	Positive	Frankic et al. (2006)

[a] An asterisk (*) indicates that the original study was not available; the data were reported in the review by Ma & Guo (2008).

2.2.5 Reproductive and developmental toxicity

(a) Effects on reproductive organs

(i) Rats

Male Sprague-Dawley rats were treated with DON (0, 0.5, 1, 2.5 or 5 mg/kg bw) daily via gastric intubation for 28 days. Epididymal (right and left) and seminal vesicle weights (expressed per gram of body weight and brain weight) were significantly reduced in animals treated with 2.5 and 5 mg/kg bw. Decreased prostate weight (expressed per gram of body weight and brain weight), spermatid numbers, cauda epididymal sperm numbers and cauda epididymal sperm numbers per gram cauda epididymis were observed in the 5 mg/kg bw dose group. Increased sperm tail abnormalities (broken tails) were also observed in the 5 mg/kg bw dose group, whereas sperm swimming speed was increased only in the 2.5 mg/kg bw dose group. Serum concentrations of follicle-stimulating hormone and luteinizing hormone were increased, whereas the testosterone concentration was decreased in a dose-dependent manner. Increases in germ cell degeneration, sperm retention and abnormal nuclear morphology were observed at doses above 2.5 mg/kg bw. A NOAEL of 1 mg/kg bw per day was derived based on reduced epididymal (right and left) and seminal vesicle weights in the next higher dose group (Sprando et al., 2005).

(b) Developmental toxicity

(i) Mice

In a review aimed at determining the relative developmental toxicity potential of DON and benomyl, both present on wheat, Hicks et al. (2000) identified a dietary NOAEL for DON for decreased body weight in mouse pups of 0.375 mg/kg bw per day from Khera et al. (1984). This study was described in the 2001 monograph (Annex 1, reference *153*). The authors also claimed that the toxic actions of DON in pregnant animals are consistent from species to species. The decrease in body weight in dams and pups at lower doses and complete resorptions at higher doses were stated to be consistent with the primary mechanism of action, which was inhibition of protein synthesis.

DON was administered to 3-month-old nulliparous female NMRI mice by intraperitoneal injection (3.3, 4.2, 5 or 10 mg/kg bw [11, 14, 17 or 34 µmol/kg bw] on gestation days 7 and 9 or 1.6, 2.5 or 3.3 mg/kg bw [5.4, 8.4 or 10 µmol/kg bw] daily on gestation days 7–10), and the mice were sacrificed on day 18 of gestation. The total numbers of implants, resorptions and dead and live fetuses were recorded. Resorption was considered as early if fetal structures were resorbed and late if some recognizable fetal tissue remained. Live fetuses were examined for external malformation, weighed and then sacrificed and prepared for histological examination of the skeleton. High maternal deaths were seen at the two highest doses in each set of doses. In embryos, the number of resorptions was dose-dependently increased in treated animals compared with controls. Skeletal abnormalities (mostly in the axial skeleton) were observed. Exencephaly was seen mainly at 2.5 or 3.3 mg/kg bw during the 4-day treatment. Neural arch defects and

fusion occurred more in the 2-day treatment than in the 4-day treatment. In both experiments, vertebral bodies showed various deformities (destruction or division), as well as hemivertebrae (except with 2.5 mg/kg bw given for 4 days) and fused, branched and/or cervical ribs. In the 2-day experiment, the effects were dose dependent, and in the 4-day experiment, the incidences were lower (Debouck et al., 2001).

(ii) Rats

Groups of 24 pregnant female Charles River Sprague-Dawley rats were gavaged once daily with purified DON at a dose of 0, 0.5, 1, 2.5 or 5 mg/kg bw per day on gestation days 6–19. At caesarean section on gestation day 20, reproductive and developmental parameters were measured. All females survived to caesarean section. DON caused a dose-related increase in excessive salivation by the pregnant females in all dose groups, statistically significant at 2.5 mg/kg bw per day, a reaction probably linked to the lack of emetic reflex in rats. At 5 mg/kg bw per day, feed consumption and mean body weight gain were significantly decreased throughout gestation, mean weight gain (carcass weight) and gravid uterine weight were significantly reduced, 52% of litters (12/23) were totally resorbed, the average number of early and late deaths per litter was significantly increased, average fetal body weight and crown–rump length were significantly decreased, the incidence of runts was significantly increased and the ossification of fetal sternebrae, centra, dorsal arches, vertebrae, metatarsals and metacarpals was significantly decreased. At 2.5 mg/kg bw per day, DON significantly decreased average fetal body weight, crown–rump length and vertebral ossification. These effects may be secondary to maternal toxicity and the reduced size of the fetuses. The incidence of misaligned and fused sternebrae was significantly increased at 5 mg/kg bw per day. No adverse developmental effects were observed at 0.5 and 1 mg/kg bw per day. Dose-related increases in maternal liver weight to body weight ratios were observed in all treated groups (significant at 1, 2.5 and 5 mg/kg bw per day). The weight changes were correlated with dose-related cytoplasmic alterations of hepatocytes. The NOAEL for maternal toxicity in this study is 0.5 mg/kg bw per day based on the dose-related increase in liver to body weight ratio at 1 mg/kg bw per day. The NOAEL for fetal toxicity is 1 mg/kg bw per day based on the general reduction in fetal development at 2.5 and 5 mg/kg bw per day. The NOAEL for teratogenicity is 2.5 mg/kg bw per day based on the increase in misaligned and fused sternebrae at 5 mg/kg bw per day (Collins et al., 2006).

(iii) In vitro

In porcine cumulus oocyte complexes, DON (0.94, 1.88, 3.75 or 7.5 µmol/l [0.28, 0.557, 1.11 or 2.2 µg/ml]) dose-dependently decreased maturation (telophase 1 and metaphase 2) rates and increased degeneration rates after 48 h culture in vitro (Alm et al., 2002).

2.2.6 Special studies on immunotoxicity

Details on the special studies on the immunotoxicity of DON are summarized in Table 2. The individual studies are described more fully below.

Table 2. Details and end-points of studies on immunotoxicity

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw)[a]	Route	Effect	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	Reference
Female BALB/c mice, 5 weeks old	DON (Sigma)	Single dose, reovirus after 2 h, effect assessment after 10 days	6		0, 2, 5, 10 or 25	Gavage	Decreased viral clearance	2	<2	Li et al. (2007)
Male and female BALB/c mice, 6 weeks old	DON (>98%)	14 or 28 days	10 + 10	0, 0.25, 0.5, 1 or 2	Eq 0, 0.038, 0.075, 0.15 or 0.3	Diet	Decreased CD19+ (B cells in peripheral blood lymphocytes)	—	No NOAEL derived	Wu et al. (2009)
Male BALB/c mice	DON (unknown)	14 days	12	0 or 2	Eq 0 or 0.3	Diet	Splenocyte proliferation suppression	0.3	<0.3	Landgren, Hendrich & Kohut (2006)
Male C57BL6 mice, 6 weeks old	DON (pure, but % unknown)	4 weeks, 3 days/week	10		0, 0.014, 0.071, 0.355 or 1.774	Gavage	Increased plasma IgA levels	—	No NOAEL derived	Gouze et al. (2006)
							Increased liver PROD activity	—	No NOAEL derived	

Table 2 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw)[a]	Route	Effect	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	Reference
Male wild-type (WT; P53N5-W) and p53+/− (TG; P53N5-T) mice, 5–7 weeks old	DON (95%)	26 weeks	20 (10 + 10)	0, 1, 5 or 10	Eq 0, 0.15, 0.75 or 1.5	Diet	Increased plasma IgA, decreased IgM in WT	1.5	0.75	Bondy et al. (2009)
							Increased kidney weight in WT	0.75	0.15	
							Increased kidney weight in TG	1.5	0.75	
							Increased IgA positive glomeruli in kidney	1.5	0.75	
Female B6, 129P2-Ptgs2tm1Smi (002181-W) mice, wild type compared with COX-2 knockout mice	DON (reagent grade or better, purity checked by HPLC)	12 weeks	3–6	0 or 10	Eq 0 or 1.5	Diet	Increased serum IgA	1.5	<1.5	Jia & Pestka (2005)

Table 2 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw)[a]	Route	Effect	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	Reference
Hybrid commercial castrated male pigs, 8 weeks old	DON (D0156, Sigma)	6 weeks	14		0 or 0.5 (1 week) and 1 (5 weeks)	Oral	No effects	—	1	Ferrari et al. (2009)
Female weaned piglets (mean body weight 9.8 kg)	DON (pure)	8 weeks	9	0, 0.3, 0.6 or 1.2	Eq 0, 0.012, 0.024 or 0.048	Diet	Increased IgA levels in serum	—	0.048	Drochner et al. (2004)
Weanling pigs, 4 weeks old, sex unknown	DON, naturally contaminated, no myocotoxins other than DON detectable	9 weeks in different age periods	6	0, 2.2 or 2.5	0, 0.088 or 0.1	Diet	Increased serum IgA, reduction mesenteric lymph node IFN-α and TGF-α mRNA	0.088	<0.088	Pinton et al. (2008)

Table 2 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw)[a]	Route	Effect	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	Reference
Male B6C3F1, B6 129-IL6tmi Kopf, L6 knockout and B6129F2l wild-type control mice	DON purified and checked	12 weeks	3–6	0 or 10	Eq 0 or 1.5	Diet	Increased serum IL-6 and IgA and increased mesangial IgA deposition in the kidney	1.5	<1.5	Pestka & Zhou (2000)
							Reduced feed intake and body weight gain	1.5	<1.5	
Female B6C3F1 mice, 8–9 weeks old	DON (purity unknown)	Treatment: 16–24 weeks Withdrawal: 8 weeks followed by control diet for remaining 8–16 weeks	12	0 or 25	Eq 0 or 3.75	Diet	Increased serum IgE from 12 weeks onwards in both treatment and withdrawal groups	3.75	<3.75	Pestka & Dong (1994)

Table 2 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw)[a]	Route	Effect	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	Reference
Female B6C3F1 mice, 3–4 weeks old	DON (unknown from Sigma labs)	Single dose, assessment each hour for 5 h	≥5		0, 0.1, 0.5, 1, 5 or 12.5	Gavage	Induction of SOCS in liver	0.1	<0.1	Amuzie, Shinozuka & Pestka (2009)
Female B6C3F1 mice, 3–4 weeks old	DON (unknown from Sigma labs)	Single dose, assessment after 2 h	4–5		0, 0.1, 0.5, 1, 5 or 12.5	Gavage	Decreased IGFALS in liver	0.5	0.1	Amuzie & Pestka (2010)
Male B3C3F1 mice, 8–10 weeks old	DON (unknown from Romer labs)	Single dose, effect assessment 2 and 3 h after exposure	3		0, 0.1, 0.5, 1, 5 or 25	Gavage in buffer	Increased cytokine mRNA in spleen and Peyer's patches	5	1	Zhou, Yan & Pestka (1997)
Male B3C3F1 mice, 8–10 weeks old	DON and LPS (from Sigma labs)	Single dose, effect assessment after 12 h	3		0 or 25 DON and/or 0.5 LPS	Gavage and/or ip injection	Enhanced LPS induction, TNF-α, IL-6 and IL-1β	5	<5	Zhou et al. (2000)
							Apoptosis	—	—	
Male B6C3F1 mice, 20 weeks old, 30–34 g	DON, purified and analysed	Single dose, effect assessment after 2 h	5		0 or 25	Gavage	Upregulation spleen cytokine and chemokine expression	25	<25	Kinser et al. (2004)

Table 2 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw)[a]	Route	Effect	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	Reference
Female B6C3F1 mice, weanling (3–4 weeks) and young adult (8–10 weeks)	DON (Sigma)	Single dose, effect assessment after 15, 30, 60 and 120 min	≥5		0 or 5	Gavage	Expression of mRNAs for TNF-α, IL-1β and IL-6 in spleen, but not in liver or lung, 2-fold higher in weanling than adult	5	<5	Pestka & Amuzie (2008)
Male B6C3F1 mice, 8 weeks old	DON (Sigma)	Single dose	Unknown		0, 12.5 or 25	Gavage	Lymphoid apoptotic depletion	12.5	<12.5	Islam et al. (2002, 2003)
Male B6C3F1 mice, 8–10 weeks old	DON (Sigma)	Single dose, effect assessment after 3 h	3		0, 1, 5 or 25	Gavage	Lymphoid apoptotic depletion	25	<25	Zhou et al. (1999)

Eq, equivalent to; IFN, interferon; IgA, immunoglobulin A; IGFALS, IGF acid-labile subunit; IgM, immunoglobulin M; ip, intraperitoneal; LOAEL, lowest-observed-adverse-effect level; LPS, lipopolysaccharides; PROD, pentoxyresorufin-*O*-deethylase; SOCS, suppressors of cytokine signalling; TG, transgenic; TGF, transforming growth factor; WT, wild type

[a] Where Eq is stated, doses were calculated from the feed using standard conversion factors.

(a) Altered host resistance and humoral and cell-mediated responses

(i) Mice

Groups of six female 5-week-old BALB/c mice were intubated with a single dose of DON at 10 mg/kg bw or with water vehicle and then intranasally instilled 2 h later with reovirus or saline vehicle. After 10 days, viral titres, virus ribonucleic acid (RNA) (L2) expression and histopathology of lungs in infected mice were determined. For a dose–response study, mice were gavaged with DON at 0, 2, 5, 10 or 25 mg/kg bw, followed by the same treatment as for the single-dose group, but effects were determined 3 days post-dosing. No pulmonary effects were seen in mice exposed to DON alone or in control mice. DON markedly exacerbated bronchopneumonia compared with exposure to reovirus alone. After 10 days, viral titres and viral L2 RNA expression of lung in infected mice were 10 times higher in the DON-treated group than in saline-treated mice (control group to reovirus). In the dose–response groups, viral L2 RNA expression in lung was increased at 2, 5, 10 and 25 mg/kg bw compared with controls. Viral-induced elevations of protein, monocyte chemotactic protein-1 (MCP-1), TNF-α and inflammatory cells in bronchoalveolar lavage fluid (BALF) were markedly enhanced at 3 days post-instillation in 10 mg/kg bw DON-exposed mice. DON exposure also upregulated induction of reovirus-specific immunoglobulin A (IgA) in BALF, faecal pellets and serum, preceded by elevated IL-6 expression and secretion in the lung. As effects on viral clearance were seen in the lowest dose group, a NOAEL could not be derived, but would be lower than 2 mg/kg bw (Li et al., 2007).

BALB/c mice (10 of each sex per dose, 6 weeks of age) were fed on a diet containing DON (purity >98%) at concentrations of 0, 0.25, 0.5, 1 and 2 mg/kg feed, equivalent to 0, 0.038, 0.075, 0.15 and 0.3 mg/kg bw per day, for 14 or 28 days. Food intake and body weights were measured weekly. After 14 or 28 days, the mice were killed, and blood was collected for haematology. Spleens were removed and used to prepare single-cell suspensions. Food intake and body weight gain were not affected by DON treatment. At day 14, but not at day 28, the percentages of CD19(+) leukocytes (in both sexes) in peripheral blood cells were statistically significantly decreased by 11%, 15% and 15% at dietary doses of 0.5, 1 and 2 mg/kg, respectively. Decreases in the percentages of mononuclear cells (up to 10%, in females only) were observed at dietary doses of 1 and 2 mg/kg at day 14 only. The percentages of CD11b(+) monocytes in peripheral blood leukocytes and total CD11b(+) splenic leukocytes were decreased (up to 32%) only in female mice fed DON at 1 and 2 mg/kg after 28 days. (Note that control levels in females had almost doubled between days 14 and 28.) The authors concluded that BALB/c mice adapted to DON exposure, as effects observed after 14 days had largely disappeared after 28 days of treatment. As effects of DON were most prominent in females, the study authors suggested that female sex hormones potentiate one potential marker of DON immunotoxicity in BALB/c mice (Wu et al., 2009). The Committee considered the transient decrease in CD19(+) leukocytes not biologically relevant.

Groups of 12 male BALB/c mice were dosed with DON at 0 or 2 mg/kg diet (equivalent to 0 or 0.3 mg/kg bw per day) for 14 days and then exercised to fatigue

on a treadmill. Mice were euthanized by decapitation, and trunk blood and spleens were collected for analysis of splenocyte proliferation, serum cytokine concentration and antibody response to sheep red blood cells. Only the non-exercised DON-fed mice showed significant suppression of splenocyte proliferation, 32.9% ± 17.9% of that of non-exercised controls (P = 0.021). Exercised controls and DON-fed exercised animals showed splenocyte proliferation of 68–75% of that of non-exercised controls. Antibody response to a T cell–dependent antigen, sheep red blood cells, was significantly less for exercised DON-fed mice than for controls (P = 0.031). Serum corticosterone levels were significantly higher for both exercised groups than for the unexercised groups (P < 0.001). IL-4 secretion from mitogen-stimulated splenocytes was elevated by DON alone (P < 0.05), whereas IL-2 concentration was elevated by DON with exercise stress (P < 0.05). As only one dose was given, a NOAEL could not be derived, but would be lower than 0.3 mg/kg bw per day (Landgren, Hendrich & Kohut, 2006).

Female BALB/c mice (n = 10) were given drinking-water containing DON (purity not reported) at 0.2–6 mg/l for 4 weeks. On day 14, the mice received a gastric inoculation with *Salmonella* Enteritides. The survival rate of mice was decreased at DON concentrations of 2 mg/l and higher. DON reduced the serum levels of TNF-α at 0.2 mg/l and increased the TNF-α levels at 2 and 6 mg/l (Sugita-Konishi, 2003).

(ii) Pigs

Two groups of 14 piglets aged 8 weeks (hybrid commercial, clinically healthy and pathogen-free castrated males) received tested control feed, devoid of mycotoxins, ad libitum or the same feed with pure DON (D0156, Sigma) daily for 6 weeks, at 0.5 mg/kg per pig (0.5 mg/kg bw per day) over the first week and 1 mg/kg per pig (1 mg/kg bw per day) over the following 5 weeks. Clinical assessment, haemochromocytometric examination, and characterization and quantification of CD3CD8+, CD4+CD8, CD4CD8+, CD8high, CD4+CD8+ and TCRγ/δ cells were performed at termination of the exposure. Histological and histochemical analyses were performed on samples of lymphoid organs (thymus, spleen, palatine tonsils, mediastinic and mesenteric lymph nodes), lungs, heart, skeletal muscle, liver, kidney, stomach and segments of the small and large intestine. The treatment of the pigs with DON did not induce alterations due to pathological effects on either clinical or cellular parameters. Although higher mean absolute values of natural killer (NK) cells and cytotoxic T lymphocytes were observed in the control group over the last experimental weeks, the treatment with DON did not significantly influence the levels of leukocyte subsets. The histopathological investigation of lymphoid tissues did not show any particular lesions of the parenchymal morphology and detected, by immunohistochemical assays, a normal composition and distribution of the lymphocyte subsets in the gut-associated lymphoid tissue. As no significant effects could be determined, the NOAEL was 1 mg/kg bw per day, the highest dose tested (Ferrari et al., 2009).

(b) Altered serum IgA levels

(i) Mice

Groups of 10 male C57BL6 mice were treated orally 3 days/week (Monday, Wednesday, Friday) for 4 weeks with "pure" DON (per cent purity not reported) in 0.150 ml 5% gum arabic solution at doses of 0, 0.014, 0.071, 0.355 or 1.774 mg/kg bw. Body weight was measured 3 times per week. After 4 weeks, the animals were killed, blood was collected and livers were weighed and stored for biochemical analysis. In the plasma, the following biochemical parameters were measured: alkaline phosphatase activity, osmolarity and levels of sodium, chlorine, carbon dioxide, phosphate, urea, glucose, IgA, immunoglobulin G (IgG) and immunoglobulin M (IgM). In liver tissue, patterns of P450 expression and activities of P450 (by measuring ethoxyresorufin-*O*-deethylase [EROD], methoxyresorufin-*O*-deethylase [MROD] and pentoxyresorufin-*O*-deethylase [PROD]) and glutathione-*S*-transferase were assessed. Body weight gain and liver weight were not affected by DON treatment. Plasma IgA levels were statistically significantly increased by 66%, 48% and 47% at DON doses of 0.071, 0.355 and 1.774 mg/kg bw, respectively. The other investigated plasma parameters were not affected by treatment. Treatment with DON at 0.014, 0.071 or 0.355 mg/kg bw increased liver microsomal PROD activity by 43%, 53% and 47%, respectively. Protein expression of the cytochrome P450 2b subfamily was increased by approximately 30% and 50% at 0.071 and 0.355 mg/kg bw, respectively. Glutathione-*S*-transferase activity was increased up to 39% and 78% by DON at doses of 0.071 and 0.355 mg/kg bw, respectively. At the highest dose, no effects on liver P450 and glutathione-*S*-transferase activity were observed. A significant competitive inhibition of 1-chloro-2,4-dinitrobenzene conjugation by DON in vitro suggests that DON may be a substrate for glutathione-*S*-transferases. The data suggest that a subchronic exposure to low (but not high) doses of DON causes changes in the normal liver metabolism of xenobiotics. The Committee noted that this specific low-dose effect of DON was not observed in other studies and concluded that these were of questionable biological relevance. As doses were given only 3 days/week, a daily dose could not be set for a NOAEL for elevated IgA in the serum (Gouze et al., 2006).

Transgenic p53+/− and corresponding wild-type mice (5–7 weeks old at the start of the study; starting body weights 23.0 ± 2.0 g for wild-type mice and 24.7 ± 1.3 g for transgenic mice) were exposed to DON (purity 95%) at 0, 1, 5 or 10 mg/kg diet (equivalent to 0, 0.15, 0.75 and 1.5 mg/kg bw per day) for 26 weeks. DON caused a significant dose-dependent reduction in body weight in wild-type and transgenic mice in the middle dose group, accompanied by declining liver fat stores. In wild-type mice, there was a significant trend towards increased plasma total IgA and decreased total IgM levels with increasing DON exposure, which was statistically significant in the highest dose group. In transgenic mice, plasma immunoglobulin levels were not affected. Kidney weights were increased in wild-type mice from the middle dose group and in transgenic mice in the highest dose group. IgA-positive glomeruli in kidney were increased in the highest dose groups in both strains. Real-time polymerase chain reaction (PCR) analyses indicated that

kidney cyclin D and cyclin E expression declined in DON-treated wild-type and transgenic mice. Overall, the effects of 26-week DON exposure on wild-type and transgenic mice were consistent with those previously seen in B6C3F1 mice exposed to DON for 2 years (Iverson et al., 1995). Based on the decreased body weight and increased kidney weight seen in the middle dose group, a NOAEL of 1 mg/kg, equivalent to 0.15 mg/kg bw per day (i.e. a similar order of magnitude as in the long-term mouse study from Iverson et al. [1995]), could be established (Bondy et al., 2009).

In a study on the mechanism of the immunotoxicity of DON, groups of 3–6 IL-6 knockout, wild-type and IL-6 sentinel mice were exposed to purified DON at 10 mg/kg bw in the diet (equivalent to 1.5 mg/kg bw per day) for 12 weeks. This dose induced serum IL-6 and IgA concentrations and increased mesangial IgA deposition in the kidney, but not in the IL-6 knockout mice. All treated groups had statistically significantly reduced feed intake and body weight gain during the study, as measured at weeks 6 and 12, which were similar for all three mouse strains (Pestka & Zhou, 2000). A later study by this group indicated that, in contrast to earlier assumptions, inhibition of cyclooxygenase-2 (COX-2, also induced by DON) expression or function did not prevent the DON-induced IgA increase but rather enhanced DON's capacity to promote IgA elevation after 16 weeks of exposure to the same dose (Jia & Pestka, 2005).

The effects of dietary treatment with purified DON (purity not reported) on serum IgE were assessed in female B6C3F1 mice (12 per group). Ingestion of DON at 25 mg/kg in the diet (equivalent to 3.75 mg/kg bw per day) resulted in 2.7-, 4-, 5- and 2.3-fold increases in serum IgE relative to controls after 12, 16, 20 and 24 weeks, respectively. When mice were fed DON at 25 mg/kg in the diet for 8 weeks and continued on toxin-free diet, serum IgE levels were 2.4-, 4-, 4.9- and 2-fold those of controls at 12, 16, 20 and 24 weeks, respectively. IgE levels were not significantly different between treatment and withdrawal groups at weeks 12–24. As only one dose was tested, a NOAEL for the reversible increase in serum IgA levels could not be determined, but would be below 3.75 mg/kg bw per day (Pestka & Dong, 1994). This study was not included in the 2001 monograph (Annex 1, reference *153*).

(ii) Pigs

Since the previous evaluation of DON, evidence for the induction of IgA concentrations in serum of pigs by DON has become available, as described below.

Groups of nine female weaned piglets (mean body weight 9.8 kg at the start of the study) were given 0, 0.3, 0.6 or 1.2 mg of isolated, pure DON per kilogram in the diet (equivalent to 0, 0.012, 0.024 and 0.048 mg/kg bw per day) for 8 weeks. Pigs were fed restrictively to allow complete feed intake by all animals. Body weight gain and biochemical and haematological values in the blood and serum, including concentrations of IgA, blood glucose, cortisol and insulin-like growth factor 1 (IGF-1), were determined. Body weight gain, food intake and feed conversion rate were not affected by DON treatment. Glucose levels tended to be decreased at the high dose throughout the treatment period (including at the start of the treatment). Cortisol and IGF-1 levels were not significantly affected. Small increases (up to

20%) in IgA levels were found at 0.6 and 1.2 mg/kg diet. As these latter effects in the two highest dose groups were not statistically significant, the NOAEL was 0.048, the highest dose tested (Drochner et al., 2004).

Effects on serum IgA levels were seen in 24 weanling pigs that were fed either control feed or feed naturally contaminated with DON at 2.2–2.5 mg/kg (equivalent to 0.088–0.1 mg/kg bw per day; other mycotoxins under LOD of 10–50 µg/kg feed) for 9 weeks. At days 4 and 15 of the experiment, the animals were subcutaneously immunized with ovalbumin. Total and specific IgA and IgG levels in serum and expression of mRNA encoding for cytokines such as TGF-α, IFN-α, IL-4 and IL-6 were also investigated in mesenteric lymph nodes, ileum and the spleen of piglets. IgA but not IgG upregulation could be observed in the serum of pigs exposed to the naturally contaminated diet. In vaccinated animals, DON also increased the concentration of ovalbumin-specific IgA and IgG. No significant effect of DON was observed in the samples from the ileum and spleen. By contrast, a significant reduction of mRNA expression encoding for both IFN-α and TGF-α was observed in mesenteric lymph nodes from DON-intoxicated animals. As only one dose was given, a NOAEL could not be derived, but would be lower than 2.2 mg/kg feed, equivalent to 0.088 mg/kg bw per day (Pinton et al., 2008).

Male and female pigs were fed DON at 0 or 3.5–5.3 mg/kg diet for 5–11 weeks in unequal group sizes. In total, six pigs were fed a DON-containing diet, but background levels were present in the control diet. Based on measured concentrations and feed intake, the DON exposure ranged from 0.051 to 0.213 mg/kg bw per day. Controls received a background concentration of DON, a maximum of 0.007 mg/kg bw per day. In vitro treatment of porcine monocyte-derived dendritic cells with DON interfered with phenotypic maturation of the dendritic cells, but also with antigen uptake and IL-10 secretion. Chronic dietary exposure of pigs to DON resulted in the generation of dendritic cells that failed to mature in response to TNF-α/lipopolysaccharides (LPS), but acquired a more mature phenotype in response to DON treatment in vitro. The study authors concluded that DON disrupts porcine dendritic cell function in vitro and in vivo. The Committee concluded that the study design is unsuitable for deriving a NOAEL (Bimczok et al., 2007).

(c) IgA-associated nephropathy

Increased mesangial IgA deposition in the kidney was found, together with induced serum IL-6 and IgA concentrations, in wild-type and IL-6 sentinel mice, but not in IL-6 knockout mice, after exposure to purified DON in the diet at 10 mg/kg bw (equivalent to 1.5 mg/kg bw per day) for 12 weeks. All treated groups had statistically significantly reduced feed intake and body weight gain during the study, as measured at weeks 6 and 12, which were similar for all three mouse strains (Pestka & Zhou, 2000).

(d) Cytokine expression

(i) Mice

Groups of 4–5 female B6C3F1 mice (3–4 weeks old) were treated with DON in phosphate-buffered saline by a single gavage at 0, 0.1, 0.5, 1, 5 or 12.5 mg/kg bw. Each hour up to 5 h after dosing, mice were euthanized, and blood, spleen, liver and muscle were sampled. In plasma, concentrations of TNF-α, IL-6, MCP-1, interferon-gamma (IFN-γ), IL-10 and IL-12p70 were determined using a bioassay kit. DON concentrations in serum were determined by ELISA. Suppressors of cytokine signalling (SOCS), some of which impair growth hormone (GH) signalling, are known to be induced by proinflammatory cytokines, which are upregulated by DON. In spleen, liver and muscle, concentrations of mRNA for four well-characterized SOCSs (cytokine-inducible SH2 domain protein [CIS], SOCS1, SOCS2 and SOCS3) were determined. The results showed that TNF-α and IL-6 mRNA and protein expression were rapidly induced (1 h) after exposure in several organs and plasma, respectively. Upregulation of mRNAs for the four SOCSs was either concurrent with (1 h) or subsequent to (2 h) cytokine upregulation. SOCS mRNAs were induced in muscle and spleen from 0.5 mg/kg bw and in liver from 0.1 mg/kg bw, with CIS, SOCS1 and SOCS2 occurring to a lesser extent than SOCS3. SOCS3 protein was detectable in the liver well after the onset of cytokine decline (5 h). Other SOCSs and cytokines were back to control levels after 5 h. DON concentration did not fully return to control levels after 5 h. Furthermore, hepatic SOCS upregulation was associated with about 75% suppression of GH-inducible IGF acid-labile subunit (IGFALS, an IGF-1-binding partner responsible for increasing the half-life of circulating IGF-1). Taken together, DON-induced cytokine upregulation corresponded to increased expression of several SOCSs and was associated with suppression of GH-inducible gene expression in the liver. As the (reversible) effect on SOCS mRNA expression in liver was seen in the lowest dose group, a NOAEL could not be derived, but would be lower than 0.1 mg/kg bw (Amuzie, Shinozuka & Pestka, 2009).

Groups of 4–5 female B6C3F1 mice (3–4 weeks old) were treated with an acute dose of DON at 0, 0.1, 0.5, 1, 5 or 12.5 mg/kg bw in phosphate-buffered saline by gavage, and liver sections were collected 2 h later. Bovine somatotropin (GH) was administered intraperitoneally at a dose of 5 mg/kg bw, at one or more time intervals (0–2 h) after DON gavage. Mice were euthanized at selected time intervals (1–4 h) after GH exposure, and the caudolateral portion of the liver's lateral lobe was collected for real-time PCR analysis of IGF-1, IGFALS, IGF binding protein 3 (IGFBP3) and SOCS3 mRNAs. In groups dosed with DON at 0.5–12.5 mg/kg bw, hepatic IGFALS mRNA levels were suppressed in a dose-dependent fashion, whereas DON at 0.1 mg/kg bw was without effect. In GH-treated mice, DON selectively suppressed hepatic IGFALS mRNA but increased IGF-1 and IGFBP3 mRNAs. The authors suggested that oral DON exposure perturbs the GH axis by suppressing two clinically relevant growth-related proteins, IGFALS and IGF-1, and therefore these effects would be related to the effects of DON on body weight. Based on the suppression of hepatic IGFALS mRNA levels in the second lowest dose group, a NOEL of 0.1 mg/kg bw could be derived (Amuzie & Pestka, 2010).

Groups of three male B3C3F1 mice (8–10 weeks old) were acclimatized for 1 week and given a single oral gavage of DON in 0.5 ml of 0.01 mol/l carbonate/bicarbonate buffer (pH 9.6). For determining dose–response effects on cytokine mRNA expression in spleen and Peyer's patches, groups received DON at 0, 0.1, 0.5, 1, 5 or 25 mg/kg bw and were euthanized 2 h post-dosing for tissue cytokine mRNA determination by reverse transcriptase PCR (RT-PCR) in combination with hybridization analysis. For determination of kinetic effects, mice were given a single dose of DON at 0 or 25 mg/kg bw and euthanized 1, 2, 4, 8 or 24 h after dosing. For serum cytokine determination, animals were given DON at 0 or 25 mg/kg bw, and blood was collected 3 h after exposure. In the two highest dose groups, statistically significantly elevated concentrations of the mRNAs for the proinflammatory cytokines IL-1β, IL-6 and TNF-α, the T helper 1 cytokines IFN-γ and IL-2, and the T helper 2 cytokines IL-4 and IL-10 were found. IL-12p40 mRNA was also induced, but not IL-12p35 mRNA. The effects were more pronounced in spleen than in Peyer's patches. IL-5 and TGF-β mRNAs were expressed constitutively in spleen and Peyer's patches but were not affected by DON. The kinetic study showed that peak levels were reached 2–4 h after exposure, and concentrations returned to control levels after 24 h in spleen and 24 h in Peyer's patches. DON treatment induced serum levels of TNF-α, IL-6 and IFN-γ 3 h after exposure to DON at 25 mg/kg bw. The NOAEL for reversible induction of cytokine mRNA in spleen and Peyer's patches in mice was 1 mg/kg bw (Zhou, Yan & Pestka, 1997). This study was also described in the 2001 monograph (Annex 1, reference *153*), but without the kinetics.

DON exposure enhanced LPS-induced expression of cytokines TNF-α, IL-6 and IL-1β in B6C3F1 mice acutely exposed to DON at 5 mg/kg bw by gavage (Zhou et al., 1999).

A single dose of purified DON at 25 mg/kg bw upregulated spleen cytokine and chemokine mRNA expression in 20-week-old B6C3F1 mice 2 h after acute exposure by gavage (Kinser et al., 2004).

In a study to test possible age differences in toxicokinetics and immune effects of DON, groups of weanling (3–4 weeks) and young adult (8–10 weeks) female mice were given a single dose of DON (Sigma) at 5 mg/kg bw by gavage. Expression of mRNAs for TNF-α, IL-1β and IL-6 in spleen, but not in liver or lung, was 2–3 times greater in weanling than in adult mice. Kinetics showed a higher uptake of DON in plasma, spleen, liver, lung and kidney in weanling mice compared with adult mice, but differences in concentrations were almost entirely diminished after 2 h. These data suggest that at these very high dose levels, young mice are modestly more susceptible than adult mice to the adverse effects of DON and that this might result from a greater toxin tissue burden resulting from differences in uptake (Pestka & Amuzie, 2008).

(e) Apoptosis in lymphoid tissue

DON potentiated LPS-induced lymphoid apoptotic depletion in B6C3F1 mice at acute oral doses of 12.5 mg/kg bw (Islam et al., 2002, 2003) and 25 mg/kg bw (Zhou et al., 2000).

2.2.7 Special studies on metabolites

The details on the special in vivo studies on metabolites of DON are summarized in Table 3 and described more fully below.

Table 3. Summary of special studies on metabolites

Species	Route	Purity	Effect	LOAEL	NOAEL	Reference
Mice, outbred albino [Crl:CDI (ICR) BR], males, weanling	Gavage	Purified 3-Ac-DON (purity not reported)	Clinical signs of toxicity, necrotic lesions in duodenal crypts, thymus and spleen, reduced mitotic activity	5 mg/kg bw per day	—	Schiefer et al. (1985)
Mice CD1 Swiss, male, 18–20 g	Diet	Purified 3-Ac-DON (purity not reported)	Increased T cell–dependent antibody response	10 mg/kg diet, equivalent to 1.5 mg/kg bw per day	5 mg/kg diet, equivalent to 0.75 mg/kg bw per day	Tomar, Blakley & Decoteau (1987)

(a) Mice

Groups of five male weanling outbred albino Crl:CDI (ICR) BR mice were given a single dose of purified 3-Ac-DON (purity not reported) at 0, 5, 10, 20 or 40 mg/kg bw in propylene glycol by intragastric administration and sacrificed 2, 4, 6, 12, 24, 48 or 96 h after dosing. The animals became clinically ill in all dose groups after 12 h, and some animals in the highest dose group died. Histological examination of duodenal crypts, thymus and spleen revealed the presence of necrotic lesions in all dose groups. As soon as 2 h after administration, mitotic activity was significantly reduced in all dose groups. No other tissues were examined. The authors concluded that the intensity of lesions in the 40 mg/kg bw group corresponded to lesions known to be caused by 4 mg/kg bw of T-2 toxin, but data supporting this conclusion were not presented. Together with the results from a rabbit skin bioassay (not summarized), the authors concluded that 3-Ac-DON was considerably less toxic than T-2 toxin, but caused acute effects in the dividing cells of the body in a manner characteristic of trichothecenes. As effects were seen in all dose groups, a NOAEL for 3-Ac-DON could not be derived, but would be lower than 5 mg/kg bw. The Committee concluded that this study could not be used for comparison of toxicity between 3-Ac-DON and DON (Schiefer et al., 1985). This study was not described in the 2001 monograph (Annex 1, reference *153*).

The effects of purified 3-Ac-DON (purity not reported) on mitogen-induced lymphocyte proliferation and antibody production were studied in male CD-I mice exposed to 3-Ac-DON at 0, 2.5, 5 or 10 mg/kg in the diet for 35 days. Concentrations were not checked after preparation of diet. The authors reported no effects on

mitogen-induced lymphocyte proliferation, but a slight (non-significant) effect was seen, and T cell–independent antibody responses to dinitrophenyl-ficoll or *Escherichia coli* were seen. The T cell–dependent antibody response to sheep red blood cells was increased in the group fed 3-Ac-DON at 10 mg/kg. In vitro, 3-Ac-DON inhibited lymphocyte proliferation in a dose-dependent manner. The authors suggested that the in vitro effects of 3-Ac-DON may not reflect its in vivo immunotoxicity. A NOAEL of 5 mg/kg diet could be derived, equivalent to 0.75 mg/kg bw per day (Tomar, Blakley & Decoteau, 1987). This study was not described in the 2001 monograph (Annex 1, reference *153*).

(b) In vitro

The cytotoxicity of the de-epoxy metabolites of trichothecenes NIV and DON was determined by DNA synthesis in 3T3 mouse fibroblasts (5-bromo-2′-deoxyuridine [BrdU] bioassay) and compared with the cytotoxicity of the respective toxin with an intact epoxy group and their acetylated derivatives. The toxicities of NIV and DON expressed as the concentration inhibiting 50% of the DNA synthesis (IC_{50}) occurred at similar micromole per litre concentrations (1.19 ± 0.06 and 1.50 ± 0.34 µmol/l). The toxicity of fusarenon X (4-acetyl-NIV) in the assay was similar to the toxicity of NIV, and the toxicity of 15-Ac-DON was equal to the toxicity of DON. 3-Ac-DON was 9 times less toxic than DON and 15-Ac-DON. The IC_{50} value for de-epoxy-DON was 54 times higher in the assay than the IC_{50} for DON, whereas the IC_{50} of de-epoxy-NIV was 55 times higher than the IC_{50} for NIV (Eriksen, Pettersson & Lundh, 2004).

DON, 3-Ac-DON and ZEA (purities not reported) were examined for their in vitro effect on mitogen-induced lymphocyte blastogenesis using rat or human peripheral blood lymphocytes as measured by incorporated [^{3}H]thymidine. Results were compiled from 20 experiments, and experiments were performed using five replicates. A dose-dependent reduction of lymphocyte proliferation was demonstrated for each mycotoxin. However, the inhibitory effect of DON was significantly higher than that of the acetylated compound. DON concentrations of 90 ng/ml and 220 ng/ml inhibited rat and human lymphocyte blastogenesis by 50%, respectively, whereas 3-Ac-DON concentrations of 450 ng/ml and 1060 ng/ml were required to produce the same effect. The amount of ZEA necessary to inhibit blastogenesis by 50% was 250 times greater than the amount of DON required. In lymphocyte cultures containing 50 ng DON, the addition of 1.5, 2.5 or 5 µg ZEA caused a depression of lymphocyte proliferation that was equal to the sum of that produced by the individual trichothecenes. When 1.5 µg ZEA was added to cultures containing 150 ng DON, a similar additive effect was observed. There was no evidence of cell death, and combinations of DON and ZEA did not alter the expected response. This study shows that 3-Ac-DON is 5 times less potent than DON in inhibiting mitogen-induced lymphocyte blastogenesis in vitro and that rat lymphocytes are approximately 2 times more sensitive to this effect than human lymphocytes. The effects of DON and ZEA were additive in this experiment. The Committee noted that the conversion of 3-Ac-DON to DON as determined in the study of Eriksen, Pettersson & Lindberg (2003) makes this in vitro study of doubtful

relevance for assessing relative potency in vivo (Atkinson & Miller, 1984). This study was not described in the 2001 monograph (Annex 1, reference *153*).

The ability of human gastrointestinal organisms to transform the trichothecenes 3-Ac-DON and NIV was investigated in vitro. Samples of human faeces were incubated under anaerobic conditions for 48 h with 10 µg/l of the toxins. They were then extracted and analysed for trichothecenes and metabolites. The recovery of the toxins in the control sample was 90–96%, and the recovery of the sum of the toxin and de-epoxide metabolite was similar to the recovery in the control sample. 3-Ac-DON was metabolized to DON during the incubation period (78% ± 30%). In contrast to what has been reported for other species such as rats, mice and pigs, no de-epoxidated metabolites were detected in the faecal incubates. The toxicological significance of the difference in the intestinal ability to transform trichothecenes between species is unknown (Eriksen & Pettersson, 2003).

2.2.8 Special studies on species differences

Pestka & Smolinski (2005) concluded from available literature that there are marked species differences in sensitivity to DON, with the pig being most sensitive, followed, in decreasing order, by rodent, dog, cat, poultry and ruminants. They stated that primate or human studies on DON-induced emesis have not been reported to date. However, they concluded that, based on the use of porcine models for human intestinal function (Nejdfors et al., 2000) and drug-induced emesis (Szelenyi, Herold & Gothert, 1994), it was not unreasonable to speculate that humans are as sensitive as pigs to DON.

In a study comparing mycotoxin cytotoxicity in several mammalian cell lines measured by metabolic activity (cleavage of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MTT) assay, sensitivities of cell lines to DON were found in the following decreasing order: Chinese hamster ovary CHO-K1 > Chinese hamster lung fibroblast (V79) > BALB/c mice keratinocyte cell line (C5-O) > human Caco-2 > human hepatocellular carcinoma (HepG2) cells, with IC_{50} values of 0.27, 0.49, 0.54, 1.02 and 8.36 µg/ml, respectively, after 48 h exposure. This could suggest that humans are less sensitive to the cytotoxicity of DON than mice and hamsters (Cetin & Bullerman, 2005). However, the Committee considered that this study did not provide a reliable basis for interspecies comparison of toxicity in vivo.

In a study using in vitro, ex vivo and in vivo approaches to determining the effects of DON on gastrointestinal epithelium, human Caco-2 cells exhibited a lower sensitivity to DON-induced increase in permeability compared with porcine IPEC-1 cells in vitro. The study is described in section 2.2.2 above (Pinton et al., 2009).

2.3 Observations in humans

The potential deleterious effects of DON on humans have been reviewed by Creppy (2002), Sudakin (2003), Pestka & Smolinski (2005), Pronk, Schothorst & van Egmond (2002) and Fokunang et al. (2006).

Since the fifty-sixth meeting of the Committee, risk assessments or reviews on DON have been performed by the Scientific Committee on Food (SCF, 1999,

2002), the European Food Safety Authority (EFSA, 2004), the Health Council of the Netherlands (2001), the Dutch National Institute for Public Health and the Environment (Pieters et al., 2001; Pieters, Bakker & Slob, 2004; Boon et al., 2009) and the French Food Safety Agency (AFFSA, 2006). All but the Health Council of the Netherlands (2001) derived the same health-based guidance value of 1 µg/kg bw per day for long-term intake of DON based on the same critical study used by the fifty-sixth meeting of the Committee (Iverson et al., 1995). The Health Council of the Netherlands (2001) derived a tolerable daily intake (TDI) of 0.5 µg/kg bw per day based on the NOAEL of 0.11 mg/kg bw per day from the study by Iverson et al. (1995), but applied a uncertainty factor of 210, composed of uncertainty factors of 10 for intraspecies differences and 3 for interspecies differences and a scaling factor of 7 for differences in energy use, as an indicator for metabolism differences between humans and mice.

An overview of available data on DON and the research needs has been compiled by the International Life Sciences Institute (Larsen et al., 2004) and the United States National Toxicology Program (NTP, 2009a). Recommendations from the latter were in line with those of the fifty-sixth meeting of the Committee (NTP, 2009b).

2.3.1 Epidemiology

Reddy & Raghavender (2008) reviewed outbreaks of mycotoxicoses in India (Reddy & Raghavender, 2008; Raghavender & Reddy, 2009), but reports relating to the DON outbreak were evaluated by the Committee previously. No new information could therefore be taken from this review.

2.3.2 Development of a urinary biomarker of exposure

Urine samples were collected from 11 female inhabitants of Linxian County, Henan Province, China (studies on occurrence data in these areas were included in the 2001 monograph; Annex 1, reference *153*), a high-risk region for oesophageal cancer and an area of potentially high DON exposure, as the staple diet consists of corn and wheat; and from 4 female inhabitants of Gejiu, Yunnan Province, a low-risk region in China, where the staple diet consists primarily of rice. Participants were selected from eligible non-smoking volunteers between the ages of 19 and 75 years. Each subject was given a sterile container, and up to 100 ml of first-voided morning urine was collected and placed in a light-protected bag. The urine samples were then kept frozen until analysis. DON was detected in all 15 samples following β-glucuronidase treatment and immunoaffinity column enrichment, with the identity of DON being confirmed by mass spectrometry. The mean levels of DON from the suspected high- and low-exposure regions of China were 37 ng/ml (range 14–94 ng/ml) and 12 ng/ml (range 4–18 ng/ml), respectively. Given that approximately 30% of the total DON consumed is excreted during a 24 h period in the animal model and assuming that a 60 kg person produces 1 litre of urine per day and that there is a 40% recovery of DON in human urine samples, the levels detected in the high- and low-risk populations were believed to represent a daily exposure ranging from 1.9 to 13.0 mg/kg bw per day and from 0.6 to 2.5 mg/kg bw per day, respectively (Meky et al., 2003).

To better assess exposure to DON at the individual level, a urinary assay was developed, incorporating immunoaffinity column enrichment and liquid chromatography–mass spectrometry (LC-MS) detection. Further refinement of this urinary assay, by inclusion of [^{13}C]DON as an internal standard, was then undertaken and tested within the United Kingdom. DON was frequently observed in urine and was associated with cereal intake. A dietary intervention study demonstrated that avoiding wheat in the diet reduced urinary levels of DON (Turner et al., 2008a).

Twenty-five volunteers from the United Kingdom (aged 21–59 years) completed semi-weighed food diaries on days 1 and 2 (normal diet), and a morning urine sample was provided on day 3. On days 3–6 (intervention), individuals restricted major sources of wheat intake following dietary guidance. Diaries were completed on days 5 and 6, and a further morning urine sample was provided on day 7. Urinary DON was measured following immunoaffinity column cleanup and analysis by LC-MS. Wheat-based food intake (mean 322 g/day, range 131–542 g/day) was significantly ($P < 0.001$) reduced during the intervention to 26 g/day (range 0–159 g/day), indicating good compliance. DON was detected in all 25 urine samples taken on day 3 (geometric mean DON concentration of 7.2 ng/mg creatinine; 95% confidence interval [CI] 4.9–10.5 ng/mg), but following the intervention, there was a significant 11-fold reduction ($P < 0.001$) to 0.6 ng/mg (95% CI 0.4–0.9 ng/mg). One individual who increased wheat intake during the intervention instead of lowering it had elevated DON levels in the urine (Turner et al., 2008b).

In another study by the same group, the United Kingdom adult National Diet and Nutrition Survey was used to compare 24 h urinary DON excretion with cereal intake. One hundred subjects were identified for each of the following cereal consumption groups: low (mean 107 g of cereal per day; range 88–125 g/day), medium (mean 179 g/day; range 162–195 g/day) and high (mean 300 g/day; range 276–325 g/day). DON was analysed in 24 h urine samples by LC-MS after purification on immunoaffinity columns. DON was detected in 296 of 300 (98.7%) urine samples. Cereal intake was significantly associated with urinary DON ($P < 0.0005$), with the geometric mean urinary levels being 6.55 µg/day (95% CI 5.71–7.53 µg/day), 9.63 µg/day (95% CI 8.39–11.05 µg/day) and 13.24 µg/day (95% CI 11.54–15.19 µg/day) for low, medium and high exposure groups, respectively. In multivariable analysis, wholemeal bread ($P < 0.0005$), white bread ($P < 0.0005$), "other" bread ($P < 0.0005$), buns/cakes ($P = 0.003$), high-fibre breakfast cereal ($P = 0.016$) and pasta ($P = 0.017$) were significantly associated with urinary DON. Wholemeal bread was associated with the greatest per cent increase in urinary DON per unit of consumption, but white bread contributed approximately twice as much as wholemeal bread to the urinary DON levels, because it was consumed in higher amounts (Turner et al., 2008c). The Committee concluded that this biomarker could be used for systemic DON exposure resulting from dietary exposure to DON and its derivatives, as DON could be metabolized from other precursors.

In a more detailed analysis of the previous study, food diary information ($n = 255$) for the day of urine collection (model I), the previous 24 h period (model II) and the day of urine collection plus the previous 24 h combined (model III) was

further examined to assess whether the recent intake of cereal correlated more strongly with urinary DON, compared with the longer-term assessment of usual cereal intake from 7-day food diaries (model IV). DON was detected in 254/255 (99.6%) urine samples (mean 12.0 µg/day; range not detected to 66 µg/day). For all the models, total cereal intake was positively associated with urinary DON ($P < 0.001$) in each model. The goodness of fit (adjusted R^2 value) was used to assess how well each model explained the variation in urinary DON. Model I provided a better goodness of fit (adjusted R^2 0.22) than did model IV (adjusted R^2 0.19), whereas model III provided the best fit (adjusted R^2 0.27). The authors suggested that the interindividual variation in urinary DON was somewhat better explained by recent cereal intake than by usual cereal intake assessed over 7 days (Turner et al., 2009).

In a study aimed at correlating urinary DON levels with one or more metabolites in the urine, the urinary metabolome of 22 adults from the United Kingdom (7 males, 15 females; age range 21–59 years) for whom urinary DON levels had been previously determined using an established LC-MS assay was analysed using a nuclear magnetic resonance (NMR)-based metabolomics approach coupled with multivariate statistical analysis. The authors suggested that, based on the metabolic profiling, hippurate levels could be used to distinguish between groups with low (3.6 ng/mg creatinine; 95% CI 2.6–5.0 ng/mg) and high (11.1 ng/mg; 95% CI 8.1–15.5 ng/mg) DON exposure, with the concentration of hippurate being significantly ($P = 0.047$) higher (1.5 times) for people with high DON exposure than for those with low DON exposure (Hopton et al., 2010).

2.3.3 Derivation of a lower dose for emetic responses to DON in humans

Analyses of two DON intoxication events in humans reported in the 2001 monograph (Luo, 1988; Guo et al., 1989) provide data that have allowed the Committee to approximate a lower dose in humans that might cause an emetic response. In one event, it was found that foodstuffs contaminated with DON at 1–40 mg/kg did not cause emesis, whereas in the second event, foodstuffs contaminated with DON at concentrations between 3 and 98 mg/kg did cause emesis. If it is assumed that food contaminated at 50 mg/kg could cause an emetic response, the following can be calculated. If a 50 g portion of food were consumed, a dose of 2.5 mg of DON would be delivered (0.05 kg × 50 mg/kg). In a 50 kg individual, this would be a dose of 0.05 mg/kg bw (2.5 mg/50 kg bw).

The use of a small portion of food and a relatively small standard body weight in the estimation above assures that the calculated emetic response level in humans is conservatively low for comparisons with pigs. For illustrative purposes, if a food portion of 200 g was necessary to cause emesis in a 20 kg child, the calculated dose would be 0.5 mg/kg bw, 10-fold higher than the above calculation.

Additional information was available on a lower no-effect dose of DON with respect to emesis. In Henan, China, in 1985, no cases of acute illness were observed among 191 peasant families who ate scabby wheat containing DON at a concentration of 0.016–3.3 mg/kg (mean 0.92 mg/kg) and NIV at a mean concentration of 0.13 mg/kg (both measured by gas chromatography with electron

capture detection [GC-ECD]). Assuming a loss of DON during processing of approximately 30% and consumption of 560 g per person, the authors estimated an intake of DON of 0.380–0.520 mg per adult, which, for a body weight of 50 kg, would give an intake of 0.0075–0.010 mg/kg bw.

3. ANALYTICAL METHODS

3.1 Chemistry

The chemistry of DON, a type B trichothecene, was summarized by the fifty-sixth meeting of the Committee (Annex 1, reference *153*).

3.2 Chemical analysis

Since the monograph for the fifty-sixth meeting of the Committee was written, considerable research has been conducted on analytical methods for the determination of DON, as well as its 3- and 15-acetyl derivatives and DON-3-glucoside. The most important development during this period has been the use of MS or tandem MS (MS/MS) coupled to HPLC (LC-MS/MS) for DON determination in a range of matrices either with or without sample extract cleanup.

The purity and stability of calibrants for mycotoxin analysis are critical issues. DON appears to be stable if kept in acetonitrile solution at 25 °C for 24 months (Widestrand & Pettersson, 2001). The European Commission (EC) funded a project to produce certified calibrants of DON, 3-Ac-DON and 15-Ac-DON in acetonitrile (Krska et al., 2007). These mycotoxins were purified from available *Fusarium* culture material and chemically characterized by ultraviolet (UV) and infrared spectroscopy, HPLC, GC with ECD, flame ionization detection (FID) and MS detection, elemental analysis and NMR. Temperature stability studies confirmed the long-term stability of the standards in acetonitrile. Molar absorptivity coefficients for DON, 3-Ac-DON and 15-Ac-DON were 6805 ± 126 litre/cm per mole, 6983 ± 141 litre/cm per mole and 6935 ± 142 litre/cm per mole, respectively, based on an interlaboratory study. The calibrator for DON produced by the project is commercially available as a sealed ampoule from the Institute for Reference Materials and Measurements in Geel, Belgium.

A number of reviews may be consulted for detailed information on analytical methods. These cover the current state of trichothecene determination in general and DON determination in particular (Krska, Baumgartner & Josephs, 2001; Mateo et al., 2001; Koch, 2004; Lattanzio, Pascale & Visconti, 2009), more general aspects of mycotoxin determinations (Krska et al., 2005, 2008; Sforza, Dall'Asta & Marchelli, 2006; Cigi & Prosen, 2009; Turner, Subrahmanyam & Piletsky, 2009), LC-MS/MS of mycotoxins (Zollner & Mayer-Helm, 2006), immunoaffinity column cleanup techniques for general food analysis, including mycotoxins (Senyuva & Gilbert, 2010), and developments in immunosensors (Ricci et al., 2007).

3.2.1 Screening tests

Various analytical techniques have been adapted and developed to screen for DON. These include thin-layer chromatography (TLC), infrared spectroscopy and a number of assays reliant on immunological principles using anti-DON antibodies, including ELISAs, test strips, surface plasmon resonance and direct fluorescence and fluorescence polarization measurements. A number of these immunological assays are commercially available and have been reviewed (Schneider et al., 2004). They should be used to analyse only matrices for which they are validated and in the test ranges set by the manufacturers. Non-commercial immunochemical assays should be carefully validated in the developing laboratory and are generally limited in use to the research laboratory. A policy on antibody characterization for conducting Association of Official Analytical Chemists (AOAC) collaborative studies for immunochemical methods stipulates that monoclonal and polyclonal antibodies should be described in terms of their purification method, avidity, specificity (cross-reactivity), matrix effects, selectivity (binding in immuno-affinity column formats) and specific capacity in the assay format (Fremy & Usleber, 2003).

ELISA is a well-established analytical format, mostly available from commercial companies with LODs and analytical ranges relevant to legislative requirements. However, useful antibodies, such as one for simultaneously detecting both DON and NIV, are still being described (Maragos, Busman & Sugita-Konishi, 2006). In addition, antibodies described before the discovery of DON-3-glucoside can now be tested for cross-reactivity with this plant metabolite, which can be responsible for overestimation of DON levels in conventional ELISA tests for the mycotoxin (Ruprich & Ostry, 2008). Similarly, commercial companies have continued to develop the concept of fluorometry for rapid (around 15 min) testing in which the cereal extract is cleaned up on proprietary columns and derivatized with fluorogenic reagent before measurement of total fluorescence on a proprietary fluorometer with dedicated software (Malone, 2001; Hafner et al., 2007). Fluorescence polarization is a technique that was first described nearly 50 years ago and has recently, with improved commercial instrumentation, been adapted for mycotoxin determination in wheat, semolina and pasta (Maragos & Plattner, 2002; Maragos, Jolley & Nasir, 2002; Lippolis, Pascale & Visconti, 2006; Maragos, 2006). Fluorescence polarization does not require separation of a bound and free label as in ELISA, but is performed purely as a solution-phase assay. It relies on the measurement of the rate of rotation of fluorescent molecules in which smaller molecules (such as a fluorescent-labelled mycotoxin substrate) rotate faster than larger molecules (such as the same fluorescent-labelled mycotoxin that has competed with unlabelled analyte for binding on the relevant antibody). These assays have been reported to have LODs of 0.1 mg/kg and recoveries above 90%, depending on the matrix and the tracer used (Maragos & Plattner, 2002; Lippolis, Pascale & Visconti, 2006). A disadvantage of fluorescence polarization is the presence of a background or matrix effect from cross-reacting compounds, which made the method unsuitable for maize and required a background correction for wheat and wheat products (Maragos & Plattner, 2002).

A number of other immunological methods have been investigated for the screening of DON. Assays based on surface plasmon resonance have been developed and tested for determination of DON in wheat by comparison with LC-MS/MS determinations (Tudos, Lucas-van den Bos & Stigter, 2003) or by comparison with GC-MS or HPLC (Schnerr, Vogel & Niessen, 2002). One of the simplest and fastest technologies is the lateral flow device, usually in the format of a strip or dipstick, which provides a simple test for contamination above or below a set level (Kolosova et al., 2008; Xu et al., 2010). DON in the sample extract interacts with colloidal gold–conjugated anti-DON antibodies at the base of the stick. Both bound and unbound antibodies are carried along the stick membrane by the extract solvent, passing a test line composed of immobilized mycotoxin, which will bind free antibody to form a visible line indicating a level of DON contamination below the test cut-off value. Typically, commercial kits contain a control line farther along the stick composed of anti-antibodies as a control for complete extract migration along the strip. Issues related to this technology, apart from the matrices for which the test is valid, include the cut-off limit set by the producer and the degree of false negatives during testing. As this is a screening technology, false positives are less serious, as such samples would normally be further tested by a fully quantitative method. This system has been commercialized for semiquantitative results by including two test lines and a proprietary photometric reader (Chrpova et al., 2008).

The desire for multiple analyses has resulted in the development of array biosensors, which can be used for simultaneous analysis of multiple samples or simultaneous analyses of multiple target analytes (Ngundi et al., 2006; Sapsford et al., 2006). The multiple targets for this technology included large pathogenic bacteria (*Campylobacter* spp.), as well as DON and other mycotoxins, such as aflatoxin B_1, ochratoxin A and fumonisin B_1. Silanized microscope slides were patterned with suitable capture species for the sandwich immunoassay used for the bacterial assay and the competitive immunoassay used for the mycotoxin assay. The glass slides acted as a waveguide for the detection system, which involved incident laser light launched into the end of the waveguide and charge coupled device (CCD) camera recording of the fluorescence of surface-bound species resulting from excitation by the evanescent wave.

All the above screening techniques require the extraction of DON from the sample before the analytical step. Considerable interest has been shown in developing a non-destructive instrumental method for detection of DON in ground wheat or maize without sample extraction by using near-infrared (10 000–4000/cm), mid-infrared (4500–650/cm) or Raman (3600–100/cm) spectroscopy combined with chemometric analysis (Pettersson & Aberg, 2003; Kos, Lohninger & Krska, 2003; Kos et al., 2004, 2007; De Girolamo et al., 2009; Liu, Delwiche & Dong, 2009). These approaches have shown potential for discrimination between wheat batches at levels that would be useful in terms of the limits set by the EC for DON, but they require large data sets for calibration.

3.2.2 Quantitative methods

The fifty-sixth meeting of the Committee summarized the basic steps for quantification of DON in cereals and food matrices, reviewing extraction, cleanup,

chromatographic separation, detection and performance characteristics. Apart from separation by TLC, both GC and HPLC have been used, and researchers have investigated two-dimensional GC (Jelen & Wasowicz, 2008). For quantification by GC, flame ionization, electron capture and MS detectors have been used. Of these three methods, FID has had limited use, and most publications have reported either ECD or MS detection (Mateo et al., 2001; Krska, Baumgartner & Josephs, 2001; Koch, 2004; Lattanzio, Pascale & Visconti, 2009). MS or MS/MS has the advantage of sensitivity, as well as providing confirmatory evidence in the form of characteristic fragment ions. DON and other trichothecenes are oxygenated polar compounds and require derivatization to increase volatility before they can be injected into a GC column. However, as the trichothecenes are structurally similar and possess similar chemical properties, GC offers the advantage of being capable of determining a range of trichothecenes simultaneously, including 3-Ac-DON and 15-Ac-DON. Common derivatization reactions at the hydroxyl moieties of DON involve the formation of trimethylsilyl ethers or trifluoroacetyl, pentafluoropropionyl or heptafluorobutyryl ester derivatives. Problems of multiple reaction products can be overcome by using mixtures of derivatization reagents, such as 1-(trimethylsilyl)-imidazole, trimethylchlorosilane and *N,O*-bis(trimethylsilyl)acetamide (Mateo et al., 2001). The silylating reagent, *N,N*-dimethyl-trimethylsilyl-carbamate, has been proposed as a suitable single reagent for silylation of DON, NIV and diacetoxyscirpenol (Eke & Torkos, 2004). The analytical method was applied to the determination of DON and NIV in maize grits and semolina (Eke, Kende & Torkos, 2004). MS detection limits of 0.05–0.35 mg/kg were slightly lower than those of 0.30–0.47 mg/kg achieved for GC-FID. For fluoroacylation of type B trichothecenes, the heptafluorobutyryl esters are preferable to trifluoroacetyl esters in terms of response, but are unsuitable for determination of 15-Ac-DON as a result of stereochemical hindrance during the derivatization reaction (Mateo et al., 2001). Other authors have found pentafluoropropionic anhydride to be preferable to heptafluorobutyric anhydride as a derivatization reagent due to its greater stability against moisture (Valle-Algarra et al., 2005). Quantitative methods have used a number of different internal standards, with mirex being used for ECD (Koch, 2004) and *n*-docosane, neosolaniol or α-chloralose being employed in FID or MS detection (Schothorst & Jekel, 2001; Eke, Kende & Torkos, 2004; Jestoi, Ritieni & Rizzo, 2004). GC-MS has been employed for trichothecene analysis and identification using electron impact ionization, negative chemical ionization and positive chemical ionization (Melchert & Pabel, 2004). Melchert & Pabel (2004) provided a list of key fragment ions of trimethylsilyl derivatives of various trichothecenes for toxin identification using the above three ionization methods in an ion trap system operating in the multiple MS mode. More recently, a fully ^{13}C-labelled DON has been used as an internal standard in GC-MS (Neuhof et al., 2009).

In order to avoid the problems of derivatization for GC, methods have been developed for the determination of DON using HPLC with UV detection at a wavelength of 220 nm. A number of methods for DON in different matrices using HPLC-UV have been validated and their performance characteristics determined by interlaboratory collaborative studies. MacDonald et al. (2005) studied an HPLC method with UV detection (220 nm) for the determination of DON in cereals (oat flour, rice flour and wheat flour) and cereal products (polenta and wheat-based

breakfast cereal). DON was extracted from samples with water by homogenization, and, after filtration, an aliquot was cleaned up on an immunoaffinity column. The column was washed with water and DON eluted with acetonitrile or methanol. Mean recoveries ranged from 78% to 87% at levels between 200 and 2000 µg/kg. Intralaboratory repeatability (relative standard deviation for within-laboratory results) was 3.1–14.1%, and interlaboratory reproducibility (relative standard deviation for between-laboratory results) was 11.3–26.3%. Horwitz ratio values were less than 1.3. The method was validated for determinations above 100 µg/kg. This method was further validated by Neumann et al. (2009) to test its applicability for the analysis of soft wheat. In this second study, repeatabilities ranged from 3.1% to 14.8% and reproducibilities from 21.0% to 32.9%, and Horwitz ratio values were 1.0–1.9. Mean recovery at 500 µg/kg was 84%.

Sugita-Konsihi et al. (2006) also studied an HPLC method with UV detection (220 nm) for the determination of DON in wheat. The method involved extraction of DON with acetonitrile–water (85:15 by volume) by shaking for 30 min. An aliquot was then cleaned up on a multifunctional column, with a portion of the eluate being collected, dried down and reconstituted in HPLC mobile phase. Intralaboratory repeatability was 5.8–11.3%, and interlaboratory reproducibility was 12.0–20.7%. Mean recovery was 100.0% at a level of 1.1 mg/kg, and Horwitz ratio values were less than 1.0. The LOD was 0.10 mg/kg. Another interlaboratory study has determined the performance characteristics of an HPLC method with UV detection for baby food and animal feed (Stroka et al., 2006). The samples were extracted with water by shaking for 1 h. Thereafter, an aliquot was cleaned up on an immunoaffinity column. Repeatability for analysis of baby food was 6.4–14.0% and for animal feed was 6.1–16.5%. Reproducibility was 9.4–19.5% for baby food and 10.5–25.2% for animal feed. Horwitz ratio values were equal to or less than 1.3. The authors recommended the method for DON at levels equal to or above 60 µg/kg for baby food and equal to or above 200 µg/kg for animal feed. Mean recoveries ranged between 89% at 120 µg/kg and 85% at 240 µg/kg for baby food and between 100% at 200 µg/kg and 93% at 400 µg/kg for animal feed.

Although the above validated methods rely on the natural UV absorption of DON, other methods involving derivatization have been developed to improve LODs using fluorescence. Precolumn derivatization with coumarin-3-carbonyl and reversed-phase HPLC with fluorescence detection allowed the determination in wheat of three type A trichothecenes and five type B trichothecenes, including DON, 3-Ac-DON and 5-Ac-DON, with LODs of 0.2–1 µg/kg (Dall'Asta et al., 2004). A post-column derivatization with methyl acetoacetate achieved the determination of DON, 3-Ac-DON and 15-Ac-DON in wheat down to an LOD of 8 µg/kg (Buttinger & Krska, 2003).

Since the fifty-sixth meeting of the Committee, the most significant advance in mycotoxin analysis has been the application of LC-MS(/MS) with atmospheric pressure ionization techniques, such as electrospray ionization, atmospheric pressure chemical ionization or atmospheric pressure photoionization, for the determination and confirmation of mycotoxins. Typically, MS detection is achieved by selected ion monitoring of a pseudo-molecular ion (frequently the protonated molecular ion in positive electrospray ionization) or ion fragment or by multiple

reaction monitoring of a given fragmentation product for quantification and of one or two other specific fragments as confirmation (Zollner & Mayer-Helm, 2006). For mycotoxin determination, triple quadrupole instruments are the most commonly used, although ion trap and time of flight instruments have also found application. LODs achieved in these assays are strongly instrument dependent, but are generally sufficient to meet the strictest legislative requirements. The most important feature (and problem) for method validation of LC-MS/MS methods is the phenomenon of enhancement or suppression of the analytical signal due to the presence of matrix components in the HPLC column eluate entering the MS ionization source. This has the effect of increasing or decreasing the slope of the calibration line (Sulyok, Krska & Schuhmacher, 2007a). The effect is generally overcome by using matrix-matched standard solutions for calibration. Alternatively, some research groups have synthesized stable isotope–labelled (generally ^{13}C) mycotoxins for use as internal standards, and these are now commercially available (Haubl et al., 2006; Asam & Rychlik, 2007). Apart from the above considerations, two distinct trends have emerged in the application of MS in HPLC systems. The MS can be viewed as a sensitive and specific detector for single or chemically similar toxins, such as its use for concurrent analysis of cereals for DON and NIV (Plattner & Maragos, 2003; Tanaka et al., 2009). Alternatively, the power of the modern MS instrument in achieving rapid mass analysis can be utilized to determine multiple toxins in a single HPLC run (Tanaka et al., 2006; Lattanzio et al., 2007; Ren et al., 2007; Sulyok, Krska & Schuhmacher, 2007b; Spanjer, Rensen & Scholten, 2008; Beltrán et al., 2009; Frenich et al., 2009; Monbaliu et al., 2009). This latter use of the MS highlights the second trend in LC-MS/MS analysis of mycotoxins—namely, the use of a "dilute-and-shoot" technique in which the latest instruments are sufficiently sensitive for the sample extract to be merely diluted without a specific cleanup and injected directly into the HPLC system (Sulyok, Krska & Schuhmacher, 2007b; Spanjer, Rensen & Scholten, 2008; Beltrán et al., 2009; Frenich et al., 2009). Where matrix effects are associated with this technique, matrix-matched standards can be used. This approach circumvents the problem of a single cleanup technique for the chemically diverse mycotoxins. Various approaches to cleanup for multitoxin analysis have been tried. Beer samples have been cleaned up by using conventional reversed-phase (C18) solid-phase extraction (Romero-González et al., 2009), sweet peppers have been analysed using multiple types of solid-phase extraction cartridges (Monbaliu et al., 2009), reversed-phase cartridges have been used for wheat, maize, barley, snacks and infant foods (Lattanzio, Solfrizzo & Visconti, 2008) and a multifunctional column has been used for maize feed samples (Ren et al., 2007). An alternative approach is the application of immunoaffinity columns containing antibodies against DON, aflatoxins, fumonisins, ochratoxin A, ZEA and T-2 toxin (Lattanzio et al., 2007). This has been incorporated in a multitoxin analysis for these legislatively important mycotoxins.

The majority of analytical method development has focused on DON and other trichothecenes, and little work has been performed specifically with the acetylated derivatives, 3-Ac-DON and 15-Ac-DON, or with the relatively newly described DON-3-glucoside, a bound form of DON. The acetylated DON mycotoxins can be determined by the GC or MS methods discussed above for DON, although careful selection of GC column that can achieve separation of the

acetylated forms of DON is required (Valle-Algarra et al., 2005). LC-MS/MS is ideal for the identification, analysis and confirmation of DON-3-glucoside, which has been identified, synthesized and determined to occur naturally in cereal samples contaminated by DON (Berthiller et al., 2005; Dall'Asta et al., 2005), as well as in beer at levels comparable to DON itself (Zachariasova et al., 2008). Alternatively, a method has been optimized for the determination of bound DON in barley grain by means of hydrolysis of bound forms in which trifluoroacetic acid is added to the extraction solvent and sample matrix (Zhou et al., 2007). The entire mixture is heated to simultaneously hydrolyse and extract total DON, and the final determination is by GC with ECD.

4. SAMPLING PROTOCOLS

The generation of meaningful analytical data requires the sampling stage to be as representative as possible. Owing to the lack of homogeneity in the distribution of mycotoxin contamination, which results from differences in fungal contamination of individual units of the raw materials, such as cereal kernels and nuts, the sampling stage of the overall mycotoxin analysis can frequently represent the greatest contribution to the overall variance of the result. Previous work on sampling plans for DON in wheat showed that for a batch level of 5.0 mg/kg, the coefficients of variation associated with the three stages of the analytical process —namely, sampling (based on a 0.45 kg sample), sample preparation (based on grinding and subsampling with a Romer mill) and chemical analysis (by Romer fluoroquant method)—were 6.3%, 10% and 6.3%, respectively (Whitaker et al., 2000). Hence, the variance introduced by sampling for the determination of DON in wheat is much less than that associated with other mycotoxin–matrix combinations. This is partly due to the relatively small kernel size of wheat, which implies that a given sample mass will represent a greater number of potentially contaminated units.

For the purpose of official control of the levels of mycotoxins, including DON, in foods, the EC has regulated sampling protocols, which stipulate, for a given batch of a commodity, the number and size of the incremental samples and size of the aggregate sample to be taken for control purposes (EC, 2006). Similarly, the regulations lay down criteria to be met by the analytical methods used in official control laboratories. Based on the EC sampling regulations, the distribution of DON and ochratoxin A within a 26-tonne truckload of wheat kernels was investigated by analysing all the incremental samples (100) taken to form an aggregate sample (Biselli, Persin & Syben, 2008). The results indicated that the variability associated with ochratoxin A (mean level 0.6 µg/kg ± 200% relative standard deviation [RSD]) was much larger than that associated with DON (mean level 1340 µg/kg ± 25% RSD); they also indicated that for multiple mycotoxin testing, the EC-regulated sampling levels could not be reduced. Recently, geostatistical analysis has been applied to the distribution of DON and ochratoxin A in a bulk lot of wheat kernels (Rivas Casado et al., 2009). The results indicated that DON presented spatial structure (possibly as it is formed pre-harvest in the field), whereas ochratoxin A was randomly distributed in the lot (possibly because of its production in "hot spots" during storage). The spatial structure of DON would indicate that the location of

sampling points as well as the number should be considered in designing sampling plans.

5. EFFECTS OF PROCESSING

Knowledge of the fate of mycotoxins during processing is important for both dietary exposure estimation and adoption of measures for its minimization. The fifty-sixth meeting of the Committee (Annex 1, reference *153*) reviewed the use of gravity separators that separate particles on the basis of differences in specific gravity, size, shape and surface texture to reduce DON concentrations. The effectiveness of milling practices, high-temperature and high-pressure cooking, baking and the use of microorganisms in reducing trichothecene concentrations was also reviewed. Additional studies conducted since the review are summarized below.

Studies conducted on the distribution of DON in wheat grains showed that effective removal of all screenings and outer layers of bran from the surface of wheat grains during the cleaning steps reduced the DON content by 50%, 55%, 41% and 47% in four samples, respectively. The highest levels of DON and the sum of 3- and 15-Ac-DON were concentrated in the waste fractions—namely, screenings and outer layers of bran (Lancova et al., 2008a).

Studies involving milling (removal of bran layer by pearling) of barley to produce white flours may lead to reductions in DON levels in the finished products (House, Nyachoti & Abramson, 2003). The distribution of DON in the wheat and processed fractions showed the concentration of the toxin in the outer portions of the kernel (bran), with lowered levels in the flour (Samar et al., 2003).

A single dry milling study to investigate the redistribution of DON and 16 other *Fusarium* toxins in maize resulted in an accumulation of toxins in fractions used mainly for the production of feedstuffs. High concentrations of DON, 3-Ac-DON and 15-Ac-DON were found in screenings, bran, germ or germ meal. 15-Ac-DON, ZEA, HT-2 toxin and T-2 toxin were detected in germ oil as a result of the higher lipophilic properties of these substances compared with the other toxins (Schollenberger et al., 2008). Recent studies conducted by Scudamore & Patel (2009a) showed similar results with mycotoxins concentrated in the feed components, such as the maize germ, meal, bran and broken maize.

The use of high-speed optical sorting for reducing the concentration of DON in *Fusarium*-infected soft red winter wheat has been reported (Delwiche, Pearson & Brabec, 2005). Commercial wheat samples of low (<1 mg/kg) to very high (>20 mg/kg) DON concentrations were sorted by the simultaneous analysis of two wavelengths (675 nm and 1480 nm) at a feed rate of 0.33 kg/(min-channel). On average, with one-pass sorting, the DON concentration of the sorted wheat was 51% of the original concentration, with successive passes further reducing the concentration of DON.

The effectiveness of detoxification procedures for specific mycotoxins depends largely on the structure and reactivity of the toxin molecule. Most chemical methods for DON reduction in cereal grains depend upon wetting with aqueous alkaline solutions, with optimal heat treatment. The fifty-sixth meeting of the

Committee noted the use of sodium carbonate solution for soaking or as a first wash for contaminated barley, maize and wheat in reducing DON levels. Since then, additional studies (Lauren & Smith, 2001; Abramson, House & Nyachoti, 2005; Ragab et al., 2007) have further confirmed the removal of DON from naturally contaminated whole barley and wheat through washing and/or soaking in water or sodium carbonate solutions. The effects of temperature, time and the use of various levels of sodium bicarbonate on naturally contaminated ground corn are also reported (Lauren & Smith, 2001). Subjecting naturally contaminated ground corn to 10% and 20% (volume by weight) 1.19 mol/l sodium bicarbonate solution at 80 °C for 2 days resulted in nearly equal reduction levels. With the same conditions, after 12 days of heating, greater DON reduction was observed at the 20% level. In heating trials with solutions containing DON at 5 µg/ml at 80 °C, 84% of the DON was destroyed with a 1.19 mol/l sodium bicarbonate solution, and 100% of DON was destroyed with a 1 mol/l solution (Lauren & Smith, 2001).

Studies by Abramson, House & Nyachoti (2005) on naturally contaminated barley sealed in polypropylene containers and subjected to heat (80 °C) and varying amounts of water or a 1 mol/l sodium carbonate solution showed reductions in DON down to near-zero values, depending on experimental conditions.

Recent studies on the fate of DON in contaminated wheat grain during the preparation of Egyptian "balila" (soaked and boiled whole wheat kernels with sugar, nuts and milk) showed that boiling contaminated wheat kernels in water reduced the DON content of grain by 70%, most probably through leaching out of DON into the boiling medium, which is subsequently discarded. Combined treatment of soaking in a 0.1 mol/l sodium carbonate solution (pH 11) with subsequent boiling reduced the DON content of the grain by 93% (Ragab et al., 2007). Further studies are required to investigate potentially harmful degradation products as well as consumers' acceptance of the product when sodium carbonate is used.

Visconti et al. (2004) studied the effects of processing and spaghetti cooking on DON levels. Nine samples of durum wheat contaminated with DON under field conditions (three samples naturally contaminated; six samples artificially inoculated with *Fusarium*) at levels ranging from 0.3 to 13.1 µg/g were processed and cooked into spaghetti. Reductions in DON levels occurred during the different steps of processing and spaghetti cooking: 23%, 63%, 67% and 80% in cleaned wheat, semolina, spaghetti and cooked spaghetti, respectively, relative to the uncleaned wheat. A repartition of DON between dry cooked spaghetti and cooking water was observed during cooking, with increasing DON leaching as the water to spaghetti ratios were increased during cooking.

Hot water treatments of *Fusarium*-infected malting barley resulted in significant ($P < 0.05$) reductions in *Fusarium* infection (Kottapalli & Wolf-Hall, 2008). One minute of treatment at 45 °C and 50 °C resulted in 41–66% and 51–69% reductions in *Fusarium* infection, respectively. After 20 min, reductions of 65–92% at 45 °C and 71–98% at 50 °C were reported. Significant reductions in DON (54–71%) were observed in malts prepared from barley treated at 45 °C or 50 °C for 1 min. The largest reductions for DON were observed in malts prepared from barley treated with hot water at 45 °C (79–93%) and 50 °C (84–88%) for 20 min.

DON is stable at 120 °C, moderately stable at 180 °C and partially unstable at 210 °C (Annex 1, reference *153*). Since the last review by the Committee, some studies on the effects of frying, baking and extrusion cooking on DON and, to some extent, 3-Ac-DON have been conducted (Cazzaniga et al., 2001; Samar et al., 2001, 2007; Lancova et al., 2008a; Scudamore et al., 2008a,b, 2009; Valle-Algarra et al., 2009). These studies are briefly reviewed below.

The effectiveness of traditional home frying of turnover pie dough cover of "empanadas" in reducing DON concentrations was studied at three ordinary frying temperatures (Samar et al., 2007). Frying flour artificially contaminated with DON at 260 µg/kg at 169, 205 and 243 °C resulted in reductions of 66%, 43% and 38%, respectively. Flour naturally contaminated at a DON level of 1200 µg/kg was also fried at similar temperatures (169, 205 and 243 °C). DON concentrations were reduced by 28%, 21%, and 20%, respectively.

The processes used for baking bread and non-yeasted products (cakes/biscuits) vary considerably in fermentation, baking conditions, time, temperatures and the inclusion of additives in the dough mixture. Available data on the effects of baking are therefore conflicting. Some studies report an increase, whereas other work observed a reduction by over 40% during dough fermentation. These have been reviewed by Pacin et al. (2010).

Recent studies have shown that baking bread at 210 °C for 14 min had no significant effect on DON levels (Lancova et al., 2008a).

A study to evaluate the stability of naturally occurring DON (150 mg/kg) during the fermentation stage of the bread-making process was conducted by Samar et al. (2001). Controlled experimental conditions were employed, and dough was fermented at 30, 40 and 50 °C according to standard procedures used in Argentinean low-technology bakeries. Fermenting dough at 50 °C resulted in a maximum reduction in DON levels by 56% for Vienna bread and 41% for French bread.

Scudamore et al. (2009) studied the effect of baking bread, cake and biscuits on DON concentrations. Baking of both white and wholemeal bread at 210 °C for 21 min from flour naturally contaminated with DON at 284 µg/kg reduced DON concentrations by 35–40%. These results are based on an "as is" basis; if moisture content and the presence of other ingredients are taken into account, the loss of DON was less than 5% or 11%, respectively, confirming the stability of DON during the processing. Reductions in concentrations of DON during baking of biscuits and cakes when compared with the concentrations in the flour were due to dilutions with other ingredients and not to processing.

Valle-Algarra et al. (2009) monitored changes in DON, 3-Ac-DON, NIV and ochratoxin A levels in wheat flour during the bread-making process. Wheat flour used was spiked at three levels (200, 750 and 1500 µg/kg) for both DON and 3-Ac-DON. Dough was fermented with *Saccharomyces cerevisiae*. Baking was at different combinations of temperature (190, 207, 223 and 240 °C) and time (50, 40, 35 and 30 min). Fermentation did not affect the levels of DON, 3-Ac-DON or NIV, but ochratoxin A levels were significantly ($P < 0.05$) reduced by between 29.8% and

33.5%, depending on the initial concentration of toxin in the flour. Reductions in all four toxin levels were reported during baking. There were significant differences ($P < 0.05$) among the different mycotoxins. The average reduction percentages for DON, 3-Ac-DON, ochratoxin and NIV were 47.9%, 65.6%, 32.9% and 76.9%, respectively. No significant differences ($P < 0.05$) in the reduction percentages of each toxin in relation to the temperature–time combination used were reported.

The effect of extrusion cooking on the stability of DON in maize flour in the presence and absence of additives has been studied by Cazzaniga et al. (2001). Detoxification levels higher than 95% were obtained using moisture contents of 15% and 30%, temperatures of 150 and 180 °C and metabisulfite concentrations of 0% and 1%.

In a study by Scudamore et al. (2008a), concentrations of NIV and ZEA were minimally changed by extrusion of wholemeal wheat grain. The amount of DON was decreased by 18.9–23.4% at the lowest moisture content of 15%. This effect was not temperature dependent and may be due to either binding or inability to extract the toxin from the extruded product (Scudamore et al., 2008a). Further extrusion studies with naturally contaminated maize grits (143 µg/kg) by Scudamore et al. (2008b) showed DON to be relatively stable. Temperature had little or no effect on DON concentration, although minor losses were reported under all conditions, probably for the same reasons noted above (binding to cereal components and/or reduced extractability). Addition of 2% sucrose (by weight) had no effect on DON levels. The presence of 2% sodium chloride resulted in slightly higher DON levels, which may be due to the fact that sodium chloride assists in extraction and is used in extraction procedures for several mycotoxins (Scudamore et al., 2008b).

The effects of superheated steam as a processing medium on *Fusarium*-infected wheat kernels with a DON concentration of 15.8 mg/kg have been studied. Reductions in DON concentrations of up to 52% were achieved at 185 °C with superheated steam and 6 min processing time. Thermal degradation was found to be the dominant factor in the destruction of DON (Cenkowski et al., 2007).

Treatment of wheat containing DON at 7.6 mg/kg with sodium metabisulfite at 10 g/kg for 15 min at 100 °C, at a moisture content of 22% and a permanently saturated steam supply under permanent mixing, reduced the DON concentration to 0.28 mg/kg (Dänicke et al., 2005).

The fate of DON during malting, mashing and fermentation has been reviewed (Hazel & Patel, 2004). Steeping lowers DON levels due to the water solubility of the toxin. Germination tends to increase DON levels because of conducive conditions created for *Fusarium* growth and toxin formation. Mashing results in increases in DON levels due to enzymatic release of the toxin from protein conjugates. During the first 20 h of fermentation, DON levels are reported to increase due to the conversion of metabolic precursors to DON. The subsequent decrease during the fermentation process has been attributed to yeast absorption or metabolism of the toxin (Hazel & Patel, 2004).

DON-3-glucoside was detected in malt and beer made from barley naturally contaminated with *Fusarium* (Lancova et al., 2008b; Kostelanska et al., 2009). Although DON conjugates with higher masses (presumably diglucosides and triglucosides) have been found in beer, significant increases in DON-3-glucoside

levels in malt over the DON plus DON-3-glucoside in the grain used have also been observed (Berthiller et al., 2009a). The possibilities are that additional mycotoxin that is conjugated is produced by the fungus during the initial steps of the malting process of *Fusarium*-infected barley or that bound mycotoxin originally present in the cell wall polymer fraction might be enzymatically released during malting.

The fate of DON and the sum of 15-Ac-DON and 3-Ac-DON during the malting and brewing processes using naturally infected barley and barley artificially inoculated with *Fusarium* spp. during the time of flowering as raw material was studied (Lancova et al., 2008b). Steeping reduced DON to levels below the limit of quantification (LOQ) of 5–10 µg/kg. There was accumulation of DON during germination to levels higher than in the original barley, but no significant change occurred during the final malting stage (kilning). Overall, DON levels were 2.1 times higher in malt than in barley. Additionally, the occurrence of DON-3-glucoside was monitored during the beer production process. DON-3-glucoside levels were 8.6 times higher in malt than in barley, with further significant increases in levels occurring during the brewing process. Concentrations of the acetylated DON derivatives increased by 1.1 times during the malting process (Lancova et al., 2008b).

The fifty-sixth meeting of the Committee reviewed the microbiological transformation of DON to less toxic metabolites using microorganisms isolated from rumen fluid and soil under anaerobic and aerobic conditions in liquid culture (Annex 1, reference *153*).

Selected strains of *Lactobacillus* (*Lactobacillus rhamnosus* strains GG and LC-705) and *Propionibacterium* (*Propionibacterium freudenreichii* ssp. *shermanii* JS) were able to remove DON and some other trichothecenes from liquid media, although their abilities varied significantly. Both viable and non-viable forms of the bacteria removed DON, whereas 3-Ac-DON was not affected. GC-MS chromatographic peaks suggesting possible degradation of the toxin were absent, implying binding rather than metabolism as the explanation for removal (El-Nezami et al., 2002).

In another study, glucomannans extracted from the external part of the cell wall of *Saccharomyces cerevisiae* showed a binding capacity of 12.6% for DON (Yiannikouris & Jouany, 2002).

In vitro screening tests to ascertain the capacity of non-nutritive adsorbent materials to bind DON at various concentrations in phosphate-buffered solutions showed binding levels generally higher than 50% with activated carbon (Avantaggiato, Solfrizzo & Visconti, 2005).

6. PREVENTION AND CONTROL

6.1 Pre-harvest control

The fifty-sixth meeting of the Committee reviewed measures to prevent and control *Fusarium* infection and DON contamination. The review covered culture techniques such as suitable crop rotation, appropriate use of fertilizers, irrigation and weed control, as well as growing resistant cultivars and the use of fungicides

or biological antagonists and decontamination procedures to reduce infection and DON formation (Annex 1, reference *153*).

Since the monograph for the fifty-sixth meeting of the Committee was prepared, several studies have concentrated on the use of fungicidal/biological antagonists to control *Fusarium* head blight (FHB) and reduce DON formation. Fewer studies are available on the use of culture techniques, use of resistant cultivars and decontamination procedures. Strategies to prevent mycotoxin contamination of food and animal feed have been reviewed (Kabak, Dobson & Var, 2006).

The use of suitable crop rotation is important and focuses on breaking the chain of production of infectious material, such as using wheat/legume rotations. The use of maize in a rotation is, however, to be avoided, as maize is also susceptible to *Fusarium* infection and can lead to carry-over onto wheat via stubble/crop residues. It is accepted that wheat that follows an alternative host for *Fusarium* pathogens is at greater risk of FHB and subsequent DON contamination of grain. Evidence is, however, conflicting that wheat following wheat is more at risk than wheat following a non-cereal crop (Edwards, 2004). It has also been observed that FHB disease severity and DON contamination of grain were significantly different when the previous crop was maize, wheat or soya bean, with the highest levels following maize and the lowest levels following soya bean (Dill-Macky & Jones, 2000). The Codex Alimentarius Commission has recommended that crops such as potato, other vegetables, clover and alfalfa that are not hosts to *Fusarium* species should be used in rotation to reduce the inoculums in the field (FAO/WHO, 2002). Applications of nitrolime to wheat plots reduced the incidence of FHB by 59% when compared with plots treated with calcium ammonium nitrate. There was, however, no significant effect on DON concentrations in harvested grain (Yi et al., 2001).

The Codex Alimentarius Commission (FAO/WHO, 2002) has further recommended that the soil must be tested to determine if there is a need to apply fertilizer and/or soil conditioners to ensure adequate soil pH and plant nutrition to avoid stress, especially during seed development. Fertilizer regimes may affect FHB incidence and severity by altering the rate of residue decomposition, by creating a physiological stress on the host plant or by altering the crop canopy structure. Lemmens et al. (2004) concluded that FHB cannot be sufficiently controlled by manipulating only the nitrogen input. Their work showed a significant increase in FHB intensity and DON contamination in the grain when the application of a mineral nitrogen fertilizer was increased from 0 to 80 kg/ha.

Irrigation is a valuable method of reducing plant stress in some growing situations. It is necessary that all plants in the field have an adequate supply of water if irrigation is used. Excess precipitation during anthesis creates conditions favourable for dissemination and infection by *Fusarium* spp., so irrigation during anthesis and during ripening of crops, specifically wheat, barley and rye, should be avoided (FAO/WHO, 2002).

There are inherent differences in the susceptibility of various cereal species to FHB, which are reflected in differences in the degree of mycotoxin contamination to which each species is susceptible. The differences in susceptibility between crop species appear to vary by country, probably due to differences in the genetic pool

within each country's breeding programme as well as the different environmental and agronomic conditions in which crops are cultivated (Edwards, 2004). Obtaining high levels of native genetic resistance in various crop types to toxigenic fungi has proven difficult. Problems in this regard have centred primarily on the lack of resistant control genotypes and the lack of involvement of single major genes (Munkvold, 2003). Kolb et al. (2001) and Ruckenbauer, Buerstmayr & Lemmens (2001) reviewed information on molecular markers associated with quantitative trait loci for resistance to FHB in wheat and barley and breeding strategies in resistance breeding against FHB, respectively.

Another factor known to increase the susceptibility of agricultural commodities to toxigenic mould invasion is injury due to insect, bird or rodent damage. These must be controlled in the vicinity of the crop by proper use of registered insecticides, fungicides and other appropriate practices within an integrated pest management control programme (FAO/WHO, 2002). The use and effects of some fungicides were reviewed by the fifty-sixth meeting of the Committee (Annex 1, reference *153*), and there was evidence that under certain conditions, fungicide use may actually stimulate toxin production. Studies on fungicides in common use have shown differential effects against toxin-forming *Fusarium* species and related non-toxin-forming pathogens, such as *Microdochium nivale* on ears (Simpson et al., 2001). The reliability of the use of fungicides seems to depend on the fungal species present, the effect the particular fungicide has on the species, the dose rate used, the time of application and even perhaps the method of application.

Recent in vitro studies on a range of *Fusarium culmorum* strains showed stimulation in DON production in the presence of epoxiconazole and propiconazole (Magan et al., 2002). Additional studies are available on the efficacy of the fungicides azoxystrobin, metconazole and tebuconazole at anthesis against *Fusarium* spp. and *Microdochium nivale* and for years on naturally infected fields of soft wheat, durum wheat and barley (Ioos et al., 2005). Infection levels of *F. graminearum, F. culmorum* and *M. nivale* were significantly reduced by the application. Tebuconazole and metconazole effectively controlled *Fusarium* spp. but had little effect on *M. nivale*. The control was, however, seasonal: tebuconazole controlled fungi in 2001 but had little effect in 2000 and 2002; metconazole significantly reduced levels in 2000 and 2001, but not in 2002. Although a few countries have recently allowed the use of several fungicides, including tebuconazole and metconazole, for the control of FHB at or about anthesis, in the European Union, fungicides must be shown to be safe to both the environment and humans before being authorized for use (Kabak, Dobson & Var, 2006).

The effects of prochloraz, tebuconazole, benomyl, carbendazim, guazatine and iminoctadine on mycelial growth of *F. graminearum* and 3-Ac-DON have been reported (Matthies, Walker & Buchenauer, 1999). Prochloraz inhibited mycelial growth and reduced 3-Ac-DON production. Tebuconazole inhibited fungal growth at 0.1, 0.5 and 1.0 µg/ml. At 0.5 µg/ml, however, 3-Ac-DON production was increased 4-fold compared with control experiments. Benomyl increased mycelial growth of *F. graminearum* by 22% and reduced 3-Ac-DON production by 22% compared with the untreated control. Carbendazim showed a dose-related inhibition

of mycelial growth and mycotoxin production when added to media at 0.5, 0.7, 1, 1.5 and 2 µg/ml. Guazatine and iminoctadine significantly reduced mycelial growth of *F. graminearum* in vitro, but increased 3-Ac-DON production by up to 200%.

The use of microorganisms is one of the most recent approaches currently employed to reduce mycotoxin contamination. Antagonistic microorganisms can reduce growth in *Fusarium* species, reduce severity of disease symptoms and reduce the levels of DON production. These microorganisms have been reviewed (Kabak & Dobson, 2009). Important factors for the successful application of FHB antagonists in the field have been listed as the potential deleterious effect of UV light, variable and sporadic arrival of pathogen inoculums on wheat heads over extended periods of head susceptibility and the phylloplane environment, with marked fluctuations in temperature, moisture and available nutrients (Schisler et al., 2002).

At the time of the fifty-sixth meeting of the Committee, only a few reports were available on biological control of FHB. Additional studies conducted since then are briefly described below.

Bacillus subtilis Ehrenberg strains NRRL B-30210 and B-30211 reduced FHB disease severity by 66% and 92% and reduced disease incidence by 35% and 78%, respectively (Schisler et al., 2002). A strain of *Fusarium equiseti* (G9) was found to be effective in controlling FHB and reducing DON formation by more than 70% on wheat (Dawson et al., 2004). Yeasts of the genus *Cryptococcus* are also reported to be effective against FHB. *Cryptococcus* sp. OH 71.4, OH 181.1 and OH 182.9 reduced FHB by up to 59% on durum wheat in the field (Khan et al., 2001). Treatment of heads of FHB-susceptible wheat with a *Streptomyces* sp. reduced both FHB disease severity and associated loss in grain weight by approximately 50% under glasshouse conditions (Nourozian, Etebarian & Khodakaramian, 2006). *Pseudomonas fluorescens* strains MKB 158 and MKB 249 and *Pseudomonas frederiksbergensis* strain 202 significantly reduced the severity of FHB disease symptoms caused by *F. culmorum* in wheat and barley grown under both glasshouse and field conditions. Treatment with either of the two strains in addition resulted in a 74–78% reduction in DON levels in wheat and barley grains in the *F. culmorum*-inoculated field trials (Khan & Doohan, 2009a). Additional studies showed that chitosan (the deacetylated derivative of chitin) was effective in reducing DON contamination of grain caused by *F. culmorum* and also reduced the severity of FHB symptom development on wheat and barley by over 74% (Khan & Doohan, 2009b). *Pseudomonas* sp. AS 64.4 isolated from wheat anthers was as effective as the fungicide tebuconazole for controlling FHB disease severity under field conditions (Kabak & Dobson, 2009). In another study, 22 bacterial strains isolated from wheat anthers in Argentina reduced the growth of *F. graminearum* and reduced the production of DON on irradiated wheat grains by 60–100% (Palazzini et al., 2007). In vitro studies on wheat and maize residues (straw/stalk and grain) showed that inoculating residues with a *Microsphaerosis* species (isolate P130A) significantly reduced *G. zeae* ascospore production by 73%. When applied to crop residues in the field, the *Microsphaerosis* species had no effect on the pattern of perithecial formation, but significantly reduced perithecial production (Bujold, Paulitz & Carisse, 2001).

Discrepancies between the performance of biocontrol agents under environmentally controlled and field conditions are an issue that is commonly observed and are a major obstacle to the development of commercial biocontrol products.

Specific tools, such as DONcast®, have been developed to assist in ameliorating mycotoxin contamination. DONcast® is a weather prediction–based tool to assist Canadian wheat farmers in deciding whether or not to apply appropriate fungicide treatments at anthesis to reduce the risk of eventual DON contamination (Weather Innovations Incorporated, 2008).

6.2 Decontamination

Numerous chemicals that have been tested for their ability to decontaminate trichothecene-contaminated grain/feed were reviewed by the fifty-sixth meeting of the Committee (Annex 1, reference *153*). These chemicals included sodium bisulfite, hypochlorite bleach and natural and modified clays, as well as treatment with moist ozone, ammonia and microwave radiation. Since the last review, no new information has been made available for review. This may be due to the fact that while some chemical treatments may destroy mycotoxins present in many foods and feeds, in many cases, they significantly decrease the nutritional value of the foods or produce toxic products or other products with undesirable effects, thus limiting their widespread use (Kabak, Dobson & Var, 2006).

7. LEVELS AND PATTERNS OF CONTAMINATION IN FOOD COMMODITIES

Information on the natural occurrence of DON was drawn from data received from a number of countries (Austria, Belgium, Brazil, China, Finland, France, Hungary, Japan, the Netherlands, Norway, Singapore and the United Kingdom), as well as surveys published in the open literature. The period of publication for incorporation of data was 2001–2009. Results of the EC's Scientific Cooperation on Questions relating to Food (SCOOP) report on mycotoxins (Schothorst & van Egmond, 2004) have been incorporated. Data gathered have been tabulated by region and country in the occurrence tables provided in Appendix 1. Data collected in the tables include information on LOD (or LOQ or both) and number of positive samples. For individual reports of surveys, the mean values reported are generally the mean of all samples, with concentrations in samples below the LOD being taken as zero. In some instances, particularly with the SCOOP data, means have been calculated based on a value of LOD/2 (or LOQ/6) for the samples without detected contamination; this is noted in the occurrence table footnote. Although the maximum level analysed in a given set of samples is recorded, information on distribution and 90th-percentile levels are mostly lacking.

It is noted that DON was a common contaminant in cereals (wheat, maize, oats, rye, barley, rice) and their products. Highest reported mean levels for raw cereals were as follows: wheat, 9900 µg/kg; maize, 4772 µg/kg; rice, 183 µg/kg; barley, 6349 µg/kg; oats, 537 µg/kg; and rye, 190 µg/kg. Contamination levels vary

widely between and within regions. Relatively lower levels were detected in processed products, such as baby food, beer, bread, biscuits, pasta, muesli, noodles, cereal-based snacks, pizza, polenta, couscous, flours and fermented soya bean, most likely due to the decrease in contamination resulting from cereal milling and processing. Mean levels of DON in samples of processed products did not exceed 1250 µg/kg. As noted by the fifty-sixth meeting of the Committee (Annex 1, reference *153*), carry-over of DON into animal products is negligible due to feed refusal, rapid metabolism and elimination in livestock species. A few reports have dealt with DON in hens' eggs and have concluded that transmission rates from feed to egg are between 15 000:1 and 29 000:1, implying that, compared with other routes of exposure, this is insignificant (Sypecka, Kelly & Brereton, 2004).

As was observed by the fifty-sixth meeting of the Committee (Annex 1, reference *153*), a range of analytical methods have been used for DON analysis. LODs and LOQs can vary with different methods and with different instrument sensitivities on the same method. This has a clear influence on the number of positive samples found in a batch. Some of these methods are applicable to DON itself, whereas certain chromatographic methods, such as HPLC-MS(/MS) or GC, are capable of determining DON derivatives, such as 3-Ac-DON, 15-Ac-Don and DON-3-glucoside. The occurrence data for the DON derivatives 3-Ac-DON and 15-Ac-DON in wheat, maize, barley, oats, rye and their products were considered by the Committee for the first time at the present meeting. In addition to data submitted by China, France, Japan and the United Kingdom, published data from studies conducted in Austria, Finland, France, Germany, the Netherlands, Norway, Sweden, the United Kingdom and the USA were also assessed. Data were available on 3-Ac-DON from 6980 samples (92% from Europe and 8% from Asia) and on 15-Ac-DON from 4300 samples (81% from Europe, 16% from Asia and 3% from the USA). Generally, these derivatives are infrequently detected, and levels were typically less than 10% of those reported for DON. Highest reported mean levels in wheat, maize and barley for 3-Ac-DON were 193 µg/kg, 27 µg/kg and 19 µg/kg, respectively; for 15-Ac-DON, the corresponding highest reported mean levels were 365 µg/kg, 236 µg/kg and 0.3 µg/kg. The Committee was aware of reports on DON-3-glucoside in cereals and beer (data on 500 samples were assessed, with 79% from China, 15% from Europe and 6% from the USA), but considered that the data were too limited for dietary exposure assessment.

For comparison of concentrations of DON and its derivatives, a separate table was compiled containing data of samples in which DON as well as (all or some of) its derivatives have been determined (see Table A9 in Appendix 1). The potential exposure to DON and its derivatives via beer consumption has been highlighted (Kostelanska et al., 2009). Levels of DON-3-glucoside have been reported to rise during brewing, and its level in beer can exceed that of DON itself (Lancova et al., 2008b).

8. FOOD CONSUMPTION AND DIETARY EXPOSURE ESTIMATES

Dietary exposure to DON was assessed according to the recommendations of a Food and Agriculture Organization of the United Nations/World Health

Organization (FAO/WHO) workshop on methods for assessing exposure to contaminants and toxins, which was held in Geneva in June 2000 (FAO/WHO, 2000). The workshop recommended that the median concentration should be given when data on individual samples are available, whereas a mean should be given when only pooled or aggregated data are available. In the case of commodities that contribute significantly to exposure, distribution curves should be generated to allow risk managers to determine the effects on dietary exposure of different maximum levels.

The workshop further recommended that international estimates of dietary exposure should be calculated by multiplying the mean or median concentration by the values for consumption of the commodity in the five Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) regional diets (WHO, 1998). In the interim, the number of regional diets has been expanded to 13 consumption cluster diets to better represent dietary tendencies around the world. They were established on the basis of information on food balance sheets compiled by FAO. As such information is available for most countries, the data are comparable across countries and regions of the world. The consumption cluster diets represent the average availability of food commodities per capita rather than actual food consumption.

The report of the workshop (FAO/WHO, 2000) noted that national exposure estimates should also be reported when available, as they may provide information about exposure by specific population subgroups or consumers with extreme exposure, which cannot be derived from GEMS/Food regional diets.

8.1 Methods

For this assessment, concentrations of DON in food commodities and in some processed foods were reported to FAO/WHO or were obtained from the literature. The quality and reporting of the data are discussed in the previous section. As the dietary exposures were based on the GEMS/Food consumption cluster diets, which include information on consumption of raw or minimally processed foods, concentrations of DON in processed foods were not used to estimate dietary exposure.

Information was available on the concentrations of six commodities: barley, maize, oats, rice, rye and wheat. Additionally, information on beer, the majority of which is produced from barley, was included. Data originating in 40 countries were analysed, representing 10 of the 13 GEMS/Food consumption cluster diets; no data were reported for the A, H and J clusters. These are primarily Central and East African countries (clusters A and J) and Central American and Caribbean countries (cluster H). The majority of the data were from European countries—more specifically, France. Of the six commodities for which data were available for the exposure assessment, data on barley, maize and wheat predominated, with limited reports on oats, rice and rye.

Most of the data available for this evaluation were pooled; that is, each data point represented the mean concentration in a number of individual samples. In calculating the mean values, samples in which the concentration was below the

LOQ or LOD were assumed to have a value of zero. The maximum analytical value was also reported for each data point. In total, 401 data points (mean values) representing 16 569 individual samples were included in the exposure assessment. Of those 401 data points, 207 were reported from cluster diet E countries, primarily Europe and the United Kingdom countries. The remaining 194 data points represented exposure to DON in the six commodities for the remaining nine reporting cluster diets.

For each commodity, the data were sorted according to the country groupings of the GEMS/Food consumption cluster diets. The number of data points reported, the number of individual samples represented, the highest maximum analytical value reported and the weighted average of all mean values are summarized in Table 4.

8.2 Concentrations in foods

The concentrations of DON used in estimating dietary exposures, summarized by commodity and region in Table 4, are described briefly below:

- *Barley:* Data on the concentrations of DON in barley were received from 10 countries. For the 1353 samples analysed, 433 (32%) had concentrations below the LOD. The weighted mean for all samples combined was 442 µg/kg, and the maximum analytical value reported was 10 000 µg/kg.
- *Beer:* Twenty-two countries reported data on a total of 727 samples of beer. Of these, 297 (41%) contained concentrations below the LOD. The weighted mean of all samples combined was 7 µg/kg, and the maximum analytical value reported was 57 µg/kg.
- *Maize:* Fourteen countries reported data on a total of 2643 samples of maize. Of these, 210 (8%) contained concentrations below the LOD. The weighted mean of all samples combined was 625 µg/kg, and the maximum analytical value reported was 13 000 µg/kg.
- *Oats:* Eight countries representing only three cluster diets submitted data on a total of 478 samples of oats. Of these, 238 (50%) contained concentrations below the LOD. The weighted mean of all samples combined was 79 µg/kg, and the maximum analytical value reported was 5004 µg/kg.
- *Rice:* Five countries representing four cluster diets submitted data on rice, including a total of 462 samples. Of these, 121 (26%) contained concentrations below the LOD. The weighted mean of all samples combined was 12 µg/kg, and the maximum analytical value reported was 34 µg/kg.
- *Rye:* Six countries reported data on a total of 909 samples of rye. Of these, 633 (70%) contained concentrations below the LOD. The weighted mean of all samples combined was 63 µg/kg, and the maximum analytical value reported was 1095 µg/kg.
- *Wheat:* Wheat was the only commodity with data reported from each of the 10 cluster diets that reported data. Twenty-nine countries reported data on 9997 samples of wheat. Of these, 2690 (27%) contained concentrations below the LOD. The weighted mean of all samples combined was 367 µg/kg, and the maximum analytical value reported was 14 000 µg/kg.

Table 4. Summary of data on concentrations of DON in commodities and beer from the GEMS/Food consumption cluster diets[a]

Commodity	Global total	B	C	D	E	F	G	I	K	L	M
Barley											
No. of data points	38			4	13	3	2			8	8
No. of individual samples	1 353			146	443	52	12			400	300
Weighted mean concentration (µg/kg)	442			570	27	60	8			142	1 475
Maximum value (µg/kg)				4 000	550	619	51			4 700	10 000
Beer											
No. of data points	32	7			18	3		2		2	
No. of individual samples	727	146			442	18		75		46	
Weighted mean concentration (µg/kg)	7	3			9	13		3		3	
Maximum value (µg/kg)		29			57	33		6		50	
Maize											
No. of data points	71	3		4	29	2	7	5		1	20
No. of individual samples	2 643	72		216	605	60	240	470		82	898
Weighted mean concentration (µg/kg)	625	3 729		209	551	143	292	843		103	582
Maximum value (µg/kg)		11 000		2 460	3 680	1 022	4 374	13 000		807	2 150
Oats											
No. of data points	18				8	9	1				
No. of individual samples	478				262	204	12				
Weighted mean concentration (µg/kg)	79				23	156	0				

Table 4 (contd)

Commodity	Global total	B	C	D	E	F	G	I	K	L	M
Maximum value (µg/kg)					530	5 004	0				
Rice											
No. of data points	17				8	1	2		6		
No. of individual samples	462				115	16	71		260		
Weighted mean concentration (µg/kg)	12				5	10	0		18		
Maximum value (µg/kg)					12	10	0		34		
Rye											
No. of data points	35			2	28	3	2				
No. of individual samples	909			187	691	28	3				
Weighted mean concentration (µg/kg)	63			0	82	15	0				
Maximum value (µg/kg)				0	1 095	178	0				
Wheat											
No. of data points	190	5	1	9	103	11	11	6	7	12	25
No. of individual samples	9 997	72	17	799	6 170	297	487	202	63	1 377	513
Weighted mean concentration (µg/kg)	367	802	27	46	304	192	399	777	303	65	2 339
Maximum value (µg/kg)		3 600	128	1 840	10 400	2 033	14 000	2 700	1 500	2 100	11 400
Total no. of data points	401										
Total no. of individual samples	16 569										

[a] Clusters A, H and J reported no data and are excluded.

8.3 Estimates of dietary exposure at the international level

The average dietary exposures to DON were calculated by multiplying the weighted mean concentration of each commodity by the corresponding amount of each commodity consumed in each of the 10 GEMS/Food consumption cluster diets that reported data (Table 5).

The total exposure to DON was estimated to range from 0.2 µg/kg bw per day (cluster C) to 14.5 µg/kg bw per day (cluster B). The main source of exposure in clusters C, D, E, F, G, K and M was wheat (56–100% of total exposure), whereas the main source in clusters B and I was maize; exposure in cluster L was evenly spread among maize, wheat and barley. The estimates of average exposure were based on the assumption that consumers choose foods randomly with respect to the distribution of concentrations of contaminants and will therefore be exposed to an approximation of the mean of that distribution over time. The Committee noted that the high estimates of DON exposure in clusters B and M were due to unusually high reported DON levels in maize and wheat in single countries for each cluster and that these data may not be representative of normal dietary exposures. The range of estimates in the remaining clusters is in agreement with those prepared at the fifty-sixth meeting.

It should be noted that any reduction in the concentration of DON as a result of processing has not been taken into consideration in this assessment.

8.4 National estimates of dietary exposure

Since the evaluation of DON at the fifty-sixth meeting of the Committee in 2001, a number of national evaluations of dietary exposure have been published. The Committee considered evaluations by the European Union (collectively) and for foods and cereal products in Argentina, Belgium, Czech Republic, Denmark, Ethiopia, France, Germany, Ireland, Lebanon, Morocco, the Netherlands, Nigeria, Republic of Korea and Thailand. Some of these reports contained overall dietary exposure assessments, whereas others assessed single commodities (or their products) considered to be the potential primary source of DON dietary exposure.

8.4.1 European Union

Following a number of years of high contamination levels of mycotoxins in grains in the 1990s, the European Union established a task for SCOOP to conduct a survey of levels of and resultant dietary exposures to mycotoxins, including DON (Schothorst & van Egmond, 2004). Austria, Belgium, Denmark, Finland, France, Germany, the Netherlands, Norway, Portugal, Sweden and the United Kingdom submitted data on DON, with appropriate food consumption data. France and the Netherlands submitted the majority of the analytical data, with wheat and wheat products having the most data points. For all of the reporting countries, mean dietary exposures were less than 1 µg/kg bw per day for all age groups considered. For France (all age groups) and Germany (young children), high-level exposure exceeded 1 µg/kg bw per day. Wheat flour and bread were the major contributors to dietary exposure.

Table 5. Commodity consumption (g/person per day) and the resulting dietary exposures (µg/day and µg/kg bw per day) for DON in the GEMS/Food consumption cluster diets[a]

	B	C	D	E	F	G	I	K	L	M
Food consumption (g/day)										
Barley	16.8	93.9	13.2	48.6	36.1	5.9	5.9	20.2	16.8	43.8
Beer	84.1	4.1	66.0	243.1	161.3	21.9	29.5	100.9	82.2	218.8
Maize	148.4	135.9	31.8	33.3	7.5	35.2	248.1	63.1	58.6	85.5
Oats	0.6	0.2	4.2	5.7	8.9	0.2	0.8	3.5	0.7	7.6
Rice	31.6	94.6	33.2	12.7	12.7	376.9	38.0	238.4	381.3	34.6
Rye	3.7	0.3	24.3	25.8	45.8	0.4	0.2	0.1	0.9	0.8
Wheat	396.3	426.5	390.2	236.3	216.0	172.9	68.1	114.1	103.4	234.2
Weighted DON concentration (µg/kg)										
Barley	N/A	N/A	570	27	60	8	N/A	N/A	142	1475
Beer	3	N/A	N/A	9	13	N/A	3	N/A	3	N/A
Maize	3729	N/A	209	551	143	292	843	N/A	103	582
Oats	N/A	N/A	N/A	23	156	0	N/A	N/A	N/A	N/A
Rice	N/A	N/A	N/A	5	10	0	N/A	18	N/A	N/A
Rye	N/A	N/A	0	82	15	0	N/A	N/A	N/A	N/A
Wheat	802	27	46	304	192	399	777	303	65	2339

Table 5 (contd)

	B	C	D	E	F	G	I	K	L	M
DON exposure (µg/day)										
Barley			7.5	1.3	2.2	0.0			2.4	64.6
Beer	0.3			2.2	2.1		0.1		0.2	
Maize	553.4		6.6	18.3	1.1	10.3	209.1		6.0	49.8
Oats				0.1	1.4	0.0				
Rice				0.1	0.1	0.0		4.3		
Rye			0.0	2.1	0.7	0.0				
Wheat	317.8	11.5	17.9	71.8	41.5	69.0	52.9	34.6	6.7	547.8
DON exposure (µg/kg bw per day)										
Barley			0.130	0.020	0.040	0.000			0.040	1.080
Beer	0.000			0.040	0.030				0.000	
Maize	9.220		0.110	0.310	0.020	0.170	3.490		0.100	0.830
Oats				0.000	0.020	0.000				
Rice				0.000	0.000	0.000		0.070		
Rye			0.000	0.040	0.010	0.000				
Wheat	5.300	0.190	0.300	1.200	0.690	1.150	0.880	0.580	0.110	9.130
Total DON exposure (µg/kg bw per day)	**14.520**	**0.190**	**0.540**	**1.610**	**0.810**	**1.320**	**4.370**	**0.650**	**0.250**	**11.040**

N/A, no data reported for this cluster

[a] Clusters A, H and J reported no data and are excluded.

The Committee noted that this report sparked a number of national evaluations by member countries to consider dietary exposure to mycotoxins in more detail. These European national DON evaluations (published) are explored individually throughout the remainder of this section.

8.4.2 Argentina

Dietary exposure to DON from bread consumption in Argentina was reported in 2010 (Pacin et al., 2010). Bread was considered to be the likely primary source of DON exposure in the Argentinean diet. It was noted that baking of French and Vienna breads resulted in 33% and 58% reductions, respectively, of DON from the levels in the wheat flour. The resulting estimations of dietary exposure were 0.065 µg/kg bw per day for French bread and 0.019 µg/kg bw per day for Vienna bread. The authors noted that consumption of other wheat-containing products, such as pizza, noodles, pasta, cookies and beer, could result in a higher total dietary exposure.

8.4.3 Belgium

The dietary exposures to DON from the consumption of beer and home-produced eggs in Belgium have been reported (Harcz et al., 2007; Tangni et al., 2009). DON exposure from consumption of conventional beer, at the 97.5th percentile, was 0.23 µg/kg bw per day. Mean exposure was less than 0.07 µg/kg bw per day for all beer types reported. DON exposure from the consumption of home-produced eggs had a maximum estimated level of 0.05 µg/kg bw per day.

8.4.4 Czech Republic

As part of a risk–risk analysis of the trade-off of use of fungicides versus mycotoxin contamination of grains, a margin of exposure (MOE) for DON in the Czech Republic was reported (Muri et al., 2009). Although dietary exposure was not reported, the lowest MOE (at the 1st percentile for the age group 4–19 years) was 11; a calculation using an effect dose of 30 µg/kg bw per day suggests that dietary exposure at the 99th percentile would be less than 3 µg/kg bw per day. The mean concentration of DON in foods was found to be 96 µg/kg.

8.4.5 Denmark

In the paper reporting the risk–risk analysis noted above for the Czech Republic, an MOE for DON in Denmark was also included (Muri et al., 2009). As for the Czech Republic, MOEs were reported. The lowest MOE (at the 1st percentile for the age group 4–19 years) was 14; a calculation using an effect dose of 30 µg/kg bw per day suggests that dietary exposure at the 99th percentile would be less than 2 µg/kg bw per day. The mean concentration of DON in foods was found to be 118 µg/kg.

Rasmussen, Petersen & Ghorbani (2007) reported on DON exposure from consumption of wheat and rye flour produced in Denmark between 1998 and 2003. The authors noted the great variability from year to year, with DON levels in wheat

flour ranging from 32 to 255 µg/kg. Although they noted reports that production of breads from contaminated wheat resulted in lower DON levels in the final products than in the raw material, they did not assume any reduction for the purposes of the dietary exposure assessments. They found mean exposure for the total population to be 0.17 µg/kg bw per day, with an exposure of 0.32 µg/kg bw per day for children using a deterministic approach. Use of the DON level in wheat flour from the year with the highest level of contamination resulted in a population mean of 0.31 µg/kg bw per day. A probabilistic evaluation of exposure to DON from consumption of wheat and rye flour found that at the 99.9th percentile for children, exposure was 0.9 µg/kg bw per day.

8.4.6 Ethiopia

A report concerning DON levels in barley, sorghum, teff and wheat from the 1999 crop year in Ethiopia stated that DON was found in only 4 of 23 wheat samples, with a mean contamination level of less than 90 µg/kg (Ayalew et al., 2006). Sorghum was found to have the highest percentage of contaminated samples, at 48% positives (mean level 360 µg/kg), but the authors noted that these were "suspect" samples, visibly damaged by *Fusarium* species. No dietary exposures were reported. The authors concluded that the levels of DON found in the grains were too low to be of concern to farmers.

8.4.7 France

Following the publication of the SCOOP task report noted above for the European Union (Schothorst & van Egmond, 2004), France undertook a total diet study including DON dietary exposure (Leblanc et al., 2005). Total diet studies examine the dietary exposure to substances (nutrients, additives or contaminants) by measuring the level of the substance of interest in all food products in a representative diet, prepared for normal consumption. These studies often give the best measure of dietary exposure to a substance because they explicitly account for any changes in the level of the substance during food production and preparation.

The French study considered the total population as well as those consuming three types of vegetarian diets (vegetarians comprised 138 of the total surveyed population of 3003). The mean dietary exposure for the adult non-vegetarian population was found to be 0.281 µg/kg bw per day, whereas that for children was 0.451 µg/kg bw per day. At the 95th percentile, the corresponding exposures were 0.571 and 0.929 µg/kg bw per day, respectively. The authors noted that all of these estimates are below the PMTDI of 1 µg/kg bw and "significantly lower" than the estimates reported in the SCOOP task report (0.46 and 0.73 µg/kg bw per day at the mean for adults and children, respectively). They suggested that this was due to reduction of DON levels in food on cooking. The range of mean DON exposures for the vegetarian diets was 0.32–0.41 µg/kg bw per day at the mean and 0.72–0.96 µg/kg bw per day at the 95th percentile. The analysis showed that 0.4% of the adult population could be above the PMTDI for DON.

The Committee noted that this analysis was the best available for this evaluation of DON dietary exposure.

8.4.8 Germany

Schollenberger, Müller & Drochner (2005) reported on consumption of trichothecene toxins for the population in south-west Germany in 1998–1999. They considered consumption of bread and pasta by infants, children and adults. DON levels in 1999 were twice those in 1998. For 1999, the DON exposure for adults was 0.45 µg/kg bw per day at the mean and 0.90 µg/kg bw per day for "high" consumption. Exposure for infants in 1998 was as high as 0.35 µg/kg bw per day. No evaluation for infants in 1999 was undertaken. The authors reported that children's exposure to DON in 1999 was 1.59 µg/kg bw per day at the mean and 3.17 for "high" consumption. The Committee believes that these estimates are reported in error. In the discussion section outlining the process used to prepare the estimates, it is stated that a 20 kg child would consume 20 g of bread and pasta per day, with a high consumption of 40 g/day. In the reported estimates, 170 g of food was used to calculate the mean, the same as for adults. The high estimates used 340 g for both populations also. If the lower food consumptions are correct, the exposures would be approximately one eighth of the reported figures, or 0.19 µg/kg bw per day at the mean and 0.38 µg/kg bw per day for "high" consumption.

8.4.9 Ireland

DON exposure from the consumption of milk in Ireland was analysed using a probabilistic assessment method employing Monte Carlo modelling (Coffey, Cummins & Ward, 2009). The authors reported DON exposure to be less than 0.001 µg/kg bw per day at the mean and 0.019 µg/kg bw per day at the 95th percentile.

8.4.10 Lebanon

The dietary exposure to DON for children and teenagers in Beirut, Lebanon, from consumption of cereal products (bread, cakes, pizza, etc.) was reported in 2009 (Soubra et al., 2009). Approximately 45% of samples had non-detectable levels, with the highest DON levels found in bread (176 µg/kg). For 8- to 13-year-olds, the mean exposure was estimated to be less than 0.55 µg/kg bw per day, with a 95th-percentile exposure of less than 1.0 µg/kg bw per day. The exposures for 14- to 18-year-olds were lower (mean 0.41 µg/kg bw per day; 95th percentile 0.66 µg/kg bw per day).

8.4.11 Morocco

DON contamination of wheat grains in Morocco was examined by Hajjaji et al. (2006). Seven of 17 samples were found to contain measurable DON levels, with a mean concentration of 27 µg/kg. Although dietary exposure was not estimated, the authors concluded that the presence of DON in wheat, even at levels below proposed regulatory limits (500 µg/kg), may constitute a risk to human health.

8.4.12 Netherlands

Three published reports have contained information concerning dietary exposure to DON in the Netherlands. The Muri et al. (2009) risk–risk analysis described above found MOEs for DON in the Netherlands to be as low as 29 (at the 1st percentile for the age group 4–19 years); a calculation using an effect dose of 30 µg/kg bw per day suggests that dietary exposure at the 99th percentile would be less than 1 µg/kg bw per day (Muri et al., 2009).

Pieters, Bakker & Slob (2004) explored the effect that government risk management efforts put in place following the high mycotoxin contamination years of the late 1990s had on dietary exposure to DON. They noted that mean DON levels in samples taken from February 2000 to December 2002 were reduced by 50% when compared with samples from the period 1998–2000. The levels were highest in maize and wheat. Using a probabilistic exposure assessment method, they estimated 95th-percentile exposure for 1-year-olds to be approximately 1.0 µg/kg bw per day (0.46 µg/kg bw per day at the mean); exposures for 5% of 1-year-olds would exceed 1.0 µg/kg bw per day. For all age groups above 10 years, the mean exposures were below 0.2 µg/kg bw per day, with 95th-percentile exposures below 0.4 µg/kg bw per day.

Schothorst et al. (2005) explored dietary DON exposures in children using a duplicate diet study, in which all portions of food prepared for normal consumption are divided in two, with half being analysed for DON; exposure is then estimated using the actual measured consumption of the food. This method is extremely effective for accurately measuring the dietary exposure to a substance. Seventy-four children were included in the study. The mean dietary exposure to DON was 0.66 µg/kg bw per day, with a maximum of 1.98 µg/kg bw per day. Nine of the 74 participants had exposures above 1.0 µg/kg bw per day.

8.4.13 Nigeria

Maize for human consumption (180 samples) was analysed for the presence of DON, among other mycotoxins (Adejumo, Hettwer & Karlovsky, 2007). Forty samples contained DON (22%) at a mean concentration of 226 µg/kg. Although no dietary exposure analysis was performed, the authors stated that since the DON levels were below the United States Food and Drug Administration's (USFDA) advisory level of 1000 µg/kg, these samples do not present a health risk for consumers. The Committee noted, however, that chronic consumption of 265 g of maize per day would result in an exposure to DON from maize at the PMTDI of 1 µg/kg bw. Nigeria is in GEMS/Food cluster diet J, with a per capita exposure to maize of 57.4 g/day. Only cluster diet H, primarily Central America and the Caribbean nations, exceeds 265 g/day (298 g/day).

8.4.14 Republic of Korea

DON exposure from numerous commodities collected from 2005 to 2008 was evaluated for the Republic of Korea (Ok et al., 2009b). DON was below the LOD in more than 60% of the samples, with dried maize having the highest levels, at a mean of 128 µg/kg. Using a probabilistic Monte Carlo model, mean exposures

for all age groups over 7 years were found to be less than 0.1 µg/kg bw per day. The 95th-percentile exposures for these groups were all below 0.2 µg/kg bw per day. For children aged 3–6 years, the mean and 95th-percentile exposures were 0.14 and 0.30 µg/kg bw per day, respectively. Polished rice consumption provided 73–91% of the DON exposure. Breads, biscuits and beer contributed less than 10% of the total for all groups.

8.4.15 Thailand

Wheat products in Thailand were examined for DON contamination (Poapolathep et al., 2008). Ninety samples were examined, 30 each of noodles, pasta and cereals. DON was detected in 18.9% of the samples (LOD 100 µg/kg), with 1 above 1 mg/kg. The mean contamination levels in quantifiable samples were 0.26, 0.37 and 0.24 mg/kg for noodles, pasta and cereals, respectively. A dietary exposure analysis was undertaken for a number of age groups. The highest exposure reported was for the 3- to 6-year-olds, with an "upper estimated exposure" of 0.0038 µg/kg bw per day. The dietary exposures to DON from bread and noodles are reported as ranging from 0.33 to 2.05 g/person per day. The Committee questions these values and could not conclude that the reported dietary exposures are valid.

8.4.16 Summary

The data used for the preparation or analyses of national estimates of dietary exposure to DON are summarized in Table 6.

The Committee concluded that all of the mean estimates of national exposure to DON were below the PMTDI of 1 µg/kg bw. In only a few cases, and typically for children only at upper percentiles, national reports showed dietary exposures that were above 1 µg/kg bw per day.

9. DOSE–RESPONSE ANALYSIS

9.1 BMD modelling for chronic effects

Since the previous evaluation, a derivation of a benchmark dose (BMD) for humans has been performed from the 2-year feeding study in mice (Iverson et al., 1995), the study on which the PMTDI for DON was based. Based on a 5% reduction in body weight, a value of 8.6 µg/kg bw per day was derived, with a lower confidence limit of 0.6 µg/kg bw per day (Slob & Pieters, 1998; Pieters et al., 2001). As the current PMTDI was not under re-evaluation at the present meeting, the Committee did not use this derived BMD.

9.2 BMD modelling for acute reference dose

The Committee considered emesis the critical end-point for acute effects, as this effect was observed consistently following DON intoxication in experimental animals and humans. Because the emetic effect was considered to be dependent on the maximum plasma concentration (C_{max}), the Committee concluded that for the

Table 6. National dietary exposures to DON[a]

Country/region	Mean exposure (µg/kg bw per day)	Upper-percentile exposure (µg/kg bw per day)
Argentina	0.02–0.06 (breads)	Not reported
Belgium	<0.07 (beer)	0.23 (97.5th, beer) 0.05 (eggs)
Czech Republic	Not reported	3 (4–19 years, 99th)
Denmark	0.02–0.03 (adults) 0.32 (children)	2 (4–19 years, 99th) 0.9 (children, 99.9th)
France	0.28 (adults) 0.45 (children) 0.32–0.45 (vegetarians)	0.57 (adults, 95th) 0.93 (children, 95th) 0.72–0.96 (vegetarians, 95th)
Germany	0.45 (adults) 0.19 (children)	0.90 (adults) 0.38 (children)
Ireland	0.001 (milk)	0.02 (milk)
Japan	Not reported	0.69 (1–6 years, 95th) 0.49 (7–14+ years, 95th) 0.24 (>19 years, 95th)
Lebanon	0.55 (8–13 years) 0.41 (14–18 years)	<1.0 (8–13 years, 95th) 0.66 (14–18 years, 95th)
Netherlands	0.46 (1 year) 0.66 (children) 0.2 (10+ years)	1 (4–19 years, 99th) 1 (1 year, 95th) 0.4 (10 years, 95th) 1.98 (children, 100th)
Republic of Korea	0.1 (7+ years) 0.14 (3–6 years)	0.2 (7+ years) 0.30 (3–6 years)
European Union	<1.0 (all age groups)	>1.0 (France, all ages, Germany, young children)
GEMS/Food clusters	0.19 (cluster C)—14.5 (cluster B)	

[a] Where no age group is specified, the dietary exposure is for the total population; when a food is specified, the dietary exposure included consumption of that food only.

purpose of establishing an ARfD, studies in which DON was administered via the diet were more appropriate than studies that used gavage dosing.

Two studies on emesis in piglets and pigs following exposure to DON via the diet (Young et al., 1983; Pollman et al., 1985) were combined for BMD modelling. Doses were calculated from the measured DON concentrations in the feed and the observed feed intake. In the first study, dietary concentrations above 3 mg/kg feed resulted in drastically reduced average feed intakes (reduced by 88–94% compared with controls) and decreases in body weights during the test period; for these

groups, it was assumed that the total feed intake over 4 or 11 days was actually all consumed on day 1. This assumption was made because it has often been observed that pigs stop eating after DON-induced vomiting on day 1. For the three dose groups in which it was reported that at least one pig vomited, it was assumed that the incidence was one. In the second study, the average feed intake was taken from the first week of exposure, although intake was decreased in the dose groups given 1.4 mg/kg feed or more, compared with controls. The initial body weights were used for the calculations, because the emesis was observed on day 1 of exposure.

The dose–response analysis was performed using the PROAST software (version 23.2). The benchmark response (BMR) was set at 10% extra risk. As Table 7 shows, the lower limit on the benchmark dose for a 10% extra risk ($BMDL_{10}$) among the accepted models ranged between 0.21 and 0.74 mg/kg bw per day. The lowest value in this range was used as a point of departure for establishing an ARfD. Figure 1 shows the dose–response data, with the fitted log-logistic model.

Table 7. Outcome of dose–response models on emesis in pigs[a]

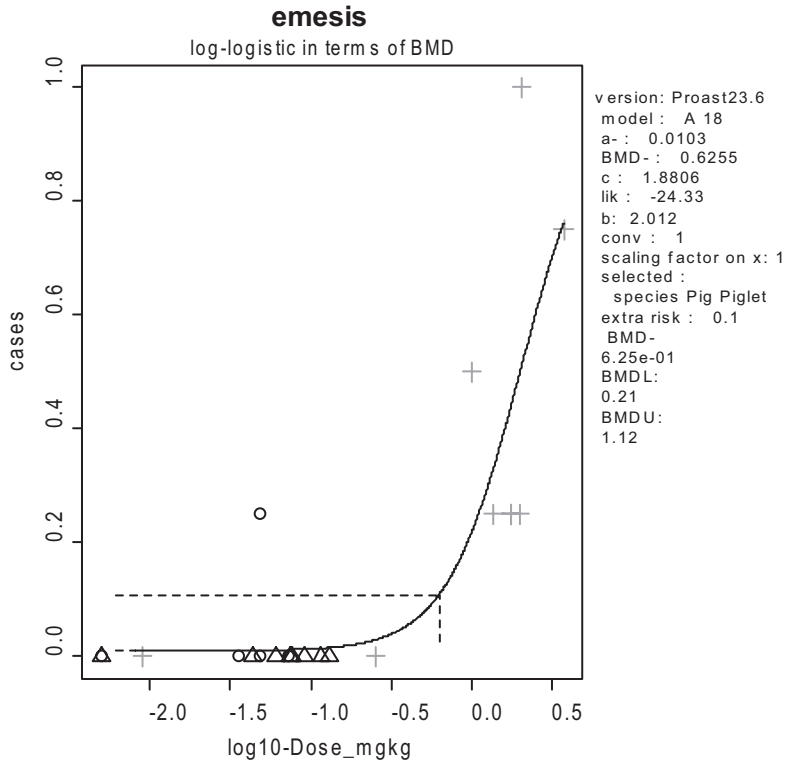
Model	npar	Log-likelihood	Accepted	BMD_{10} (mg/kg bw per day)	$BMDL_{10}$ (mg/kg bw per day)	$BMDU_{10}$ (mg/kg bw per day)
Null	1	−43.23	—	—	—	—
Full	22	−16.27	—	—	—	—
One-stage	2	−24.78	Yes	0.34	0.22	0.55
Two-stage	3	−24.54[b]	No	0.49	—	—
Log-logistic	3	−24.33	Yes	0.63	0.21	1.12
Weibull	3	−24.43	Yes	0.57	0.21	1.06
Log-probit	3	−24.21	Yes	0.61	0.21	1.09
Gamma	3	−24.36	Yes	0.62	0.21	1.10
Logistic	2	−26.14	Yes	0.99	0.74	1.29
Probit	2	−25.75	Yes	0.93	0.69	1.22

$BMDU_{10}$, upper limit on the benchmark dose for a 10% extra risk; npar = number of parameters in dose–response model

[a] No constraint; *P*-value goodness-of-fit test: 0.05.

[b] Not accepted for not being significantly better than the one-stage model.

Figure 1. Dose–response data on emesis in piglets and pigs after exposure to DON via the diet, with fitted log-logistic model



Note: Circles: Pollman et al. (1985) (pigs); triangles: Pollman et al. (1985) (piglets); plus signs: Young et al. (1983) (piglets). Note that the outlying circle (at response fraction 0.25) reflects one out of four animals.

10. COMMENTS

10.1 Absorption, distribution, metabolism and excretion (ADME)

The additional studies on metabolism in mice, rats and pigs confirmed that DON and its acetyl derivatives are rapidly and extensively absorbed from the upper gastrointestinal tract and cleared with a short plasma half-life. After absorption of 3-Ac-DON, DON was the principal metabolite observed in plasma, and acetylated DON was not detected, indicating that deacetylation is an extensive and rapid metabolic process. De-epoxidation of DON is a microbial pathway that occurs in the lower gut and does not appear to be a significant route of detoxification in the pig and other monogastric animals.

The Committee noted that the new ADME studies addressed the request made at the fifty-sixth meeting for data from comparative studies on toxicokinetics.

10.2 Toxicological data

As concluded at the previous meeting, emesis is the most sensitive functional manifestation of acute toxicity in the pig, dog and cat after either oral or parenteral administration. This is a systemic effect and is believed to arise from increased central serotonergic activity. The lowest doses that did not induce emesis in the pig were 0.025 mg/kg bw by gavage and 0.25 mg/kg bw by exposure via the diet. The Committee took note of the fact that much higher doses were tolerated when DON was given in the diet than by gavage.

New toxicological studies in mice, rats and pigs have provided insights into the mode of action of DON in causing reduced weight gain, which was the basis for the PMTDI established at the fifth-sixth meeting, and into its immunological and related effects in single-dose and repeated-dose studies. These studies indicated that the effects were largely due to the induction of suppressors of cytokine signalling and to effects on the pituitary GH axis. Changes in these parameters are observable very soon after acute dosing in vivo and are rapidly reversible in parallel with the decline in DON concentrations in plasma. At the levels likely to be encountered in the diet (described below), sustained exposure would be necessary to cause functional effects on growth or the immune system.

At its previous evaluation, the Committee concluded that DON is not mutagenic in bacteria but gave rise to chromosomal aberrations both in vitro and in vivo, but their overall significance remained equivocal. The limited new information regarding the potential genotoxicity of DON did not alter the Committee's previous conclusion.

Despite the request from the fifty-sixth meeting of the Committee, no new long-term study in a species other than mouse has become available, and the support for the lack of carcinogenic potential in humans remains dependent on a single mouse study.

One study on reproductive toxicity in rats became available, from which a NOAEL of 1 mg/kg bw per day was derived for reduced epididymal and seminal vesicle weights in rats, as well as increased sperm swimming speed. An additional developmental toxicity study in rats was available in which the NOAELs were 0.5 mg/kg bw per day for maternal toxicity, 1 mg/kg bw per day for fetal toxicity and 2.5 mg/kg bw per day for teratogenicity.

Results from studies on immunotoxicity in mice and pigs showed that low doses of DON increase IgA levels in the blood. The Committee noted, however, that there were insufficient data with which to establish a threshold for IgA nephropathy. Most mechanistic studies on immunological end-points in mice and pigs were unsuitable for deriving a NOAEL, but in one study, an acute NOEL of 0.1 mg/kg bw was derived based on suppression of hepatic mRNA for IGFAL. However, the toxicological significance of this finding is unknown.

The Committee considered the toxicity data on derivatives of DON. A few new studies have been published on the toxicity of acetylated DON, and these were considered together with the derivative studies in the previous evaluation. Given the results from the ADME studies, the toxicity of the acetylated DON compounds is likely to arise from conversion to DON. In vitro cytotoxicity and immunotoxicity studies of the relative potencies of DON and its acetylated derivatives are not considered to provide a reliable indication of relative potency in vivo, as they generally do not take account of this conversion. LD_{50} studies indicated their toxicity in mouse to be similar to that of DON. The acetylated DON compounds were therefore considered to be as toxic as DON.

No toxicological studies were found on DON-3-glucoside, a fungal metabolite recently detected in wheat and beer. The Committee considered it possible that this compound would be hydrolysed in the body and the DON would become bioavailable, but noted that ADME studies would be necessary to confirm this.

10.3 Observations in humans

No new epidemiological studies were found. With respect to possibilities for derivation of a NOAEL from outbreaks of mycotoxicosis in humans, recent studies indicate that urinary biomarkers may be used for assessing human exposure to DON. As DON can be formed from its acetylated derivatives, the Committee considered that these biomarkers could provide an indication of total dietary exposure to DON and its derivatives. Using the limited information on outbreaks from epidemiological studies summarized for the previous evaluation, the Committee noted that the calculated level that was not likely to elicit acute intoxication in humans was around 50 µg/kg bw.

10.4 Analytical methods

Since the fifty-sixth meeting, when the Committee reviewed the range of screening and quantitative methods available for the determination of DON in various foods, a number of advances have been made in the analysis of both DON and its derivatives, and certified standard solutions for DON, 3-Ac-DON and 15-Ac-DON have been made available.

Immunoassays for screening purposes for DON have been further developed and, in some instances, commercialized. These methods include lateral flow devices, fluorescence polarization and direct fluorometry after extract cleanup and derivatization. New antibodies continue to be developed. Possible cross-reactivity between DON and its derivatives in ELISA has been demonstrated in comparative studies and possibly accounts for the previously noted higher levels of naturally occurring DON determined by ELISA as opposed to chromatographic methods. Commercialized screening methods are usually developed with LODs targeted to meet legislative or other requirements.

Major advances have been made in DON determination by HPLC in which analytical methods using UV detection for DON in cereals (oat flour, wheat flour and rice flour), cereal products (polenta and wheat-based breakfast cereals), soft wheat

and baby food have been validated by international collaborative studies. These methods, using either immunoaffinity column or multifunctional column cleanup, have been validated down to 60 µg/kg for baby foods and to 100 µg/kg for all other products. The application of HPLC coupled to MS has enabled multi-mycotoxin analysis to be undertaken. The major problem of LC-MS and LC-MS/MS—namely, matrix effects in which signal enhancement or suppression occurs—is generally overcome by the use of isotope-labelled internal standards or matrix-matched standard solutions. These methods can be used for a limited range of mycotoxins for which a common cleanup, such as by multi-mycotoxin immunoaffinity column, is available; alternatively, a more diverse analysis can be performed by an injection of an aliquot of diluted sample extract without prior cleanup.

Based on current knowledge, the main derivatives of DON that might contribute to exposure are 3-Ac-DON, 15-Ac-DON and DON-3-glucoside. The analysis of these compounds requires chromatographic separation. They can be determined simultaneously with DON by LC-MS/MS. Alternatively, the acetyl derivatives have been determined by GC after suitable derivatization.

10.5 Sampling protocols

Owing to the lack of homogeneity in the distribution of mycotoxins, the sampling stage of the overall mycotoxin analysis can frequently represent the greatest contribution to the overall variance of the result. This was noted by the fifty-sixth meeting of the Committee. Specific sampling protocols for DON should be followed, such as the one provided by the EC, which regulates the number and size of incremental samples as well as the size of the aggregate sample to be taken for control purposes.

10.6 Effects of processing

The fifty-sixth meeting of the Committee reviewed the effects of gravity separation, milling, washing, soaking in water or sodium carbonate solutions, baking, extrusion cooking, fermentation and the use of microorganisms on DON levels. These are documented in the monograph of the fifty-sixth meeting of the Committee (Annex 1, reference *153*). Milling redistributes DON, with the highest amounts appearing in the bran, which is sometimes used in human food and most often in animal feed. Additional studies conducted since then have shown that removal of screenings and bran from wheat grains reduced DON levels by 41–50%. Current data have also confirmed the efficacy of washing or soaking in water or sodium carbonate solutions in reducing DON levels in barley and wheat. Although results of frying and baking studies have been conflicting, the use of extrusion cooking indicated a reduction of DON levels by between 18% and 95%, depending on the moisture content and temperature. It is, however, suggested that apparent reductions may be due to binding or the inability to extract the toxin from the extruded matrix using current analytical techniques. Few studies exist on the effects of malting and brewing processes on DON levels. Steeping lowered DON levels as a result of the water solubility of the toxin. During germination, DON levels increased 2-fold because of the conditions conducive for *Fusarium* growth and toxin formation. A subsequent decrease in DON levels during fermentation was observed, which

was attributed to yeast absorption. Additional studies are required to confirm these changes as well as the effects of processing on the acetyl derivatives of DON.

10.7 Prevention and control

Prevention and control practices include the use of suitable crop rotation, appropriate use of fertilizers, irrigation and weed control, and the use of resistant cultivars and decontamination procedures. The use of microorganisms is a recent approach employed to reduce growth of *Fusarium* species, severity of disease symptoms and DON levels. Strains of *Bacillus subtilis*, *Fusarium equiseti* and *Cryptococcus* sp. have given encouraging results (controlling FHB and reducing DON formation) in field studies with wheat. Experimental studies under glasshouse conditions with *Streptomyces* sp., *Pseudomonas fluorescens* and *Pseudomonas frederiksbergensis* strains similarly reduced both the severity of FHB symptoms caused by *Fusarium culmorum* in wheat and barley and DON levels under both glasshouse and field conditions. The use of chitosan (deacetylated derivative of chitin) for reducing DON levels as well as the severity of FHB symptom development in wheat and barley has been studied, but additional data are required to confirm the effects.

No new data are available on the use of chemicals such as sodium bisulfite, hypochlorite bleach, ammonia, moist ozone, and natural and modified clays to decontaminate grain.

10.8 Levels and patterns of contamination in food commodities

Information on the occurrence of DON was drawn from data received from a number of countries (Austria, Belgium, Brazil, China, Finland, France, Hungary, Japan, the Netherlands, Norway, Singapore and the United Kingdom), surveys published in the open literature from 42 countries, as well as the EC's SCOOP report on mycotoxins. Only DON data published since the previous evaluation were included in this assessment. In total, data on 23 980 samples analysed for DON were collected (68% from Europe, 17% from Asia, 6% from North America, 5% from South America and 3% from Africa). It was noted that DON remains a common contaminant in cereals (wheat, maize, oats, rye, barley, rice) and their products. Highest reported mean levels for raw cereals were as follows: wheat, 9900 µg/kg; maize, 4772 µg/kg; rice, 183 µg/kg; barley, 6349 µg/kg; oats, 537 µg/kg; and rye, 190 µg/kg. Contamination levels vary widely between and within regions. Relatively lower levels were detected in processed products, such as baby food, beer, bread, biscuits, pasta, muesli, noodles, cereal-based snacks, pizza, polenta, couscous, flours and fermented soya bean, most likely due to the decrease in contamination resulting from cereal milling and processing. Mean levels of DON in samples of processed products did not exceed 1250 µg/kg. As noted by the fifty-sixth meeting of the Committee, carry-over of DON into animal products is negligible due to feed refusal, rapid metabolism and elimination in livestock species.

The occurrence data for the DON derivatives 3-Ac-DON and 15-Ac-DON in wheat, maize, barley, oats, rye and their products were considered by the Committee for the first time at the present meeting. In addition to data submitted by

China, France, Japan and the United Kingdom, published data from studies conducted in nine countries were also assessed. Data were available on 3-Ac-DON from 6980 samples (92% from Europe and 8% from Asia) and on 15-Ac-DON from 4300 samples (81% from Europe, 16% from Asia and 3% from the USA). Generally, these derivatives are infrequently detected, and levels were typically less than 10% of those reported for DON. Highest reported mean levels in wheat, maize and barley for 3-Ac-DON were 193 µg/kg, 27 µg/kg and 19 µg/kg, respectively; for 15-Ac-DON, the corresponding highest reported mean levels were 365 µg/kg, 236 µg/kg and 0.3 µg/kg. The Committee was aware of reports on DON-3-glucoside in cereals and beer (data on 500 samples were assessed, with 79% from China, 15% from Europe and 6% from the USA), but considered that the data were too limited for dietary exposure assessment.

10.9 Food consumption and dietary exposure assessment

Dietary exposure to DON was evaluated at the fifty-sixth meeting of the Committee. Using the then-available five regional diets from GEMS/Food, the total dietary exposure to DON was estimated to range from 0.77 µg/kg bw per day in the African diet to 2.4 µg/kg bw per day in the Middle Eastern diet. The major source of dietary exposure in three of the five regional diets (European, Latin American and Middle Eastern) was wheat (64–88% of total exposure), whereas the sources in the other two regional diets were more varied (wheat, rice and maize in the African diet and wheat and rice in the Far Eastern diet).

At the current meeting, the Committee prepared updated international estimates using the consumption cluster diets from GEMS/Food and occurrence data reported in the literature or supplied to the Committee by Member States. Information was available on the concentrations of DON in six commodities: barley, maize, oats, rice, rye and wheat. Additionally, information on beer, the majority of which is produced from barley, was included. Data originating in 42 countries were analysed, representing 10 of the 13 GEMS/Food consumption cluster diets; no data were reported for the A, H and J clusters. Of the six commodities for which information was available for the exposure assessment, data on DON concentrations in barley, maize and wheat predominated, with limited reports on concentrations in oats, rice and rye. In total, 401 data points (mean values) representing 16 569 individual samples sorted by specific cluster diet were included in the exposure assessment. As the acetylated derivatives of DON are, in general, found at levels less than 10% of those for DON, they were not included in the dietary exposure estimates. Their inclusion would not be expected to change the estimates significantly.

The average dietary exposures to DON were calculated by multiplying the weighted mean concentration of each commodity by the corresponding amount of each commodity consumed in each of the 10 GEMS/Food consumption cluster diets for which occurrence data were available. The total dietary exposure to DON was estimated to range from 0.2 µg/kg bw per day (cluster C) to 14.5 µg/kg bw per day (cluster B). The main source of exposure in clusters C, D, E, F, G, K, L and M was wheat (56–100% of total exposure), whereas the main source in clusters B and I was maize. Three of the clusters had dietary exposure estimates above the PMTDI

of 1 µg/kg bw established previously. The Committee noted that the high estimates of dietary exposure to DON in clusters B and M were due to unusually high reported DON levels in maize and wheat in single countries for each cluster and that these data may not be representative of chronic dietary exposures. The range of estimates in the remaining clusters is in agreement with those prepared at the fifty-sixth meeting. It should be noted that any reduction in the concentration of DON as a result of processing has not been taken into consideration in this assessment.

Since the evaluation of DON at the fifty-sixth meeting of the Committee, a number of national evaluations of dietary exposure to DON have been published. The Committee considered evaluations of dietary exposure to DON from Argentina, Belgium, Czech Republic, Denmark, Ethiopia, France, Germany, Ireland, Japan, Lebanon, Morocco, the Netherlands, Nigeria, Republic of Korea and Thailand. Some of these reports contained overall dietary exposure assessments, whereas others assessed single commodities (or their products) considered to be the potential primary source of dietary exposure to DON in the population assessed. The evaluations that contained numerical estimates are summarized in Table 6 in section 8.4.16.

For risk characterization, the Committee chose a dietary exposure of 0.5 µg/kg bw per day for an average exposure and 1.0 µg/kg bw per day for a high exposure.

The Committee was asked to consider the need for an ARfD for DON. In this regard, the Committee prepared an estimate of acute dietary exposure to DON. The Committee chose to use a high-percentile daily consumption (97.5th, taken from the WHO GEMS/Food database) with a high concentration of DON (and its acetyl derivatives) in food (the highest mean value taken from the review of occurrence data at the present meeting). The consumptions for the foods most likely to be contaminated with DON were as follows: maize, 4.06 g/kg bw per day; wheat flour, 9.17 g/kg bw per day; white bread, 9.08 g/kg bw per day; and wheat, 13.46 g/kg bw per day. Considering that breads were the mostly likely foods to be regularly consumed, the Committee used a figure of 9 g/kg bw per day in making the estimate. Combining this with a DON contamination level of 10 mg/kg of wheat gives an acute dietary exposure estimate of 90 µg/kg bw per day. The Committee noted that regulatory limits for DON in foods in various countries range up to 1 mg/kg food. Using this limit with the high consumption figure would result in an acute dietary exposure of 9 µg/kg bw per day.

10.10 Dose–response analysis

The Committee was aware that acute exposure to high doses of DON and its derivatives has resulted in emesis in humans and considered it appropriate to establish an ARfD. Although developmental toxicity might be considered a potential effect of acute intoxication during critical periods of embryogenesis, the NOAEL for teratogenicity in the rat was 1 order of magnitude greater than the level found not to induce emesis in the pig; therefore, emesis in pigs was chosen to derive an acute health-based guidance value. Because the emetic effect was considered to be dependent on C_{max}, the Committee concluded that for the purpose of establishing

an ARfD, studies in which DON was administered via the diet were more appropriate than studies that used gavage dosing.

Data on DON-induced emesis in pigs, cats and dogs were available; although the effect was noted at similar concentrations in the three species, the dog and cat data were deemed not suitable for dose–response modelling. Two studies on emesis in piglets and pigs following exposure to DON via the diet (Young et al., 1983; Pollman et al., 1985) were combined for BMD modelling. Doses were calculated from the measured DON concentrations in the feed and the observed feed intake. In the first study, dietary concentrations above 3 mg/kg of feed resulted in drastically reduced average feed intakes (reduced by 88–94% compared with controls) and decreases in body weights during the test period; for these groups, it was assumed that the total feed intake over 4 or 11 days was actually all consumed on day 1. This assumption was made because it has often been observed that pigs stop eating after DON-induced vomiting on day 1. For the three dose groups in which it was reported that at least one pig vomited, it was assumed that the incidence was one. In the second study, the average feed intake was taken from the first week of exposure, although intake was decreased in the dose groups given 1.4 mg/kg of feed or more compared with controls. The initial body weights were used for the calculations, because the emesis was observed on day 1 of exposure.

The dose–response analysis was performed using the PROAST software (version 23.2). The BMR was set at 10% extra risk. The $BMDL_{10}$s among the accepted models ranged between 0.21 and 0.74 mg/kg bw per day. The lowest value in this range was used as a point of departure for establishing an ARfD.

11. EVALUATION

At its fifty-sixth meeting, the Committee established a PMTDI of 1 µg/kg bw for DON on the basis of the NOEL[2] of 100 µg/kg bw per day based on decreased body weight gain from a 2-year feeding study in mice and application of a safety factor of 100. Repeated-dose short-term studies considered in the present evaluation indicated that this NO(A)EL remains appropriate.

Since 3-Ac-DON is converted to DON and therefore contributes to the total DON-induced toxicity, the Committee decided to convert the PMTDI for DON to a group PTMDI of 1 µg/kg bw for DON and its acetylated derivatives (3-Ac-DON and 15-Ac-DON). In this regard, the Committee considered the toxicity of the acetylated derivatives equal to that of DON. The Committee concluded that, at this time, there was insufficient information to include DON-3-glucoside in the group PMTDI.

The Committee derived a group ARfD for DON and its acetylated derivatives using the lowest $BMDL_{10}$ of 0.21 mg/kg bw per day for emesis in pigs. The Committee considered that because DON-induced emesis is a systemic effect and more dependent on C_{max} than on area under the plasma concentration–time curve (AUC), it would be appropriate to apply an uncertainty factor of 25, which is the value

[2] At its sixty-eighth meeting (Annex 1, reference *187*), the Committee decided to differentiate between NOAEL and NOEL. This NOEL would now be considered a NOAEL.

used by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) for acute C_{max}-dependent effects. The Committee established a group ARfD for DON and its acetylated derivatives of 8 µg/kg bw. Limited data from human case reports indicated that dietary exposures to DON up to 50 µg/kg bw per day are not likely to induce emesis.

Estimation of dietary exposure was made using data from 42 countries, representing 10 of the 13 GEMS/Food consumption cluster diets, and was therefore considered to be more globally representative than the previous evaluation. The Committee concluded that all of the mean estimates of national exposure to DON were below the group PMTDI of 1 µg/kg bw. National reports showed dietary exposures that were above 1 µg/kg bw per day in only a few cases, only for children at upper percentiles. For acute dietary exposure, the estimate of 9 µg/kg bw per day, based on high consumption of bread and a regulatory limit for DON of 1 mg/kg food, was close to the group ARfD.

The acetylated derivatives have not been included in the estimates of dietary exposure to DON prepared at this meeting. The Committee noted that in general they are found at levels less than 10% of those for DON, and inclusion would not be expected to significantly change the estimates of dietary exposure to DON. Data are limited on the occurrence of DON-3-glucoside, which might be an important contributor to dietary exposure; this derivative was also not included in the dietary exposure estimates.

11.1 Recommendations

- As DON-3-glucoside has been detected in cereals and beers and might therefore contribute to systemic exposure to DON, the Committee recommended that ADME studies be conducted on this substance.
- Additional data on the occurrence of and the effects of processing on 3-Ac-DON, 15-Ac-DON and DON-3-glucoside are needed, as well as their co-occurrence with DON.

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Appendix 1:

DON occurrence tables

Table A1. Occurrence data for DON (Africa)

Country/ region	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Kenya	Beer (Tusker)		39	1.045				3.29/6.40			Mbugua & Gathumbi (2004)
Kenya	Beer (Pilsner)		36	1.045				3.57/4.35			Mbugua & Gathumbi (2004)
Kenya (Nakuru)	Wheat	2004	48			12		132.7/303			Muthomi et al. (2008)
Kenya (Nyandura)	Wheat	2004	34			20		113/289			Muthomi et al. (2008)
Morocco	Wheat		17	50	85		10	27.1/128			Hajjaji et al. (2006)
South Africa	Maize	2003–2004	90	500				200/13 000			Southern African Grain Laboratory (http://www.sagl.co.za/)
	Maize	2004–2005	100	500				600/3260			Southern African Grain Laboratory (http://www.sagl.co.za/)
	Maize	2005–2006	90	500				2740/6200			Southern African Grain Laboratory (http://www.sagl.co.za/)
	Maize	2006–2007	90	500				530/3100			Southern African Grain Laboratory (http://www.sagl.co.za/)
	Maize	2007–2008	100	250				240/1700			Southern African Grain Laboratory (http://www.sagl.co.za/)
	Wheat	2004–2005	30	500				1060/1800			Southern African Grain Laboratory (http://www.sagl.co.za/)

Table A1 (contd)

Country/ region	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat	2005–2006	30	500				1010/1500			Southern African Grain Laboratory (http://www.sagl.co.za/)
	Wheat	2006–2007	30	500				1460/2400			Southern African Grain Laboratory (http://www.sagl.co.za/)
	Wheat	2007–2008	30	500				1360/2700			Southern African Grain Laboratory (http://www.sagl.co.za/)
Tunisia	Durum wheat	2007	65	10	30		11	7200/54 000			Bensassi et al. (2010)

Table A2. Occurrence data for DON (Americas)

Country	Commodity	Year/season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Argentina	Maize	2003	12	9	12		8	118.5/834.4			Broggi et al. (2007)
	Maize	2004	9	9	12		9	nd			Broggi et al. (2007)
	Maize, Bt & non-Bt	2002–2003		50		0.44		1700			Barros et al. (2009)
	Maize, Bt & non-Bt	2003–2004		50		0.28		2150			Barros et al. (2009)
- Miramar	Wheat	?	10	50		0		1300/3040			Lori et al. (2003)
- La Dulce	Wheat	?	10	50		1		900/2400			Lori et al. (2003)
- Barrow	Wheat	?	10	50		7		100/570			Lori et al. (2003)
- Bordenave	Wheat	?	10	50		10		nd			Lori et al. (2003)
- Balcarce	Wheat	Consecutive	12	50		0		2600/8440			Lori et al. (2003)
- Miramar	Wheat	Consecutive	12	50		0		2000/4220			Lori et al. (2003)
- La Dulce	Wheat	Consecutive	12	50		0		1600/3200			Lori et al. (2003)
- Barrow	Wheat	Consecutive	12	50		1		2100/5330			Lori et al. (2003)
- Bordenave	Wheat	Consecutive	12	50		12		nd			Lori et al. (2003)

Table A2 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Brazil	Wheat	2005	5			0		1321			Miranda et al. (2006)
	Maize-based foods	November 2001–January 2002	78	20–60	70–300	77	78	nd/167			Milanez, Valente-Soares & Baptista (2006)
	Whole rice	2003–2008	16	10			16	nd			Nunes, Garda & Furlong (2001)
	Parboiled rice	2003–2008	16	10			16	nd			Nunes, Garda & Furlong (2001)
	Polished rice	2003–2008	24	10			22	190/300			Nunes, Garda & Furlong (2001)
	Rice by-products	2006	24	10			21	183/250			Nunes, Garda & Furlong (2001)
	Wheat grain	2002–2003	14	80	100		9	?/1500			Lamardo, Navas & Sabino (2006)
	Wheat flour	2002–2003	28	80	100		14	296/600			Lamardo, Navas & Sabino (2006)
	Sandwich cookie	2008	5	<80	80		0	461/594			Unpublished
	Wheat flour	2008	2	<80	80		0	834/1450			Unpublished

Table A2 (contd)

Country	Commodity	Year/season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat bran	2008	6	<80	80		0	386/863			Unpublished
	Rice	November 2007	101	30	100		88	108/192			Unpublished
	Rice	January–December 2008	65	30	100		52	172/244			Unpublished
	Rice bran	June 2008	11	30	100		11	nd			Unpublished
	Wheat grain	July 2008	7	30	100		5	293/378			Unpublished
	Wheat flour	March 2009	1	30	100		1	nd			Unpublished
	Filled biscuit wafer	October 2009	5				0	1246/1348			Unpublished
Canada	Maize, Bt	1996	17	200		7		450			Schaafsma et al. (2002)
	Maize, non-Bt	1996	17	200		1		1250			Schaafsma et al. (2002)
	Maize, Bt	1997	27	200		16		360			Schaafsma et al. (2002)
	Maize, non-Bt	1997	27	200		9		510			Schaafsma et al. (2002)

Table A2 (contd)

Country	Commodity	Year/season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Maize, Bt	1998	31	200		8		690			Schaafsma et al. (2002)
	Maize, non-Bt	1998	31	200		3		1150			Schaafsma et al. (2002)
	Maize, Bt	1999	27	200		4		1060			Schaafsma et al. (2002)
	Maize, non-Bt	1999	27	200		4		1190			Schaafsma et al. (2002)
	Infant cereal, oat-based	1997–1999	53		20		20	32/90			Lombaert et al. (2003)
	Infant cereal, barley-based	1997–1999	50		20		21	150/980			Lombaert et al. (2003)
	Infant cereal, soya-based	1997–1999	8		20		0	116/240			Lombaert et al. (2003)
	Infant cereal, rice-based	1997–1999	9		20		9	nd			Lombaert et al. (2003)
	Infant cereal, multigrain	1997–1999	86		20		24	83/400			Lombaert et al. (2003)
	Infant teething biscuits	1997–1999	24		20		6	45/120			Lombaert et al. (2003)

Table A2 (contd)

Country	Commodity	Year/season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Infant soya formula	1997–1999	1		20		1	nd			Lombaert et al. (2003)
	Infant creamed corn	1997–1999	6		20		6	nd			Lombaert et al. (2003)
	Breakfast cereal, maize-based	1999–2001	34	10	20		22	30/420			Roscoe et al. (2008)
	Breakfast cereal, multigrain	1999–2001	36	10	20		15	80/770			Roscoe et al. (2008)
	Breakfast cereal, oat-based	1999–2001	27	10	20		10	20/80			Roscoe et al. (2008)
	Breakfast cereal, rice-based	1999–2001	29	10	20		28	1.4/40			Roscoe et al. (2008)
	Breakfast cereal, wheat-based	1999–2001	29	10	20		8	110/940			Roscoe et al. (2008)
	Breakfast cereal, buckwheat	1999–2001	1	10	20		1	nd			Roscoe et al. (2008)
Canada, eastern	Maize	1991	54		10		25	370/1070			Campbell et al. (2002)
	Maize	1992	145		10		25	500/17 500			Campbell et al. (2002)

Table A2 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Maize	1993	93		10		5	550/2360			Campbell et al. (2002)
	Maize	1994	109		10		6	490/2090			Campbell et al. (2002)
	Maize	1995	89		10		10	400/2640			Campbell et al. (2002)
	Maize	1996	69		10		0	640/2550			Campbell et al. (2002)
	Maize	1997	74		10		0	750/5880			Campbell et al. (2002)
	Maize	1998	40		10		2	470/1710			Campbell et al. (2002)
	Wheat	1991	4		10		2	170/200			Campbell et al. (2002)
	Wheat	1992	12		10		3	1370/6990			Campbell et al. (2002)
	Wheat	1993	12		10		4	520/1080			Campbell et al. (2002)
	Wheat	1994	21		10		4	1870/9160			Campbell et al. (2002)
	Wheat	1995	11		10		1	730/1320			Campbell et al. (2002)
	Wheat	1996	10		10		0	3210/9070			Campbell et al. (2002)
	Wheat	1997	20		10		5	1150/3370			Campbell et al. (2002)
	Wheat	1998	9		10		1	1680/5440			Campbell et al. (2002)
Uruguay	Barley	1996	100		500		70	326/2100			Pan et al. (2007)
	Barley	1997	59		500		19	794/3000			Pan et al. (2007)
	Barley	1998	45		500		33	294/1900			Pan et al. (2007)

Table A2 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Barley	1999	26		500		19	411/2600			Pan et al. (2007)
	Barley	2000	8		500		1	3592/5000			Pan et al. (2007)
	Barley	2001	25		500		0	6349/10 000			Pan et al. (2007)
	Barley	2002	37		500		0	4098/10 000			Pan et al. (2007)
	Wheat	1997	13		500		5	1539/3400			Pan, Graneri & Bettucci (2009)
	Wheat	1998	10		500		10	nd			Pan, Graneri & Bettucci (2009)
	Wheat	1999	10		500		10	nd			Pan, Graneri & Bettucci (2009)
	Wheat	2000	10		500		2	3233/5000			Pan, Graneri & Bettucci (2009)
	Wheat	2001	81		500		0	6593/11 400			Pan, Graneri & Bettucci (2009)
	Wheat	2002	20		500		0	5880/8800			Pan, Graneri & Bettucci (2009)
	Wheat	2003	142		500		59	1360/7500			Pan, Graneri & Bettucci (2009)

Table A2 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
USA	Durum wheat, Montana	2001	23		50			nd			Manthey et al. (2004)
	Durum wheat, NW North Dakota	2001	27		50			2700/13 000			Manthey et al. (2004)
	Durum wheat, NC North Dakota	2001	24		50			9100/23 000			Manthey et al. (2004)
	Durum wheat, NE North Dakota	2001	15		50			9900/22 000			Manthey et al. (2004)
	Durum wheat, SW North Dakota	2001	17		50			400/4100			Manthey et al. (2004)
	Durum wheat, SE North Dakota	2001	17		50			2900/9100			Manthey et al. (2004)
	Wheat, hard red spring	2005	28	1	0.5		10	1400/10 000			Sasanya, Hall & Wolf-Hall (2008)

Bt, *Bacillus thuringiensis*; NC, north-central; nd, not detected; NE, north-east; NW, north-west; SE, south-east; SW, south-west

Table A3. Occurrence data for DON (Asia)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
China	Maize	2008	203	0.1	0.3		103	144/4374			GEMS/Food database
	Wheat	2008	162	0.1	0.3		23	63/591			GEMS/Food database
	Wheat flour	2008	30	0.1	0.3		0	52/3425			GEMS/Food database
China, Henan Province											
- Puyang	Wheat	1998	31	10		1		2850/14 000			Li et al. (2002)
- Zhumadian	Wheat	1998	28	10		3		223/1240			Li et al. (2002)
- Puyang	Wheat	1999	34	10		5		294/941			Li et al. (2002)
Japan	Wheat	2002	199		50		118	180/2100		550	GEMS/Food database
	Wheat	2003–2004	213		50		136	67/580		260	GEMS/Food database
	Wheat	2004	226	20	50		145	44/930		130	GEMS/Food database
	Wheat	2005	200	4	10		108	18/230		42	GEMS/Food database
	Wheat	2006	100	3	10		13	130/880	42	410	GEMS/Food database
	Wheat	2007–2008	100	3	9		43	23/290	10	55	GEMS/Food database
	Wheat	2008	120	5	13		39	33/460	14	53	GEMS/Food database
	Barley	2002	50		50		28	280/4800		720	GEMS/Food database
	Barley	2003–2004	54		50		34	290/3700		910	GEMS/Food database

Table A3 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Barley	2004	56	2	50		23	250/1800	90	670	GEMS/Food database
	Barley	2005	50	4	10		20	60/460	11	190	GEMS/Food database
	Barley	2006	10	3	10		0	550/2500	370	1000	GEMS/Food database
	Barley	2007	10	3	7		3	64/320	16	190	GEMS/Food database
	Barley	2008	100	3	7		22	32/560	15	61	GEMS/Food database
	Bread	2007	50	20	40		49	1/40			GEMS/Food database
	Uncooked noodles	2007	20	20	40		19	2/40			GEMS/Food database
	Dried noodles	2007	20	20	40		20	nd			GEMS/Food database
	Boiled noodles	2007	20	20	40		20	nd			GEMS/Food database
	Uncooked noodles	2007	10	20	40		10	nd			GEMS/Food database
	Steamed noodles	2007	10	20	40		10	nd			GEMS/Food database
	Boiled noodles	2007	10	20	40		10	nd			GEMS/Food database
	Instant noodles	2007	20	20	40		19	2/40			GEMS/Food database
	Macaroni	2007	20	20	40		17	9/100			GEMS/Food database
	Baked goods	2007	10	20	40		10	nd			GEMS/Food database
	Wheat flour	2007	79	2	5		8	60/630		170	GEMS/Food database

Table A3 (contd)

Country	Commodity	Year/season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat flour	2008	50	20	40		48	2/40			GEMS/Food database
	Bean paste	2009	20	30	60		20	nd			GEMS/Food database
	Soya sauce	2009	20	20	50		20	nd			GEMS/Food database
	Beer	2009	20	20	50		19	3/50			GEMS/Food database
	Barley tea	2009	20	20	50		20	nd			GEMS/Food database
	Bread	2007	35		1		0	8.6/25.2			Sugiyama et al. (2009)
	Wheat flour	2007	12		5		1	31.3/113			Sugiyama et al. (2009)
	Biscuits (wheat)	2004–2006	201	0.9	3.0	5		23/791			Tanaka et al. (2010)
Lebanon	Biscuits	2005	20	30		10		31/70			Soubra et al. (2009)
	Bread	2005	40	30		22		176/700			Soubra et al. (2009)
	Cakes	2005	20	30		15		60/100			Soubra et al. (2009)
	Cornflakes	2005	20	30		14		58/100			Soubra et al. (2009)
	Croissant	2005	20	30		10		50/120			Soubra et al. (2009)
	Doughnuts	2005	20	30		12		60/130			Soubra et al. (2009)
	Kaak assrounieh	2005	20	30		10		50/130			Soubra et al. (2009)
	Kaak tea	2005	20	30		10		70/220			Soubra et al. (2009)
	Lahm bi ajin	2005	20	30		11		88/240			Soubra et al. (2009)

Table A3 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Manakeesh	2005	20	30		10		88/300			Soubra et al. (2009)
	Pizza	2005	20	30		10		85/200			Soubra et al. (2009)
	Toast	2005	20	30		10		52/120			Soubra et al. (2009)
Malaysia	Wheat-based noodles	?									Moazami & Jinap (2009)
	- Instant, domestic	?	42	0.63		18		<1.0			Moazami & Jinap (2009)
	- Instant, imported	?	48	0.63		24		<1.0			Moazami & Jinap (2009)
	- Yellow alkaline, domestic	?	21	0.63		9		<1.0			Moazami & Jinap (2009)
	- Yellow alkaline, imported	?	9	0.63		9		nd			Moazami & Jinap (2009)
	- White salted	?	15	0.63		15		nd			Moazami & Jinap (2009)
Nepal											
- Bagmati	Maize	2004	12	100		8		663/4070			Desjardins et al. (2008)
- Bheri	Maize	2004	5	100		4		4150			Desjardins et al. (2008)
- Dhaulagiri	Maize	2004	6	100		3		440/1040			Desjardins et al. (2008)
- Gandaki	Maize	2004	3	100		2		3130			Desjardins et al. (2008)

Table A3 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Republic of Korea	Corn breakfast cereal	2007–2008	18	2.2	5.6	12		8.0/36.5			Ok et al. (2009a)
	Dried corn	2007–2008	82	2.2	5.6	18		103.2/807.3			Ok et al. (2009a)
	Canned corn	2007–2008	25	2.2	5.6	25		nd			Ok et al. (2009a)
	Barley	2007–2008	70	2.2	5.6	32		9.4/36.8			Ok et al. (2009a)
	Barley beer	2007–2008	26	2.2	5.6	23		2.4/28.6			Ok et al. (2009a)
	Biscuits	2007–2008	8	2.2	5.6	5		9.4/35.2			Ok et al. (2009a)
	Bread	2007–2008	8	2.2	5.6	5		19.6/78.1			Ok et al. (2009a)
	Wheat	2007–2008	41	2.2	5.6	17		32.7/353.6			Ok et al. (2009a)
	Wheat flour	2007–2008	37	2.2	5.6	21		18.8/172.9			Ok et al. (2009a)
	Glutinous rice	2007–2008	43	2.2	5.6	41		1.4/37.4			Ok et al. (2009a)
	Rice-based mixed cereal	2007–2008	50	2.2	5.6	31		5.9/57.5			Ok et al. (2009a)
	Polished rice	2007–2008	62	2.2	5.6	57		0.8/16.0			Ok et al. (2009a)
	Unpolished rice	2007–2008	44	2.2	5.6	38		6.0/127.9			Ok et al. (2009a)

Table A3 (contd)

Country	Commodity	Year/season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Singapore	Buckwheat	2005	1		13		1	nd			GEMS/Food database
	Buckwheat	2008	3	4	13		3	nd			GEMS/Food database
	Wheat	2002, 2005	2		13		2	nd			GEMS/Food database
	Cereal grains	2005	21		13		18	17/259			GEMS/Food database
	Cereal grains	2008	16	4	13		12	16/137			GEMS/Food database
	Spices	2005	2		13		1	81			GEMS/Food database
	Rye	2002, 2005	2		13		2	nd			GEMS/Food database
	Rye	2008	1	4	13		1	nd			GEMS/Food database
	Popcorn	2002, 2005	2		13		2	nd			GEMS/Food database
	Sweet corn kernels	2002	9		13		9	nd			GEMS/Food database
	Tapioca	2005	1		13		1	nd			GEMS/Food database
	Vegetable spaghetti	2005	23		13		19	8.8/102			GEMS/Food database
	Corn flour	2008	2		13		2	nd			GEMS/Food database
	Noodles	2008	26	4	13		23	7.9/89			GEMS/Food database
	Wheat, whole meal	2008	1	4	13		1	nd			GEMS/Food database

Table A3 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat, bulgur	2008	1	4	13		1	nd			GEMS/Food database
Thailand	Noodles	2007	30	100		28		4.3/350			Poapolathep et al. (2008)
	Breads	2007	30	100		25		62/1130			Poapolathep et al. (2008)
	Cereals	2007	30	100		20		80/390			Poapolathep et al. (2008)
Turkey	Beer	2002–2003	50	125 µg/l		50		nd			Omurtag & Beyoğlu (2007)
	Wheat	?	27	100		27		nd			Omurtag & Beyoğlu (2003)
	Corn starch	?	3	100		3		nd			Omurtag & Beyoğlu (2003)
	Corn flour	?	7	100		3		517/2670			Omurtag & Beyoğlu (2003)
	Dried corn	?	11	100		10		650			Omurtag & Beyoğlu (2003)
	Home-made macaroni	?	3	100		2		470			Omurtag & Beyoğlu (2003)
	Other cereals / processed food	?	17	100		17		nd			Omurtag & Beyoğlu (2003)
	Processed pulses	?	15	100		15		nd			Omurtag & Beyoğlu (2003)

nd, not detected

Table A4. Occurrence data for DON (Europe)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Austria, Germany, Slovakia	Wheat	2005	23	8	20			1500/4130			Berthiller et al. (2009b)
Austria	Maize	2006	54	16	40			753/3680			Berthiller et al. (2009b)
	Beer	2000–2002	33	3.7[a]		8		10.2/29.5[a]	8.7[a]		Papadopoulou-Bouraoui et al. (2004)
	Oat	2000	96		100	83		43.5/530[b]	2000		Schothorst & van Egmond (2004)
	Oat	2001	40		100	36		29.3/200[b]	15		Schothorst & van Egmond (2004)
	Wheat	2000	62		100	24		744/6090[b]	2000		Schothorst & van Egmond (2004)
	Wheat	2001	36		100	18		175.6/1230[b]	72.5		Schothorst & van Egmond (2004)
Belgium	Wheat	2001	33		250	28		87/504[b]	<250		Food Inspection Service and Institute for Public Health
	Wheat	2002	14		50	14		8/<50[b]	<50		Food Inspection Service and Institute for Public Health

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Biscuits	2000	5		250	5		42/<250[b]	<250		Food Inspection Service and Institute for Public Health
	Baby food	2000	5		250	5		42/<250[b]	<250		Food Inspection Service and Institute for Public Health
	Baby food	2002	5		50	5		8/<50[b]	<50		Food Inspection Service and Institute for Public Health
	Bread	2000	10		250	6		187/560[b]	<250		Food Inspection Service and Institute for Public Health
	Bread	2001	5		200	4		97/350[b]	<200		Food Inspection Service and Institute for Public Health
	Bread	2001	5		175	5		29/<175[b]	<175		Food Inspection Service and Institute for Public Health
	Bread	2002	18		50	18		8/<50[b]	<50		Food Inspection Service and Institute for Public Health
	Pasta	2000	10		250	7		159/559[b]	<250		Food Inspection Service and Institute for Public Health
	Pasta	2001	10		250	7		193/716[b]	<250		Food Inspection Service and Institute for Public Health
	Pasta	2002	9		50	8		16/74[b]	<50		Food Inspection Service and Institute for Public Health

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat bran	2000	5		250	4		128/475[b]	<250		Food Inspection Service and Institute for Public Health
	Muesli bars	2000	5		250	5		42/<250[b]	<250		Food Inspection Service and Institute for Public Health
	Beer	2003–2004	80	2	6	21	36	5/22	4		Anselme et al. (2006)
	Beer	2000–2002	47	3.7[a]		0		18.1/56.7[a]	15.1[a]		Papadopoulou-Bouraoui et al. (2004)
	Eggs	2006	10	0.6	2	2	6	2.3/7.5	2		Tangni et al. (2009)
	Eggs	2007	10	0.6	2	1	4	4.5/17.9	3		Tangni et al. (2009)
Cyprus	Beer	2000–2003	6	3.7[a]		1		8/12.2[a]	7.8		Papadopoulou-Bouraoui et al. (2004)
Czech Republic	Beer	2000–2003	17	3.7[a]		0		21.5/55.3	18.8[a]		Papadopoulou-Bouraoui et al. (2004)
	Winter wheat	2003	42		250	0		330/3500			Ostry et al. (2005)
Denmark	Wheat	2000	28	20		2		59/330	33		Rasmussen, Ghorbani & Berg (2003)
	Wheat	2001	30	20		11		32/204	10		Rasmussen, Ghorbani & Berg (2003)
	Wheat flour	2000	28	20	20	2		59/33[b]	33		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat flour	2001	30	20	20	11		32/204[b]	10		Schothorst & van Egmond (2004)
	Rye flour	2000	17	20	20	9		23/84[b]	10		Schothorst & van Egmond (2004)
	Rye flour	2001	20	20	20	9		17/55[b]	10		Schothorst & van Egmond (2004)
	Durum wheat flour	2000	23	20	20	0		1157/2591[b]	1242		Schothorst & van Egmond (2004)
	Durum wheat flour	2001	10	20	20	0		1153/1619[b]	1224		Schothorst & van Egmond (2004)
	Rye	2000	17	20		9		23/84	10		Rasmussen, Ghorbani & Berg (2003)
	Rye	2001	20	20		9		17/55	10		Rasmussen, Ghorbani & Berg (2003)
	Beer	2000–2002	9	3.7[a]		0		19.9/47.1	19.9[a]		Papadopoulou-Bouraoui et al. (2004)
Finland	Wheat	2000	35		50	10		169.9/1026[b]	111		Schothorst & van Egmond (2004)
	Wheat	2001	39		25/40	25		36.3/376[b]	4.17		Schothorst & van Egmond (2004)
	Barley	2000	20		50	12		57/293[b]	8.33		Schothorst & van Egmond (2004)
	Barley	2001	20		25/40	10		78.8/619[b]	17.4		Schothorst & van Egmond (2004)
	Barley malt	2000	25		50	18		45.7/394[b]	8.33		Schothorst & van Egmond (2004)
	Barley malt	2001	25		25/40	15		28.4/144[b]	4.17		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Oats	2000	25		50	10		537.2/5004[b]	97.6		Schothorst & van Egmond (2004)
	Oats	2001	30		25/40	4		236.1/1560[b]	89.9		Schothorst & van Egmond (2004)
	Rye	2000	15		50	14		19.6/178[b]	8.33		Schothorst & van Egmond (2004)
	Rye	2001	12		25/40	10		9.18/37.4[b]	4.17		Schothorst & van Egmond (2004)
	Rye organic	2001	1		25/40	1		6.67[b]			Schothorst & van Egmond (2004)
	Beer	2000–2002	4	3.7[a]		1		7.4/10.6	6.3[a]		Papadopoulou-Bouraoui et al. (2004)
France	Wheat	2001	30		20			132.15/2125[b]	3.3		Schothorst & van Egmond (2004)
	Wheat	2001	22		20			10.88/170[b]	3.3		Schothorst & van Egmond (2004)
	Wheat	2000	1	100		1		50/0[b]	50		Schothorst & van Egmond (2004)
	Wheat	2001	1	50		0		105/85[b]	40		Schothorst & van Egmond (2004)
	Wheat	2002	3	100		1		100.33/120[b]	50		Schothorst & van Egmond (2004)
	Corn	2001	29		20			494.12/8850[b]	50		Schothorst & van Egmond (2004)
	Corn	2000	25		125	0		895/2000[b]			Schothorst & van Egmond (2004)
	Corn	2001	25	30		1		1056/4800[b]			Schothorst & van Egmond (2004)
	Corn	2000	59		60–100	0		475/3390[b]	300		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Corn	2001	107		50–60	0		903/5400[b]	650		Schothorst & van Egmond (2004)
	Corn	2001	9	25	50	5		19/36[b]	12		Schothorst & van Egmond (2004)
	Barley	2001	9		20			6.47/35[b]	3.3		Schothorst & van Egmond (2004)
	Wheat bran	2001	1		100	1		16.67/[b]	16.67		Schothorst & van Egmond (2004)
	Wheat bran	2002	6		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Wheat bran	2001	3		200	1		33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Wheat bran	2000	9	50		0		525.56/2000[b]	240		Schothorst & van Egmond (2004)
	Wheat bran	2001	8	20		1		59.38/170[b]	13		Schothorst & van Egmond (2004)
	Wheat bran	2002	13	20		4		222.23/915[b]	140		Schothorst & van Egmond (2004)
	White wheat flour	2000	15	60	200	6		155.62/500[b]	125		Schothorst & van Egmond (2004)
	White wheat flour	2000	1		100			125/[b]	125		Schothorst & van Egmond (2004)
	White wheat flour	2001	170		200	14		143.14/1213[b]	33.3		Schothorst & van Egmond (2004)
	White wheat flour	2002	11		200			45.5/136[b]	33.3		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	White wheat flour	2002	46		100	13		63.08/595[b]	16.7		Schothorst & van Egmond (2004)
	White wheat flour	2000	55	51		9		992.89/50 000[b]	45		Schothorst & van Egmond (2004)
	White wheat flour	2001	38	50		16		105/400[b]	40		Schothorst & van Egmond (2004)
	White wheat flour	2002	33	50		16		100.33/330[b]	50		Schothorst & van Egmond (2004)
	White wheat flour	2000	10	50		3		153/300[b]	45		Schothorst & van Egmond (2004)
	White wheat flour	2001	4	50		2		105/280[b]	40		Schothorst & van Egmond (2004)
	White wheat flour	2002	3	100		3		50/0[b]	50		Schothorst & van Egmond (2004)
	White wheat flour	2000	37		50	0		304/2100[b]	220		Schothorst & van Egmond (2004)
	White wheat flour	2001	101		20–50	0		53/328[b]	25		Schothorst & van Egmond (2004)
	Corn fractions	2000	29	60	200	11		30/[b]	30		Schothorst & van Egmond (2004)
	Corn fractions	2001	1					340/340[b]	340		Schothorst & van Egmond (2004)
	Corn fractions	2001	1					620/620[b]	620		Schothorst & van Egmond (2004)
	Corn fractions	2000	1	100		1		50/[b]	50		Schothorst & van Egmond (2004)
	Corn fractions	2001	17	100		3		105/1400[b]	40		Schothorst & van Egmond (2004)
	Corn fractions	2002	7	20		0		100.33/825[b]	50		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Corn meal	2001	1		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Corn meal	2001	2					435/450[b]	435		Schothorst & van Egmond (2004)
	Corn meal	2001	3		200			331.1/1400[b]	340		Schothorst & van Egmond (2004)
	Corn meal	2002	1					245/245[b]	245		Schothorst & van Egmond (2004)
	Wheat products	2000	3	60		3		30/[b]	30		Schothorst & van Egmond (2004)
	Wheat products	2001	5		200	1		33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Wheat products	2001	75		200	8		286.4/1826[b]	235		Schothorst & van Egmond (2004)
	Wheat products	2002	2		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Wheat products	2002	8		200	2		348.28/502[b]	33.3		Schothorst & van Egmond (2004)
	Wheat products	2000	1	50		0		289/289[b]	289		Schothorst & van Egmond (2004)
	Wheat products	2001	15	50		13		105/250[b]	40		Schothorst & van Egmond (2004)
	Wheat products	2002	7	100		6		100.33/220[b]	50		Schothorst & van Egmond (2004)
	Wheat products	2000	12	50		1		992.89/1000[b]	45		Schothorst & van Egmond (2004)
	Wheat products	2001	15	50		3		105/410[b]	40		Schothorst & van Egmond (2004)
	Wheat products	2002	3	100		2		100.33/220[b]	50		Schothorst & van Egmond (2004)
	Pasta	2001	1		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Pizza	2000	1					150/150[b]	150		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Pizza	2001	1					216/216[b]	216		Schothorst & van Egmond (2004)
	Pizza	2001	2					33.3/611[b]	33.3		Schothorst & van Egmond (2004)
	Corn products	2001	1					33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Corn products	2001	8			2		237.59/320[b]	225.95		Schothorst & van Egmond (2004)
	Polenta	2001	1					33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Polenta	2002	3		100			63.9/[b]	25		Schothorst & van Egmond (2004)
	Sweet corn	2002	4			1		33.3/222[b]	33.3		Schothorst & van Egmond (2004)
	Sweet corn	2002	9		200			57.49/224[b]	33.3		Schothorst & van Egmond (2004)
	Soft wheat	2001	31		20			22 068/230[b]	3.3		Schothorst & van Egmond (2004)
	Soft wheat	2000	82	30	60	7		270/1500[b]	190		Schothorst & van Egmond (2004)
	Soft wheat	2001	72	30	60	48		62/700[b]	15		Schothorst & van Egmond (2004)
	Soft wheat	2002	71	30	60	15		216/1900[b]	100		Schothorst & van Egmond (2004)
	Soft wheat	2000	276	30–250	40–100	47		283/1520[b]	190		Schothorst & van Egmond (2004)
	Soft wheat	2001	252	25–30	10–100	67		95/1038[b]	25		Schothorst & van Egmond (2004)
	Soft wheat	2001	8	25	50	5					Schothorst & van Egmond (2004)
	Durum wheat	2000	16	30	60	7		372/1600[b]	175		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Durum wheat	2001	13	30	60	4		169/730[b]	110		Schothorst & van Egmond (2004)
	Durum wheat	2002	52	30	60	9		689/3600[b]	470		Schothorst & van Egmond (2004)
	Grain fractions	2000–2001	145	200	200	50					Schothorst & van Egmond (2004)
	Grain fractions	2002	69	200	200	24					Schothorst & van Egmond (2004)
	Rye flour	2000	1					120/120[b]	120		Schothorst & van Egmond (2004)
	Rye flour	2001	2		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Rye flour	2002	1		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Rye flour	2002	3		200	1		174/595[b]	33		Schothorst & van Egmond (2004)
	Rice flour	2001	1		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Rice flour	2002	1		100	1		16.7/[b]	16.7		Schothorst & van Egmond (2004)
	Buckwheat	2000	1	60		1		30/[b]	30		Schothorst & van Egmond (2004)
	Buckwheat	2000	5	60		5		30/[b]	30		Schothorst & van Egmond (2004)
	Buckwheat	2001	3		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Buckwheat	2001	15		200	6		33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Buckwheat	2002	1		100			16.07/[b]	16.07		Schothorst & van Egmond (2004)
	Buckwheat	2002	3		100	2		34.5/70[b]	16.07		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Oat products	2000	1	60		1		30/[b]	30		Schothorst & van Egmond (2004)
	Oat products	2001	11		200	1		33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Oat products	2001	3		200	1		33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Oat products	2002	5		50	4		8.3/[b]	8.3		Schothorst & van Egmond (2004)
	Barley products	2000	1	60		1		30/[b]	30		Schothorst & van Egmond (2004)
	Barley products	2001	9		200	3		33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Rice products	2000	1	60		1		30/[b]	30		Schothorst & van Egmond (2004)
	Rice products	2001	7		200	1		33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Rice products	2002	1		100	1		16.7/[b]	16.7		Schothorst & van Egmond (2004)
	Rice products	2002	3		200	1		33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Rye flour	2001	11		200	1		103.76/350[b]	33.3		Schothorst & van Egmond (2004)
	Breakfast cereals	2001	14		200			63/235[b]	33		Schothorst & van Egmond (2004)
	Breakfast cereals	2002	1		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Breakfast cereals	2000	9	50		3		993/250[b]	45		Schothorst & van Egmond (2004)
	Breakfast cereals	2001	1	50		0		105/0[b]	40		Schothorst & van Egmond (2004)
	Breakfast cereals	2002	4	50		3		100/80[b]	50		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Malting barley	2001	30	30		30		15/[b]	15		Schothorst & van Egmond (2004)
	Malting barley	2001	52	30		50		9.76/200[b]	5		Schothorst & van Egmond (2004)
	Malting barley	2002	44	30		32		5/500[b]	5		Schothorst & van Egmond (2004)
	Malting barley	2002	68	30		55		21.1/310[b]	5		Schothorst & van Egmond (2004)
	Malting barley	2000	50		20			46/550[b]	10		Schothorst & van Egmond (2004)
	Malting barley	2000	64		20			65/350[b]	37		Schothorst & van Egmond (2004)
	Conventional wheat		11		10		1	215 (maximum)	55		Malmauret et al. (2002)
	Organic wheat		11		10		5	494 (maximum)	106		Malmauret et al. (2002)
	Conventional barley		5		10		1	73 (maximum)	41		Malmauret et al. (2002)
	Organic barley		5		10		2	209 (maximum)	69		Malmauret et al. (2002)
	Beer	2000–2002	27	3.7[a]		3		11/30.2	8.4[a]		Papadopoulou-Bouraoui et al. (2004)
	Baby food	2001	2		200		2				National monitoring programmes
	Baby food	2004	13		60–200		9	291.8/507			National monitoring programmes
	Baby food	2006	12		100		10	242.5/360			National monitoring programmes
	Baby food	2006	9	30		8	1				National monitoring programmes

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Baby food	2007	13	30	100	13					National monitoring programmes
	Baby food	2008	8	30	100	7	1				National monitoring programmes
	Biscuit	2003	4					131.7/151.3			National monitoring programmes
	Biscuit	2004	2	30		1		437/			National monitoring programmes
	Biscuit	2006	9		100			116.7/139			National monitoring programmes
	Biscuit	2006	5	30		5	6				National monitoring programmes
	Biscuit	2007	5	30	100	3	1	167/			National monitoring programmes
	Biscuit	2008	5	30	100	1	3	129/			National monitoring programmes
	Biscuit	2008	8	10–20	20	2	1	168/263.4			National monitoring programmes
	Breakfast cereal	2001	9		200		7	242/250			National monitoring programmes
	Breakfast cereal	2003	10		50		9	65.1/			National monitoring programmes
	Breakfast cereal	2003	3	30		3					National monitoring programmes
	Breakfast cereal	2003	3		100		3				National monitoring programmes
	Breakfast cereal	2004	4	30		2	1	99/			National monitoring programmes
	Breakfast cereal	2004	3		100–200		3				National monitoring programmes
	Breakfast cereal	2006	7	30	100	2	3	167/244			National monitoring programmes
	Breakfast cereal	2007	2	30	100	2					National monitoring programmes

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Breakfast cereal	2008	9	30	100	5	2	360/615			National monitoring programmes
	Breakfast cereal (maize)	2002	11		200		8	168.7/224.3			National monitoring programmes
	Breakfast cereal (maize)	2003	6		50–100		4	174.5/191			National monitoring programmes
	Breakfast cereal (maize)	2004	14	30	60–200	3	11				National monitoring programmes
	Breakfast cereal (maize)	2006	2		100		1	160/			National monitoring programmes
	Breakfast cereal (maize)	2007	3	30	90–100		2	160/			National monitoring programmes
	Breakfast cereal (maize)	2008	4	30	100	3	1				National monitoring programmes
	Breakfast cereal (wheat)	2003	6	30	50–100	2	2	191.9/255.8			National monitoring programmes
	Breakfast cereal (wheat)	2006	3	30	100	1	1	218.8/			National monitoring programmes
	Breakfast cereal (wheat)	2007	1	30	100			240/			National monitoring programmes
	Breakfast cereal (wheat)	2008	3	30	100	1		286.5/341			National monitoring programmes
	Breakfast cereal (oats)	2001	5		200		5				National monitoring programmes
	Breakfast cereal (oats)	2002	5	30		4	1				National monitoring programmes
	Breakfast cereal (oats)	2003	3		50		3				National monitoring programmes

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Buckwheat flour	2001	9		200		9				National monitoring programmes
	Buckwheat flour	2003	4	30		4					National monitoring programmes
	Buckwheat flour	2005	1	30		1					National monitoring programmes
	Buckwheat flour	2008	5	30	100	5					National monitoring programmes
	Cereal bar	2008	3	30	100	2					National monitoring programmes
	Couscous	2001	7		200	1	2	304/371			National monitoring programmes
	Couscous	2002	2		200	1	1	234/			National monitoring programmes
	Couscous	2003	1		50	1	1				National monitoring programmes
	Couscous	2004	1		200	2	1				National monitoring programmes
	Cupcake	2998	3	30	100	10	2	140/			National monitoring programmes
	Durum wheat flour	2001	26	30	200		10	785/1780			National monitoring programmes
	Durum wheat flour	2002	6					461/632			National monitoring programmes
	Durum wheat flour	2003	9			4		334/653			National monitoring programmes
	Durum wheat flour	2005	12	30	50–70		4	127/184			National monitoring programmes
	Durum wheat flour	2006	1	30		1					National monitoring programmes
	Durum wheat flour	2007	5	30	100	1	1	427/443			National monitoring programmes
	Durum wheat flour	2008	13	30	100		1	330/631			National monitoring programmes

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Muesli	2001	1		200		1				National monitoring programmes
	Muesli	2003	3		50		3				National monitoring programmes
	Muesli	2004	7	30	200	2	4	116/			National monitoring programmes
	Muesli	2006	6		100		4	227/330			National monitoring programmes
	Muesli	2007–2008	2	30	100	2					National monitoring programmes
	Pasta	2003	9		50–100		5	166/238			National monitoring programmes
	Wheat bread	2002	1		200		1				National monitoring programmes
	Wheat bread	2003	8	30	50–200	2	4	96/110			National monitoring programmes
	Wheat bread	2005	2	30	50		2				National monitoring programmes
	Wheat bread	2006	14	30	100	3	10				National monitoring programmes
	Wheat bread	2007	6	30	90–100	2	2	146/154			National monitoring programmes
	Wheat bread	2008	5		100			328/540			National monitoring programmes
	Malt	2001	7		200		7				National monitoring programmes
	Malt	2003	1	30	100		1				National monitoring programmes
	Malt	2007	4	30	100	1		457/500			National monitoring programmes
	Maize fecula	2002	1	30		1					National monitoring programmes
	Maize starch	2004	3		200	2	1				National monitoring programmes

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Maize bread	2006	4		100		4				National monitoring programmes
	Dough	2006	2		100		2				National monitoring programmes
	Almond cake	2006	1		100		1				National monitoring programmes
	Coconut balls cookie	2008	1								National monitoring programmes
	Rice flour	2005	1	30		1					National monitoring programmes
	Crepe	2005–2006	3	30		2		67.8/			National monitoring programmes
	Chocolate cake	2006	2	30		3					National monitoring programmes
	Chocolate biscuit	2006	1	30		1					National monitoring programmes
France (imported)	Pasta	2006	6	30	200	3	2	50/			National monitoring programmes
	Pasta	2007–2008	6	30	100	4	1	131/			National monitoring programmes
	Rye bread	2006	1		200		1				National monitoring programmes
	Rye biscuit	2006	1		200		1				National monitoring programmes
	Fermented soya bean	2006	1					372/			National monitoring programmes
	Panettone	2008	1					164.9/			National monitoring programmes
	Rice pancake	2002	1	30		1					National monitoring programmes

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Germany	Beer	2000–2002	46	3.7[a]		1		4.7/40.5	4.0[a]		Papadopoulou-Bouraoui et al. (2004)
	Soft wheat	2006	78			10		217 (maximum)	28		Curtui et al. (2006)
	Durum wheat semolina	2006	6			10		203 (maximum)	38		Curtui et al. (2006)
	Durum wheat pasta	2006	49			10		119 (maximum)	24		Curtui et al. (2006)
	Wheat and wheat products	2005–2006	130	0.038	0.11	0		57/1160	23		Gottschalk et al. (2009)
	Rye and rye products	2005–2006	61	0.038	0.11	0		28/288	15		Gottschalk et al. (2009)
	Oat and oat products	2005–2006	98	0.038	0.11				0.53		Gottschalk et al. (2009)
	Cereal food products	2001	333	20–100				251/	142		Majerus et al. (2002)
	Wheat (integrated cultivation)	2000	47	50		44		140/280	80		Meister (2005)
	Wheat (integrated cultivation)	2001	47	50		35		310/1350	90		Meister (2005)
	Wheat (integrated cultivation)	2002	46	50		15		470/4870	240		Meister (2005)
	Wheat (integrated cultivation)	2003	46	50		37		140/540	90		Meister (2005)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat (integrated cultivation)	2004	41	50		26		195/1120	70		Meister (2005)
	Wheat (integrated cultivation)	2005	42	50		22		212/1730	98		Meister (2009)
	Wheat (integrated cultivation)	2006	43	50		23		200/1020	88		Meister (2009)
	Wheat (integrated cultivation)	2007	43	50		3		1211/10 400	428		Meister (2009)
	Wheat (ecological cultivation)	2000	16	50		16		n/a	n/a		Meister (2005)
	Wheat (ecological cultivation)	2001	12	50		11		70/70	70		Meister (2005)
	Wheat (ecological cultivation)	2002	14	50		8		100/200	90		Meister (2005)
	Wheat (ecological cultivation)	2003	10	50		10		n/a	n/a		Meister (2005)
	Wheat (ecological cultivation)	2004	16	50		10		100/220	80		Meister (2005)
	Wheat (organic cultivation)	2005	13	50		12		87 (value for lone sample)	n/a		Meister (2009)
	Wheat (organic cultivation)	2006	14	50		13		147 (value for lone sample)	n/a		Meister (2009)
	Wheat (organic cultivation)	2007	15	50		5		262/782	133		Meister (2009)
	Rye (integrated cultivation)	2000	43	50		33		90/200	80		Meister (2005)
	Rye (integrated cultivation)	2001	44	50		35		150/420	60		Meister (2005)
	Rye (integrated cultivation)	2002	48	50		32		190/750	120		Meister (2005)
	Rye (integrated cultivation)	2003	45	50		40		60/80	50		Meister (2005)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Rye (integrated cultivation)	2004	39	50		23		175/1095	90		Meister (2005)
	Rye (integrated cultivation)	2005	39	50		21		89/261	72		Meister (2009)
	Rye (integrated cultivation)	2006	41	50		17		84/271	67		Meister (2009)
	Rye (integrated cultivation)	2007	38	50		13		156/677	74		Meister (2009)
	Rye (ecological cultivation)	2000	18	50		18		n/a	n/a		Meister (2005)
	Rye (ecological cultivation)	2001	18	50		17		50 (value for lone sample)	50		Meister (2005)
	Rye (ecological cultivation)	2002	20	50		20		n/a	n/a		Meister (2005)
	Rye (ecological cultivation)	2003	20	50		19		50 (value for lone sample)	50		Meister (2005)
	Rye (ecological cultivation)	2004	27	50		21		105/290	70		Meister (2005)
	Rye (organic cultivation)	2005	24	50		22		85/119	85		Meister (2009)
	Rye (organic cultivation)	2006	23	50		9		61/86	60		Meister (2009)
	Rye (organic cultivation)	2007	23	50		22		80 (value for lone sample)	n/a		Meister (2009)
	Wheat flour	2000	29	111	111	11		198/690[b]	166		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat flour	2001	108	25	25	34		120/640[b]	86		Schothorst & van Egmond (2004)
	Wheat	2000	27	20	40	9		112/402[b]	115		Schothorst & van Egmond (2004)
	Pasta	2000	8	20	40	1		145/370[b]	123		Schothorst & van Egmond (2004)
	Pasta	2001–2002	102	30	30	6		292/3200[b]	177		Schothorst & van Egmond (2004)
	Baby food	2000	32	20	40	10		134/1075[b]	102		Schothorst & van Egmond (2004)
	Baby food	2001–2002	132	30	30	37		70/220[b]	46		Schothorst & van Egmond (2004)
Greece	Beer	2000–2002	4	3.7[a]		0		17.0/16.8	16.5		Papadopoulou-Bouraoui et al. (2004)
Hungary	Beer	2000–2002	2	3.7[a]		0		10.8/11.1	10.8		Papadopoulou-Bouraoui et al. (2004)
	Wheat	2001	10		100		1	182.2/340	182.2		GEMS/Food database
	Cereal grains	2001	16		220		16	/220			GEMS/Food database
	Wheat flour	2001	16		100		11	/455			GEMS/Food database
Ireland	Beer	2000–2002	2	3.7[a]		0		8.7/9.6	8.7		Papadopoulou-Bouraoui et al. (2004)
Italy	Cereals and related foods	2001–2002	202	7		32		7–930 (range)	65		Cirillo et al. (2003)
	Ground wheat	2005–2006	57	2.8				7.7/17.3			Lattanzio, Solfrizzo & Visconti (2008)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Infant semolina	2005–2006	1	5.3				7.1			Lattanzio, Solfrizzo & Visconti (2008)
	Infant biscuits	2005–2006	1	4.6				nd			Lattanzio, Solfrizzo & Visconti (2008)
	Bacon biscuits	2005–2006	1	3				191			Lattanzio, Solfrizzo & Visconti (2008)
	Cocoa wafers	2005–2006	1	4				38.4			Lattanzio, Solfrizzo & Visconti (2008)
	Coconut snack	2005–2006	1	3.8				9.5			Lattanzio, Solfrizzo & Visconti (2008)
	Beer	2000–2002	16	3.7[a]		0		10.5/29.4	8.4		Papadopoulou-Bouraoui et al. (2004)
Lithuania	Winter wheat	1999	23	10				29/142			Garaleviciene, Pettersson & Agnedal (2002)
	Barley	1999	12	10				34/66			Garaleviciene, Pettersson & Agnedal (2002)
	Oats	1999	5	10				20/40			Garaleviciene, Pettersson & Agnedal (2002)
Netherlands	Beer	2000–2002	4	3.7[a]		1		8.0/9.7	8.5		Papadopoulou-Bouraoui et al. (2004)
	Beer	2000–2001	32	8[a]	25[a]	24	24	41 (value for lone sample)	n/a		Schothorst & Jekel (2003)
	Beer (imported from Germany)	2000–2001	9	8[a]	25[a]	8	8	n/a			Schothorst & Jekel (2003)
	Beer (imported from Belgium)	2000–2001	9	8[a]	25[a]	7	7	31/36			Schothorst & Jekel (2003)

Table A4 (contd)

Country	Commodity	Year/season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Beer (imported from Ireland)	2000–2001	1	8[a]	25[a]	1	1	n/a			Schothorst & Jekel (2003)
	Infant food	2000	5	10	20	1		131/270[b]	130		Inspectorate for Health Protection and Veterinary Public Health (unpublished results)
	Barley	2000–2001	4	10	20	4		5/0[b]	<220		Inspectorate for Health Protection and Veterinary Public Health (unpublished results)
	Barley	2000	6	110		3		113/230[b]	<50		Anonymous
	Barley	2001	12	220		10		153/510[b]	<50		Anonymous
	Oat	2000–2001	8	10	20	8		5/0[b]			Inspectorate for Health Protection and Veterinary Public Health (unpublished results)
	Corn	2000	23	110		2		379/1300[b]	293		Anonymous
	Corn	2001	84	220		10		761/3920[b]	640		Anonymous
	Rye	2000	8	110		1		150/220[b]	142.5		Anonymous
	Rye	2001	20	220		20		110/[b]	<220		Anonymous

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat	2000	175	50	100	107		145/3280[b]	<10		Inspectorate for Health Protection and Veterinary Public Health (unpublished results)
	Wheat	2001	98	10	20	43		206/1200[b]	110		Inspectorate for Health Protection and Veterinary Public Health (unpublished results)
	Wheat	2000	939	110		150		315/5000[b]	220		Anonymous
	Wheat	2001	765	220		583		206/2300[b]	<220		Anonymous
	Beer	2000	51	12.5	25	48		7.9/41[b]	<12.5		National Institute for Public Health and the Environment
	Puffed wheat	2001	12	220		11		121/240[b]	240		Anonymous
	Wheat bio	2001	14	220		12		144/430[b]	345		Anonymous
	Wheat durum	2000	22	110	110	2		370/1700[b]	230		Anonymous
	Wheat durum	2001	19	220		14		204/680[b]	460		Anonymous
	Wheat durum bio	2000	4	110	110	1		183/235[b]	220		Anonymous
	Wheat durum bio	2001	8	220		8		110/[b]			Anonymous
	Couscous/ semolina	2003	2	20	60			690/700			Food and Consumer Product Safety Authority - Survey

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Couscous/ semolina	2003	3	20	60			473/510			Food and Consumer Product Safety Authority - Monitoring
	Wheat	2003	5	20	60			324/480			Food and Consumer Product Safety Authority - Survey
	Wheat	2003	51	20	60			119/610			Food and Consumer Product Safety Authority - Monitoring
	Wheat flour brown	2003	13	20	60			104/242			Food and Consumer Product Safety Authority - Monitoring
	Wheat loaves/ rolls, wholemeal	2003	1	20	60			70/70			Food and Consumer Product Safety Authority - Monitoring
	Pasta, egg-free	2003	5	20	60			28.2/87			Food and Consumer Product Safety Authority - Monitoring
	Rye	2003	2	20	60	2		0/0			Food and Consumer Product Safety Authority - Monitoring
	Barley	2003	5	20	60			51.6/138			Food and Consumer Product Safety Authority - Monitoring
	Maize	2003	2	20	60			117.5/170			Food and Consumer Product Safety Authority - Monitoring
	Maize milled	2003	2	20	60			260/300			Food and Consumer Product Safety Authority - Monitoring

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Popcorn	2003	2	20	60			110/110			Food and Consumer Product Safety Authority - Survey
	Rice	2003	2	20	60	2		0/0			Food and Consumer Product Safety Authority - Monitoring
	Peanut	2003	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Survey
	Baking flour	2004	2	20	60			55/110			Food and Consumer Product Safety Authority - Monitoring
	Couscous/ semolina	2004	7	20	60			212.8/400			Food and Consumer Product Safety Authority - Monitoring
	Wheat	2004	20	20	60			107.2/340			Food and Consumer Product Safety Authority - Survey
	Wheat	2004	42	20	60			147.7/580			Food and Consumer Product Safety Authority - Monitoring
	Wheat flour, brown	2004	2	20	60			86/107			Food and Consumer Product Safety Authority - Survey
	Wheat flour, brown	2004	36	20	60			120.9/234			Food and Consumer Product Safety Authority - Monitoring
	Wheat loaves/rolls, brown	2004	1	20	60			110/110			Food and Consumer Product Safety Authority - Monitoring

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Pasta, egg-free	2004	2	20	60			57.5/60			Food and Consumer Product Safety Authority - Survey
	Pasta, egg-free	2004	32	20	60			157.5/630			Food and Consumer Product Safety Authority - Monitoring
	Rye	2004	2	20	60			110/110			Food and Consumer Product Safety Authority - Monitoring
	Barley	2004	1	20	60			325/325			Food and Consumer Product Safety Authority - Monitoring
	Maize	2004	1	20	60			210/210			Food and Consumer Product Safety Authority - Monitoring
	Maize milled	2004	4	20	60			53.7/90			Food and Consumer Product Safety Authority - Survey
	Maize milled	2004	10	20	60			69.2/150			Food and Consumer Product Safety Authority - Monitoring
	Maize semolina	2004	4	20	60			175/360			Food and Consumer Product Safety Authority - Monitoring
	Popcorn	2004	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Cereals, mixed grains	2004	1	20	60			63/63			Food and Consumer Product Safety Authority - Monitoring

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Cashew nut	2004	1	20	60			140/140			Food and Consumer Product Safety Authority - Monitoring
	Hazelnut	2004	1	20	60			2.5/2.5			Food and Consumer Product Safety Authority - Monitoring
	Honey	2004	1	20	60	1		0/0			Food and Consumer Product Safety Authority - National Plan Animal Products
	Toast	2004	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Baking flour	2005	3	20	60			106/110			Food and Consumer Product Safety Authority - Monitoring
	Breadcrumbs	2005	3	20	60			93/110			Food and Consumer Product Safety Authority - Monitoring
	Couscous/semolina	2005	5	20	60			112/350			Food and Consumer Product Safety Authority - Monitoring
	Gluten	2005	1	20	60			164/164			Food and Consumer Product Safety Authority - Monitoring
	Wheat	2005	16	20	60			186.2/451			Food and Consumer Product Safety Authority - Survey
	Wheat	2005	22	20	60			164.4/420			Food and Consumer Product Safety Authority - Monitoring

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat flour, brown	2005	26	20	60			92.9/460			Food and Consumer Product Safety Authority - Monitoring
	Wheat flour, white	2005	2	20	60			40/80			Food and Consumer Product Safety Authority - Monitoring
	Wheat germ	2005	1	20	60			100/100			Food and Consumer Product Safety Authority - Monitoring
	Wheat loaves/rolls, mixed flour	2005	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Wheat starch	2005	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Biscuits	2005	6	20	60			54.1/140			Food and Consumer Product Safety Authority - Monitoring
	Crackers	2005	2	20	60			85/110			Food and Consumer Product Safety Authority - Monitoring
	Pasta, egg-free	2005	4	20	60			86.2/150			Food and Consumer Product Safety Authority - Survey
	Pasta, egg-free	2005	52	20	60			88.6/550			Food and Consumer Product Safety Authority - Monitoring
	Rye	2005	4	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Barley	2005	5	20	60			915/3184			Food and Consumer Product Safety Authority - Monitoring
	Maize	2005	1	20	60			140/140			Food and Consumer Product Safety Authority - Monitoring
	Maize milled	2005	12	20	60			50/100			Food and Consumer Product Safety Authority - Survey
	Maize milled	2005	18	20	60			173/550			Food and Consumer Product Safety Authority - Monitoring
	Maize semolina	2005	12	20	60			70/140			Food and Consumer Product Safety Authority - Survey
	Maize semolina	2005	1	20	60			210/210			Food and Consumer Product Safety Authority - Monitoring
	Popcorn	2005	2	20	60			102.5/110			Food and Consumer Product Safety Authority - Survey
	Popcorn	2005	2	20	60			80/160			Food and Consumer Product Safety Authority - Monitoring
	Rice	2005	2	20	60	2		0/0			Food and Consumer Product Safety Authority - Monitoring
	Unripe spelt flour	2005	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Cereals, mixed grains	2005	1	20	60			106/106			Food and Consumer Product Safety Authority - Monitoring
	Almond	2005	1	20	60			50/50			Food and Consumer Product Safety Authority - Monitoring
	Fig	2005	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Survey
	Wine, alcohol > 9%	2005	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Grape juice	2005	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Toast	2005	1	20	60			70/70			Food and Consumer Product Safety Authority - Monitoring
	Wheat	2006	2	20	60			29/58			Food and Consumer Product Safety Authority - Survey
	Wheat	2006	10	20	60	10		0/0			Food and Consumer Product Safety Authority - Monitoring
	Wheat flour, brown	2006	4	20	60	4		0/0			Food and Consumer Product Safety Authority - Monitoring
	Pasta, egg-free	2006	11	20	60			5.8/64			Food and Consumer Product Safety Authority - Monitoring

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Maize	2006	2	20	60	2		0/0			Food and Consumer Product Safety Authority - Monitoring
	Maize milled	2006	2	20	60	2		0/0			Food and Consumer Product Safety Authority - Survey
	Maize milled	2006	4	20	60	4		0/0			Food and Consumer Product Safety Authority - Monitoring
	Maize semolina	2006	3	20	60	3		0/0			Food and Consumer Product Safety Authority - Survey
	Maize semolina	2006	3	20	60	3		0/0			Food and Consumer Product Safety Authority - Monitoring
	Spelt	2006	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Cereals, mixed grains	2006	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Paranut	2006	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Walnuts	2006	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Honey	2006	1	20	60	1		0/0			Food and Consumer Product Safety Authority - National Plan Animal Products

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Beer, alcohol > 5%	2006	8	20	60	8		0/0			Food and Consumer Product Safety Authority - Monitoring
	Wheat	2007	25	20	60			972/3400			Food and Consumer Product Safety Authority - Survey
	Pasta, egg-free	2007	9	20	60			126.4/300			Food and Consumer Product Safety Authority - Survey
	Rye	2007	1	20	60			79/79			Food and Consumer Product Safety Authority - Survey
	Barley	2007	3	20	60			388/630			Food and Consumer Product Safety Authority - Survey
	Maize	2007	18	20	60			287.6/1000			Food and Consumer Product Safety Authority - Survey
	Buckwheat	2007	1	20	60			250/250			Food and Consumer Product Safety Authority - Survey
	Cereals, mixed grains	2007	11	20	60			160.6/380			Food and Consumer Product Safety Authority - Survey
	Pistachio nuts	2007	2	20	60			85.5/88			Food and Consumer Product Safety Authority - Survey
	Honey	2007	1	20	60	1		0/0			Food and Consumer Product Safety Authority - National Plan Animal Products

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Beer, alcohol > 5%	2007	2	20	60			34.9/36.5			Food and Consumer Product Safety Authority - Survey
Norway	Wheat (imported)	2001	64	20	60	30		87.1/464[b]	35		Schothorst & van Egmond (2004)
	Wheat	2001	44	20	60	40		12.1/51[b]	10		Schothorst & van Egmond (2004)
	Wheat	2001	39	20	60	26		27.8/183[b]	10		Schothorst & van Egmond (2004)
	Oats	2001	24	20	60	24		10/[b]	10		Schothorst & van Egmond (2004)
	Oats	2001	58	20	60	38		26.2/220[b]	10		Schothorst & van Egmond (2004)
	Oats	2001	28	20	60	28		10/[b]	10		Schothorst & van Egmond (2004)
	Maize	2001	19	20	60	1		450.9/1022[b]	609		Schothorst & van Egmond (2004)
	Rice	2001	16	20	60	16		10/[b]	10		Schothorst & van Egmond (2004)
	Cereal fraction	2001	19	20	60	9		25.4/86[b]	21		Schothorst & van Egmond (2004)
	Beer	2000–2002	4	3.7[a]		1		7.7/9.9	7.3		Papadopoulou-Bouraoui et al. (2004)
	Wheat flour (organic)	2008	2	5	15	1		352/			GEMS/Food database
	Wheat flour (spelt)	2008	2	5	15			23.5/33			GEMS/Food database
	Wheat flour	2008	60	5	15			210.8/639			GEMS/Food database

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat flour (wholemeal)	2008	52	5	15	7		162.9/430			GEMS/Food database
	Wheat (wholemeal, spelt)	2008	1	5	15			49/			GEMS/Food database
	Wheatbran (processed)	2008	23	5	15			382.6/1093			GEMS/Food database
	Maize (baby porridge, organic)	1999–2002	10	20	60			819/1022			GEMS/Food database
	Maize (baby porridge)	1999–2002	12	20	60	4		47.4/78			GEMS/Food database
	Maize (baby porridge)	2008	2	5	15			46/46			GEMS/Food database
	Wheat (baby porridge)	2008	5	5	15	2		17/23			GEMS/Food database
	Oats (baby porridge)	2008	4	5	15	1		33.3/70			GEMS/Food database
	Oats	2008	30	5	15			282/520			GEMS/Food database
	Oatbran	2008	1	5	15			372/			GEMS/Food database
	Rice (baby porridge)	1999–2002	4	20	60	4					GEMS/Food database
Norway (imported)	Cereal grain (baby porridge)	2008	4	5	15	1		15.3/31			GEMS/Food database
	Cereal grain (baby porridge with fruits)	2008	2	5	15			6.5/8			GEMS/Food database
	Barley (baby porridge)	2008	2	5	15	1		15/			GEMS/Food database

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Maize (baby porridge)	2008	1	5	15			34/			GEMS/Food database
Poland	Beer	2000–2002	10	3.7[a]		0		17.2/32.9	18		Papadopoulou-Bouraoui et al. (2004)
Portugal	Corn flour		41	100		41					Martins et al. (2008)
	Popcorn		49	100		49					Martins et al. (2008)
	Cornflakes		15	100		15					Martins et al. (2008)
	Wheat	2002	3	25	50	2		256.3/744[b]			Schothorst & van Egmond (2004)
	White wheat flour	2002	3	25	50	2		119.3/333[b]			Schothorst & van Egmond (2004)
	Wheat bran	2002	4	25	50	2		761/1821[b]			Schothorst & van Egmond (2004)
	Cereal breakfast	2002	10	25	50			161.6/426[b]	161		Schothorst & van Egmond (2004)
Romania	Maize	2002–2004	54	25				4772/11 000			Tabuc et al. (2009)
	Wheat	2002–2004	35	25				1531/3600			Tabuc et al. (2009)
	Barley	2002–2004	21	25				3923/4000			Tabuc et al. (2009)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Russian Federation	Stored wheat grain	2000	40	50		30		40/420	0	170	Tutelyan (2004)
	Stored wheat grain	2001	167	50		118		80/1590	0	0	Tutelyan (2004)
	Stored wheat grain	2002	59	50		56		10/390	0	0	Tutelyan (2004)
	Wheat grain	2000	222	50		216		10/770	0	0	Tutelyan (2004)
	Wheat grain	2001	252	50		240		10/620	0	0	Tutelyan (2004)
	Barley	2000	57	50		56		5/280	0	0	Tutelyan (2004)
	Barley	2001	64	50		64		0/0	0	0	Tutelyan (2004)
	Rye grain	2000	99	50		99		0/0	0	0	Tutelyan (2004)
	Rye grain	2001	88	50		88		0/0	0	0	Tutelyan (2004)
Serbia	Soya bean and soya bean meal	2004	13		40			110/110	110		Jajić et al. (2008)
	Soya bean and soya bean meal	2005	11		40			100/100	100		Jajić et al. (2008)
	Sunflower and sunflower meal	2004	9		40			155/304	138		Jajić et al. (2008)
	Sunflower and sunflower meal	2005	10		40			447/788	467		Jajić et al. (2008)
	Barley	2005	4		40			140/140	140		Jajić et al. (2008)

Table A4 (contd)

Country	Commodity	Year/season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Maize	2004	10		40			536/2460	50		Jajić et al. (2008)
	Maize	2005	66		40			363/2210	213		Jajić, Jurić & Abramović (2008)
	Maize	2006	21		40			426/1340	260		Jajić et al. (2008)
	Maize	2007	119	25				58/172	40		Jajić, Jurić & Abramović (2008)
	Wheat	2004	4		40			1235/1840	1235		Jajić, Jurić & Abramović (2008)
	Wheat	2005	12		40			182/423	124		Jajić, Jurić & Abramović (2008)
	Wheat	2006	34		40			223/410	223		Jajić, Jurić & Abramović (2008)
	Wheat	2007	9		40			177/208	182		Jajić, Jurić & Abramović (2008)
Slovakia	Beer	2000–2002	12	3.7[a]		3		13.5/36.9	10.4		Papadopoulou-Bouraoui et al. (2004)
	Wheat	2004	139					560/1500			Šliková, Šudyová & Gregová (2008)
	Wheat	2005	139					230/300			Šliková, Šudyová & Gregová (2008)
	Wheat	2006	139					730/1300			Šliková, Šudyová & Gregová (2008)
	Wheat	2004	139					910/7200			Šliková, Šudyová & Gregová (2008)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat	2005	139					390/900			Šliková, Šudyová & Gregová (2008)
	Wheat	2006	139					650/1100			Šliková, Šudyová & Gregová (2008)
	Wheat	2004	139					1000/7300			Šliková, Šudyová & Gregová (2008)
	Wheat	2005	139					490/2200			Šliková, Šudyová & Gregová (2008)
	Wheat	2006	139					1100/1700			Šliková, Šudyová & Gregová (2008)
Spain	Corn-based breakfast cereals	2005	55	14.4	25.4	33		30.1–121.1 (range)	44.5		Castillo et al. (2008)
	Baked corn snacks	2005	57	14.4	25.4	44		36.4–131.7 (range)	62.5		Castillo et al. (2008)
	Fried corn snacks	2005	63	14.4	25.4	51		26.1–80.4 (range)	55.5		Castillo et al. (2008)
	Fried corn snacks	2002–2005	446		10			19/1416	11	35	Castillo et al. (2008)
	Beer	2000–2002	13	3.7[a]		7		7.3/12.2	6.3		Papadopoulou-Bouraoui et al. (2004)
	Beer	2000–2002	7	3.7[a]		3		5.1/14.6	3.6		Papadopoulou-Bouraoui et al. (2004)
Sweden	Durum wheat flour	2000	23	20		0		1157/2591	1242		Rasmussen, Ghorbani & Berg (2003)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Durum wheat flour	2001	10	20		0		1153/1619	1224		Rasmussen, Ghorbani & Berg (2003)
	Processed cereals	?	68	100		62		2670 (maximum)			Omurtag & Beyoğlu (2003)
	Wheat	2001	27	2	10	2		61/333[b]	26		Schothorst & van Egmond (2004)
	Wheat	2001	17	2	10			1427/2033[b]	1367		Schothorst & van Egmond (2004)
	Oat	2001	3	2	10			87/174[b]	66		Schothorst & van Egmond (2004)
United Kingdom	Barley grain	2001–2005	1624		10			230/20 333	42	368	Edwards (2009)
	Beer	2000–2002	33	3.7[a]		8		10.9/30.8	10.2		Papadopoulou-Bouraoui et al. (2004)
	Maize (imported from France)	2004–2007	25	5	10			139/444	63		Scudamore & Patel (2009b)
	Maize (imported from France)	2004–2007	24	5	10			271/932	254		Scudamore & Patel (2009b)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Maize (imported from Argentina)	2004–2007	15	5	10			89/220	74		Scudamore & Patel (2009b)
	Rice	2000	100		10	99		1.8/12[b]	<10		Food Standards Agency (2002a)
	Cereal fractions	2000	16		10	15		27.6/61[b]	<10		Food Standards Agency (2002b)
	Cereal fractions	2000	11		10	1		106/531[b]	50		Food Standards Agency (2002b)
	Barley	2000	50		5	46		1.5/20[b]	<5		Schothorst & van Egmond (2004)
	Barley	2001	49		5	29		5.9/53[b]	<5		Schothorst & van Egmond (2004)
	Beer	2000	28		5	28		0.8/<5[b]	<5		Schothorst & van Egmond (2004)
	Corn products	2000	15		10	0		132.7/683[b]	46		Food Standards Agency (2002b)
	Corn products	2000	30		10	4		257.3/879[b]	88		Food Standards Agency (2002b)
	Corn products	2000	1		10	0		16/16[b]	16		Food Standards Agency (2002b)
	Corn products	2001	24		10	3		109.5/275[b]	127		Food Standards Agency (2002b)
	Wheat products	2000	14		10	3		3.5/31[b]	24		Food Standards Agency (2002b)
	Wheat products	2000	14		10	0		235.6/2261[b]	54		Food Standards Agency (2002b)
	Wheat products	2000	40		10	21		12.4/67[b]	<10		Food Standards Agency (2002b)
	Wheat products	2000	6		10	0		75.7/156[b]	67		Food Standards Agency (2002b)
	Wheat products	2001	13		10	0		47.5/199[b]	25		Food Standards Agency (2002b)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat products	2000	8		10	1		53.5/177[b]	36		Food Standards Agency (2002b)
	Wheat flour	2000	29		10	3		35.1/99[b]	32		Food Standards Agency (2002b)
	Flour	2000	8		10	8		1.7/<10[b]	<10		Food Standards Agency (2002b)
	Polenta	2000	8		10	1		153.3/466[b]	140		Food Standards Agency (2002b)
	Biscuits	2000	54		10	8		29.1/315[b]	18.5		Food Standards Agency (2002b)
	Bread	2000	40		10	2		47.8/366[b]	25.5		Food Standards Agency (2002b)
	Bread	2000	16		10	0		79.9/198[b]	82		Food Standards Agency (2002b)

n/a, not applicable; nd, not detected

[a] µg/l.

[b] Arithmetic mean value of all samples (both positive and negative samples). Concentration in samples less than LOD is considered as LOD/2. Where only LOQ is available, then values less than LOQ are considered as LOQ/6.

Table A5. Occurrence data for DON-3-glucoside (Americas, Asia and Europe)

Region/country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Americas											
USA	Wheat, hard red spring	2005	28	1	0.5		22	200/5400			Sasanya, Hall & Wolf-Hall (2008)
Asia											
China	Maize	2008	203	1	3		134	22/499			GEMS/Food database
China	Wheat	2008	162	1	3		82	26/238			GEMS/Food database
China	Wheat flour	2008	30	1	3		9	7.3/39			GEMS/Food database
Europe											
Austria, Germany, Slovakia	Wheat	2005	23	4	10			393/1070			Berthiller et al. (2009b)
Austria	Maize	2006	54	4	10			141/763			Berthiller et al. (2009b)
Belgium (De-epoxy-DON)	Eggs	2006	10	0.6	2	7	7	1.2/4.8	2		Tangni et al. (2009)
	Eggs	2007	10	0.6	2	9	9	2.4/23.7	3		Tangni et al. (2009)

Table A6. Occurrence data for 15-Ac-DON (Americas)

Region/ country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
USA	Durum wheat, Montana	2001	23	50				nd			Manthey et al. (2004)
	Durum wheat, NW North Dakota	2001	27	50				0.0/0.5 mg/kg			Manthey et al. (2004)
	Durum wheat, NC North Dakota	2001	24	50				100/800			Manthey et al. (2004)
	Durum wheat, NE North Dakota	2001	15	50				0.0/0.5			Manthey et al. (2004)
	Durum wheat, SW North Dakota	2001	17	50				nd			Manthey et al. (2004)
	Durum wheat, SE North Dakota	2001	17	50				nd			Manthey et al. (2004)

NC, north-central; nd, not detected; NE, north-east; NW, north-west; SE, south-east; SW, south-west

Table A7. Occurrence data for 3-Ac-DON and 15-Ac-DON (Asia)

Country/compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
3-Ac-DON											
China	Maize	2008	203	0.1	0.3		131	6.6/368			GEMS/Food database
China	Wheat	2008	162	0.1	0.3		120	1.8/35			GEMS/Food database
Japan	Wheat	2008	120	6	16		114	0.5/18			GEMS/Food database
Japan	Barley	2008	100	3	8		81	2.9/53			GEMS/Food database
15-Ac-DON											
China	Maize	2008	203	0.1	0.3		106	75/1734			GEMS/Food database
China	Wheat	2008	162	0.1	0.3		118	1.7/71			GEMS/Food database
China	Wheat flour	2008	30	0.1	0.3		15	1.5/5			GEMS/Food database
China, Henan Province											
- Puyang	Wheat	1998	31	10		11		365/1800			Li et al. (2002)
- Zhumadian	Wheat	1998	28	10		28		nd			Li et al. (2002)
- Pujang	Wheat	1999	34	10		34		nd			Li et al. (2002)
Japan	Wheat	2008	120	3	8		120	nd			GEMS/Food database
Japan	Barley	2008	100	2	7		92	0.3/8.8			GEMS/Food database

nd, not detected

Table A8. Occurrence data for 3-Ac-DON and 15-Ac-DON (Europe)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
Austria (3-Ac-DON)	Corn	1996	46		50	39		20.9/125[a]	<LOQ		Schothorst & van Egmond (2004)
	Corn	1997	58		50	58		8.3/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Corn	1998	48		50	48		8.3/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Oat	1999	96		100	96		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Oat	2000	96		100	96		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Oat	2001	40		100	40		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Wheat	1999	68		100	68		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Wheat	2000	62		100	62		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Wheat	2001	36		100	36		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
Austria (15-Ac-DON)	Corn	1996	46		50	17		170.3/975[a]	97.5		Schothorst & van Egmond (2004)
	Corn	1997	58		50	48		23.2/190[a]	<LOQ		Schothorst & van Egmond (2004)
	Corn	1998	48		50	22		122.8/830[a]	57.5		Schothorst & van Egmond (2004)
	Oat	1999	96		100	96		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Oat	2000	96		100	96		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Oat	2001	40		100	40		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Wheat	1999	68		100	68		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Wheat	2000	62		100	60		19.5/110[a]	<LOQ		Schothorst & van Egmond (2004)
	Wheat	2001	36		100	36		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
Finland (3-Ac-DON)	Wheat	1998	27	5		27		2.5/[a]	2.5		Schothorst & van Egmond (2004)
	Wheat	1999	37	25		37		12.5/[a]	12.5		Schothorst & van Egmond (2004)
	Wheat	2000	35		50	35		8.33/[a]	8.33		Schothorst & van Egmond (2004)
	Wheat	2001	35		25/40	35		4.17/[a]	4.17		Schothorst & van Egmond (2004)
	Barley	1998	7	5		7		2.5/[a]	2.5		Schothorst & van Egmond (2004)
	Barley	1999	30	25		25		19/70.8[a]	12.5		Schothorst & van Egmond (2004)
	Barley	2000	20		50	20		8.33/[a]	8.33		Schothorst & van Egmond (2004)
	Barley	2001	20		25/40	19		4.17/101[a]	4.17		Schothorst & van Egmond (2004)
	Barley malt	1999	18	25		18		12.5/[a]	12.5		Schothorst & van Egmond (2004)
	Barley malt	2000	25		50	25		8.33/[a]	8.33		Schothorst & van Egmond (2004)
	Barley malt	2001	25		25/40	25		4.17/[a]	4.17		Schothorst & van Egmond (2004)
	Oats	1998	7	5		6		8.4/79[a]	8.4		Schothorst & van Egmond (2004)
	Oats	1999	10	25		10		12.5/[a]	12.5		Schothorst & van Egmond (2004)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Oats	2000	25		50	19		53.7/438[a]	197.4		Schothorst & van Egmond (2004)
	Oats	2001	30		25/40	22		25.5/183[a]	84.3		Schothorst & van Egmond (2004)
	Rye	1998	6	5		6		2.5/[a]	2.5		Schothorst & van Egmond (2004)
	Rye	1999	2	25		2		12.5/[a]	12.5		Schothorst & van Egmond (2004)
	Rye	2000	15		50	15		8.33/[a]	8.33		Schothorst & van Egmond (2004)
	Rye	2001	10		25/40	10		4.17/[a]	4.17		Schothorst & van Egmond (2004)
	Rye organic	2001			25/40						Schothorst & van Egmond (2004)
France (3-Ac-DON)	Organic wheat		11		10		10	/17	10		Malmauret et al. (2002)
	Wheat	2001	30		20			4.69/45[a]	3.3		Schothorst & van Egmond (2004)
	Wheat	2001	22		20			3.33/[a]	3.33		Schothorst & van Egmond (2004)
	Corn	2001	29		20			26.75/520[a]	3.3		Schothorst & van Egmond (2004)
	Corn	2001	25	30		25		15/15[a]			Schothorst & van Egmond (2004)
	Corn	2000	10		10–30	0		11/18[a]	15		Schothorst & van Egmond (2004)
	Corn	2001	55	20–25		28		21/172[a]	12		Schothorst & van Egmond (2004)
	Barley	2001	9		20			3.33/[a]	3.33		Schothorst & van Egmond (2004)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Soft wheat	2001	31		20			3.33/[a]	3.33		Schothorst & van Egmond (2004)
	Soft wheat	2000	82	30	60	82		15/[a]	15		Schothorst & van Egmond (2004)
	Soft wheat	2001	72	30	60	72		15/[a]	15		Schothorst & van Egmond (2004)
	Soft wheat	2002	71	30	60	71		15/[a]	15		Schothorst & van Egmond (2004)
	Soft wheat	2000	204	30	10–60	185		15/30[a]	15		Schothorst & van Egmond (2004)
	Soft wheat	2001	112	25–30	10–60	85		19/50[a]	15		Schothorst & van Egmond (2004)
	Durum wheat	1999	16	30	60	16		15/[a]	15		Schothorst & van Egmond (2004)
	Durum wheat	2000	16	30	60	16		15/[a]	15		Schothorst & van Egmond (2004)
	Durum wheat	2001	13	30	60	13		15/[a]	15		Schothorst & van Egmond (2004)
	Durum wheat	2002	52	30	60	52		15/[a]	15		Schothorst & van Egmond (2004)
	Malting barley	2001	30	30		30		15/[a]	15		Schothorst & van Egmond (2004)
	Malting barley	2001	52	30		52		15/[a]	15		Schothorst & van Egmond (2004)
	Malting barley	2002	44	30		44		15/[a]	15		Schothorst & van Egmond (2004)
	Malting barley	2002	68	30		68		15/[a]	15		Schothorst & van Egmond (2004)
	Muesli		3	30		3		nd			National monitoring programmes
	Wheat bread		5	30		5		nd			National monitoring programmes

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Breakfast cereal (wheat)		1	30		1		nd			National monitoring programmes
	Breakfast cereal (wheat)		4	30		4		nd			National monitoring programmes
	Pasta		2	30		2		nd			National monitoring programmes
	Wheat bread		1	30		1		nd			National monitoring programmes
	Viennese pastry		5	30		5		nd			National monitoring programmes
	Brioche		1	30		1		nd			National monitoring programmes
	Brownie		1	30		1		nd			National monitoring programmes
	Fruit cake		1	30		1		nd			National monitoring programmes
	Shell-shaped cookie		1	30		1		nd			National monitoring programmes
	Chocolate biscuit		1	30		1		nd			National monitoring programmes
	Boiled beef		1	30		1		nd			National monitoring programmes
	Stuffed crepe		1	30		1		nd			National monitoring programmes
	Pastry		1	30		1		nd			National monitoring programmes
	Fruit tart		2	30		2		nd			National monitoring programmes
	Doughnut		1	30		1		nd			National monitoring programmes

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Cream cake		1	30		1		nd			National monitoring programmes
	Omelette		3	30		3		nd			National monitoring programmes
	Hazel-based sweet spread		1	30		1		nd			National monitoring programmes
	Bean curd		1	30		1		nd			National monitoring programmes
	Beer		2	30		2		nd			National monitoring programmes
	Alcoholic beverage		1	30		1		nd			National monitoring programmes
	Pizza		1	30		1		nd			National monitoring programmes
	Quiche Lorraine		1	30		1		nd			National monitoring programmes
	Vegetable tart		1	30		1		nd			National monitoring programmes
	Burger		3	30		3		nd			National monitoring programmes
	Sandwich		3	30		3		nd			National monitoring programmes
	Couscous		3	30		3		nd			National monitoring programmes
	Paella		3	30		3		nd			National monitoring programmes

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Lasagna		3	30		3		nd			National monitoring programmes
	Pasta		1	30		1		nd			National monitoring programmes
	Toasted ham and cheese sandwich		1	30		1		nd			National monitoring programmes
	Stuffed crepe		1	30		1		nd			National monitoring programmes
	Spring roll		1	30		1		nd			National monitoring programmes
	Tabbouleh		1	30		1		nd			National monitoring programmes
	Soya dessert		1	30		1		nd			National monitoring programmes
	Rice or semolina pudding		2	30		2		nd			National monitoring programmes
	Biscuit		2	30		2		nd			National monitoring programmes
	Wheat	2001	30		20			4.86/50[a]	3.33		Schothorst & van Egmond (2004)
	Wheat	2001	22		20			3.33/[a]	3.33		Schothorst & van Egmond (2004)
	Corn	2001	29		20			76.88/1320[a]	3.33		Schothorst & van Egmond (2004)
	Corn	2001	25	30		3		236/700[a]			Schothorst & van Egmond (2004)
	Corn	2000	10		30	0		52/152[a]	28		Schothorst & van Egmond (2004)
	Corn	2001	54	20–25	30	4		197/883[a]	160		Schothorst & van Egmond (2004)
	Barley	2001	9		20			3.33/[a]	3.33		Schothorst & van Egmond (2004)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Soft wheat	2001	31		20			3.33/[a]	3.33		Schothorst & van Egmond (2004)
	Soft wheat	2001	31		20			3.33/[a]	3.33		Schothorst & van Egmond (2004)
	Soft wheat	2000	82	30	60	82		15/[a]	15		Schothorst & van Egmond (2004)
	Soft wheat	2001	72	30	60	72		15/[a]	15		Schothorst & van Egmond (2004)
	Soft wheat	2002	71	30	60	71		15/[a]	15		Schothorst & van Egmond (2004)
	Soft wheat	2000	203	30	10–60	185		19/50[a]	15		Schothorst & van Egmond (2004)
	Soft wheat	2001	112	25–30	10	84		15/[a]	15		Schothorst & van Egmond (2004)
	Durum wheat	1999	16	30	60	16		15/[a]	15		Schothorst & van Egmond (2004)
	Durum wheat	2000	16	30	60	16		15/[a]	15		Schothorst & van Egmond (2004)
	Durum wheat	2001	13	30	60	13		15/[a]	15		Schothorst & van Egmond (2004)
	Durum wheat	2002	52	30	60	52		15/[a]	15		Schothorst & van Egmond (2004)
France (15-Ac-DON)	Muesli		3	30		3		nd			National monitoring programmes
	Wheat bread		5	30		5		nd			National monitoring programmes
	Breakfast cereal (wheat)		1	30		1		nd			National monitoring programmes
	Breakfast cereal (wheat)		4	30		4		nd			National monitoring programmes

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Pasta		2	30		2		nd			National monitoring programmes
	Wheat bread		1	30		1		nd			National monitoring programmes
	Viennese pastry		5	30		5		nd			National monitoring programmes
	Brioche		1	30		1		nd			National monitoring programmes
	Brownie		1	30		1		nd			National monitoring programmes
	Fruit cake		1	30		1		nd			National monitoring programmes
	Shell-shaped cookie		1	30		1		nd			National monitoring programmes
	Chocolate biscuit		1	30		1		nd			National monitoring programmes
	Boiled beef		1	30		1		nd			National monitoring programmes
	Stuffed crepe		1	30		1		nd			National monitoring programmes
	Pastry		1	30		1		nd			National monitoring programmes
	Fruit tart		2	30		2		nd			National monitoring programmes
	Doughnut		1	30		1		nd			National monitoring programmes
	Cream cake		1	30		1		nd			National monitoring programmes
	Omelette		3	30		3		nd			National monitoring programmes
	Hazel-based sweet spread		1	30		1		nd			National monitoring programmes

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Bean curd		1	30		1		nd			National monitoring programmes
	Beer		2	30		2		nd			National monitoring programmes
	Alcoholic beverage		1	30		1		nd			National monitoring programmes
	Pizza		1	30		1		nd			National monitoring programmes
	Quiche Lorraine		1	30		1		nd			National monitoring programmes
	Vegetable tart		1	30		1		nd			National monitoring programmes
	Burger		3	30		3		nd			National monitoring programmes
	Sandwich		3	30		3		nd			National monitoring programmes
	Couscous		3	30		3		nd			National monitoring programmes
	Paella		3	30		3		nd			National monitoring programmes
	Lasagna		3	30		3		nd			National monitoring programmes
	Pasta		1	30		1		nd			National monitoring programmes
	Toasted ham and cheese sandwich		1	30		1		nd			National monitoring programmes
	Stuffed crepe		1	30		1		nd			National monitoring programmes

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Spring roll		1	30		1		nd			National monitoring programmes
	Tabbouleh		1	30		1		nd			National monitoring programmes
	Soya dessert		1	30		1		nd			National monitoring programmes
	Rice or semolina pudding		2	30		2		nd			National monitoring programmes
	Biscuit		2	30		2		nd			National monitoring programmes
Germany (3-Ac-DON)	Wheat and wheat products	2005–2006	130	0.14	0.42			0.57/15	0.21		Gottschalk et al. (2009)
	Oat and oat products	2005–2006	98	0.14	0.42			0.43/8.2	<LOD		Gottschalk et al. (2009)
	Rye and rye products	2005–2006	61	0.14	0.42			0.39/5	0.21		Gottschalk et al. (2009)
	Wheat and wheat products	2005–2006	130	0.033	0.1			0.9/26	0.17		Gottschalk et al. (2009)
	Oat and oat products	2005–2006	98	0.033	0.1			0.11/1.4	<LOD		Gottschalk et al. (2009)
	Rye and rye products	2005–2006	61	0.033	0.1			0.73/8.6	0.35		Gottschalk et al. (2009)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
Netherlands (3-Ac-DON)	Beer	2000–2001	32	8[a]	25[a]	32	32	n/a			Schothorst & Jekel (2003)
	Beer (imported from Germany)	2000–2001	9	8[a]	25[a]	9	9	n/a			Schothorst & Jekel (2003)
	Beer (imported from Belgium)	2000–2001	9	8[a]	25[a]	9	9	n/a			Schothorst & Jekel (2003)
	Beer (imported from Ireland)	2000–2001	1	8[a]	25[a]	1	1	n/a			Schothorst & Jekel (2003)
Norway (3-Ac-DON)	Wheat	1990	138	30	90	138		15/[a]	15		Schothorst & van Egmond (2004)
	Wheat (imported)	1990	27	30	90	27		15/[a]	15		Schothorst & van Egmond (2004)
	Wheat	1991	107	30	90	106		16/120[a]	15		Schothorst & van Egmond (2004)
	Wheat	1992	112	30	90	109		16.5/96[a]	15		Schothorst & van Egmond (2004)
	Wheat (imported)	1992	16	30	90	16		15/[a]	15		Schothorst & van Egmond (2004)
	Wheat	1993	101	30	90	101		15/[a]	15		Schothorst & van Egmond (2004)
	Wheat (imported)	1993	29	30	90	28		15.9/40[a]	15		Schothorst & van Egmond (2004)
	Wheat	1994	112	30	90	112		15/[a]	15		Schothorst & van Egmond (2004)
	Wheat (imported)	1994	30	30	90	30		15/[a]	15		Schothorst & van Egmond (2004)
	Wheat	1995	26	30	90	26		15/[a]	15		Schothorst & van Egmond (2004)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Wheat (imported)	1995	13	30	90	13		15/[a]	15		Schothorst & van Egmond (2004)
	Barley	1990	20	30	90	20		15/[a]	15		Schothorst & van Egmond (2004)
	Rye	1990	2	30	90	2		15/[a]	15		Schothorst & van Egmond (2004)
	Rye (imported)	1990	18	30	90	18		15/[a]	15		Schothorst & van Egmond (2004)
	Rye (imported)	1993	11	30	90	11		15/[a]	15		Schothorst & van Egmond (2004)
	Rye (imported)	1994	12	30	90	12		15/[a]	15		Schothorst & van Egmond (2004)
	Rye (imported)	1995	11	30	90	11		15/[a]	15		Schothorst & van Egmond (2004)
	Oat	1990	40	30	90	34		19.1/60[a]	15		Schothorst & van Egmond (2004)
	Oat	1993	3	30	90	1		35.3/52[a]	15		Schothorst & van Egmond (2004)
	Oat	1994	3	30	90	2		34/72[a]	15		Schothorst & van Egmond (2004)
	Oat	1995	26	30	90	26		15/[a]	15		Schothorst & van Egmond (2004)
Sweden (3-Ac-DON)	Wheat	1996–1997	57	2	10	53		3/86[a]	1		Schothorst & van Egmond (2004)
	Wheat	1999	75	2	10	74		1/10[a]	1		Schothorst & van Egmond (2004)
	Wheat	2001	17	2	10			193/239[a]	191		Schothorst & van Egmond (2004)
	Oat	1996–1997	23	2	10	23		0/0	0		Schothorst & van Egmond (2004)
	Oat	1999	10	2	10	10		0/0	0		Schothorst & van Egmond (2004)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Rye	1996–1997	28	2	10	27		2/19[a]	1		Schothorst & van Egmond (2004)
	Rye	1999	19	2	10	19		0/0	0		Schothorst & van Egmond (2004)
United Kingdom (3-Ac-DON)	Barley grain	2002–2005	446		10			<10/15	<10	<10	Edwards (2009)
	Wheat	2001–2005	1292		10			<10/44	<10	<10	Edwards (2009)
	Rice	2000	100		10	100		1.67/<10[a]	<10		Food Standards Agency (2002a)
	Cereal fractions	2000	16		10	16		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Cereal fractions	2000	11		10	11		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Barley	1999	54		5	51		1.18/8[a]	<5		Schothorst & van Egmond (2004)
	Barley	2000	50		5	50		0.83/<5[a]	<5		Schothorst & van Egmond (2004)
	Barley	2001	49		5	44		2.07/37[a]	<5		Schothorst & van Egmond (2004)
	Beer	2000	28		5	25		1.64/10.2[a]	<5		Schothorst & van Egmond (2004)
	Corn products	2000	15		10	14		2.29/11[a]	<10		Food Standards Agency (2002b)
	Corn products	2000	30		10	26		3.04/15[a]	<10		Food Standards Agency (2002b)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Corn products	2001	24		10	24		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Corn products	2000	1		10	1		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	14		10	14		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	14		10	13		4.12/36[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	40		10	40		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	6		10	6		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2001	13		10	13		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	8		10	8		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat flour	2000	29		10	29		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Flour	2000	8		10	8		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Polenta	2000	8		10	8		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Biscuits	2000	54		10	54		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Bread	2000	40		10	40		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Bread	2000	16		10	16		1.67/<10[a]	<10		Food Standards Agency (2002b)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
United Kingdom (15-Ac-DON)	Barley grain	2002–2005	446		10			<10/35	<10	<10	Edwards (2009)
	Wheat	2001–2005	1292		10			<10/217	<10	<10	Edwards (2009)
	Rice	2000	100		10	100		1.67/<10	<10		Food Standards Agency (2002a)
	Cereal fractions	2000	16		10	16		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Cereal fractions	2000	11		10	11		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Barley	2001	49		10	48		0.94/6[a]	<5		Schothorst & van Egmond (2004)
	Corn products	2000	15		10	9		20.6/157[a]	17		Food Standards Agency (2002b)
	Corn products	2000	30		10	14		51.48/214[a]	17		Food Standards Agency (2002b)
	Corn products	2001	24		10	10		22.15/59[a]	<10		Food Standards Agency (2002b)
	Corn products	2000	1		10	1		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	14		10	14		1.67/<10[a]			Food Standards Agency (2002b)
	Wheat products	2000	14		10	13		62.21/806[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	40		10	40		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	6		10	6		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	13		10	13		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2001	8		10	8		1.67/<10[a]	<10		Food Standards Agency (2002b)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Wheat products	2000	29		10	29		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Flour	2000	8		10	8		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Polenta	2000	8		10	4		16.96/46[a]	<10		Food Standards Agency (2002b)
	Biscuits	2000	54		10	54		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Bread	2000	40		10	40		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Bread	2000	16		10	16		1.67/<10[a]	<10		Food Standards Agency (2002b)

n/a, not applicable; nd, not detected

[a] Arithmetic mean value of all samples (both positive and negative samples). Concentration in samples less than LOD is considered as LOD/2. Where only LOQ is available, then values less than LOQ are considered as LOQ/6.

[b] µg/l.

Table A9. Comparative data for DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside

Sample	LOR (µg/kg)	*n* < LOR	Mean (µg/kg)	Maximum (µg/kg)
Austria, maize, 2006, *n* = 54				
DON	40		753	3 680
DON-3-glucoside	10		141	763
Austria, Germany, Slovakia, wheat, 2005, *n* = 23				
DON	20		1 500	4 130
DON-3-glucoside	10		393	1 070
China, maize, 2008, *n* = 203				
DON	0.3	103	144	4 374
3-Ac-DON	0.3	131	6.6	368
15-Ac-DON	0.3	106	75	1 734
DON-3-glucoside	3	134	22	499
China, wheat, 2008, *n* = 162				
DON	0.3	23	63	591
3-Ac-DON	0.3	120	1.8	35
15-Ac-DON	0.3	118	1.7	71
DON-3-glucoside	3	82	26	238
China, wheat, flour, 2008, *n* = 30				
DON	0.3	0	52	3 425
15-Ac-DON	0.3	15	1.5	5
DON-3-glucoside	3	9	7.3	39
China, Henan Province, Puyang, wheat, 1998, *n* = 31				
DON	10	1	2 850	14 000
15-Ac-DON	10	11	365	1 800
China, Henan Province, Zhumedian, wheat, 1998, *n* = 28				
DON	10	3	223	1 240
15-Ac-DON	10	28	nd	
China, Henan Province, Puyang, wheat, 1999, *n* = 34				
DON	10	5	294	941
15-Ac-DON	10	34	nd	nd
Japan, wheat, 2008, *n* = 120				
DON	13	39	33	460
3-Ac-DON	16	114	0.5	18
15-Ac-DON	8	120	nd	nd

Table A9 (contd)

Sample	LOR (µg/kg)	*n* < LOR	Mean (µg/kg)	Maximum (µg/kg)
Japan, barley, 2008, *n* = 100				
DON	7	22	32	560
3-Ac-DON	8	81	2.9	53
15-Ac-DON	7	92	0.3	8.8
USA, Hard Red Spring Wheat, 2005, *n* = 28				
DON	0.5	10	1 400	10 000
DON-3-glucoside	0.5	22	200	5 400
USA, durum wheat, North Dakota, 2001, *n* = 24				
DON	50		9 100	23 000
15-Ac-DON	50		100	800

The following cereals had no or very few samples with detectable Ac-DON:

Austria, oats, *n* = 136	
DON	
No 3-Ac-DON or 15-Ac-DON detected	530
Austria, wheat, *n* = 98	
DON	6 090
No 3-Ac-DON or 15-Ac-DON detected	
Finland, wheat, *n* = 35	
DON	1 026
No 3-Ac-DON detected	
Finland, barley, *n* = 20	
DON	619
One sample: 3-Ac-DON at 101 µg/kg	
Finland, oats, *n* = 55	
DON	5 004
14 samples: 3-Ac-DON maximum 438 µg/kg	
Finland, rye, *n* = 15	
DON	37
No 3-Ac-DON detected	
France, maize, *n* = 25	
DON	4 800
No 3-Ac-DON detected	
France, barley, *n* = 9	
DON	35
No 3-Ac-DON detected	

Table A9 (contd)

Sample	LOR (µg/kg)	*n* < LOR	Mean (µg/kg)	Maximum (µg/kg)
France, soft wheat, *n* = 225				
DON				1 500
No 3-Ac-DON detected				
France, durum wheat, *n* = 81				
DON				3 600
No 3-Ac-DON detected				
United Kingdom, rice, *n* = 100				
DON				12
No 3-Ac-DON detected				
United Kingdom, barley, *n* = 99				
DON				53
Five samples: 3-Ac-DON maximum 37 µg/kg				

FURAN

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1. EXPLANATION

Furan (C_4H_4O) (Chemical Abstracts Service [CAS] No. 110-00-9) is a highly volatile cyclic ether that can be formed unintentionally in foods during processing from precursors that are natural food components. Information available to the Committee at its present meeting suggested that the major route of exposure to

furan in the human population is through consumption of heat-treated foods and beverages.

Furan has not been evaluated previously by the Committee. The request for a full evaluation of furan originated from the Second Session of the Codex Committee on Contaminants in Food (FAO/WHO, 2008).

2. BIOLOGICAL DATA

2.1 Biochemical aspects

Searches for relevant publications were performed utilizing various search engines and databases, including, but not limited to, RefWorks, PubMed, Google, SpringerLink.com, Wiley Interscience and Elsevier. Methods involved keyword searches, journals, article titles, authors and previously cited references in related material.

2.1.1 Absorption, distribution and excretion

The disposition of [^{14}C]furan has been studied in male F344 rats. Furan (>99% pure) in corn oil was administered by the intragastric route at a dose of 8 mg/kg body weight (bw) once per day for between 1 and 8 consecutive days. This corresponded to the highest dose tested in the F344 rat in the United States National Toxicology Program (NTP) 2-year assay described below in section 2.2.3. Following dosing, animals were placed in metabolism cages for the separate collection of urine and faeces, as well as the trapping of carbon dioxide and volatiles (animals receiving a single dose only). Animals were sacrificed at time points ranging from 1 to 8 days after dosing, and each of the major tissues was removed for analysis of radioactivity and covalent binding to liver macromolecules.

In the first 24 h after dosing, expired air, urine and faeces were all significant routes of elimination of furan-derived radioactivity. Approximately 14% of the administered dose was expired as unchanged furan, most (11%) within the first hour after dosing. In addition, 26% of the administered dose was expired as carbon dioxide, virtually all of which was eliminated in the first 12 h. Radioactivity in urine and faeces accounted for about 20% and 22% of the administered dose, respectively. By 24 h, 19% of the administered radioactivity remained in the tissues, suggesting that 81% had been eliminated in expired air, urine and faeces. The highest concentration of radioactivity was found in the liver (13% of the dose); the next highest concentrations were found in the kidney, blood and small and large intestines, each accounting for less than 1% of the administered dose (0.45%, 0.42%, 0.15% and 0.13%, respectively). The concentrations in lung tissue were very low, accounting for only 0.02% of the administered dose. Elimination of radioactivity from the liver appeared to follow first-order kinetics, with a half-life of 1.8 days. In contrast, the elimination kinetics for kidney and blood were more complex, with the blood concentration of radioactivity remaining mostly constant for 8 days following a single dose of 8 mg/kg bw. The concentrations of furan-derived radioactivity were higher in the liver, kidney and blood with multiple doses of furan compared with a

single dose by about 6-fold in kidney and blood and 4-fold in the liver. The percentage of administered radioactivity eliminated in the faeces was the same for eight daily doses as for a single dose followed for 8 days. Elimination of radioactivity in the urine, in contrast, increased as a percentage of the total administered dose from 20% of a single dose to 33% of 8 days' cumulative dose.

At 24 h, high-performance liquid chromatographic (HPLC) analysis of plasma or an extract of liver showed no unchanged furan. Approximately 80% of the radioactivity retained in the liver was not extractable by organic solvents; the remaining radioactivity was assumed to be covalently bound to tissue macromolecules. With the techniques available at the time, the authors concluded that this radioactivity was associated with protein and that either there was no binding to deoxyribonucleic acid (DNA) or furan–DNA adducts were not stable to the isolation procedure. Repeated daily administration of [^{14}C]furan resulted in an increase in covalent binding over 4 days, at which point the amount of non-extractable radioactivity reached a plateau. At least 10 metabolites of furan were isolated from the urine, but were not identified. The authors noted that the large amount of administered radioactivity expired as carbon dioxide (26%) indicated that a considerable portion of furan metabolism involves ring opening and oxidation to carbon dioxide. The ring-opened product was predicted to be *cis*-2-butene-1,4-dial (BDA, also known as maleic anhydride), with subsequent hydration to maleic acid and rapid excretion as carbon dioxide in rats. The generation of this citric acid intermediate was considered to be a possible explanation for some of the tissue non-extractable radioactivity in liver protein (Burka, Washburn & Irwin, 1991).

A physiologically based pharmacokinetic (PBPK) model was developed for furan in rats. Model simulations of furan metabolism in the rat, following an oral dose of 8 mg/kg bw, predicted metabolism of 84% of the dose and 16% exhaled as the parent compound (Kedderis et al., 1993). Furan biotransformation kinetics determined with freshly isolated rat hepatocytes in vitro were found to accurately predict furan pharmacokinetics in vivo, suggesting that furan biotransformation kinetics determined with freshly isolated mouse or human hepatocytes could be used to develop species-specific pharmacokinetic models.

The PBPK models were used to compare the bioactivation and liver dosimetry of furan between rodents and humans by the inhalation route. The absorbed dose of furan was approximately 3.5-fold and 10-fold greater in rats and mice, respectively, than in humans following the same inhalation exposure. Similar species differences were observed for the integrated exposure of the liver to BDA, the toxic metabolite of furan, over the same inhalation exposure. Comparison of the initial rate of furan metabolism and the rate of liver perfusion indicated that for each of the three species, furan oxidation occurred at rates that were approximately 13- to 37-fold higher than the rate of furan delivery to the liver via blood flow. This suggested that hepatic uptake and clearance and the hepatic concentration of the toxic metabolite of furan as a function of furan exposure concentration would be limited by hepatic blood flow. As a consequence, the authors concluded that the toxic metabolite formed in the liver would be unaffected by increases in the maximum rate (V_{max}) due to the induction of cytochrome P450 (CYP) 2E1 and that interindividual variations observed in CYP2E1 activity in human populations would

not be expected to have a significant effect on the extent of furan bioactivation in humans (Kedderis & Held, 1996). The hepatic blood flow limitation of biotransformation has also been observed after oral bolus dosing of rapidly metabolized compounds, such as ethanol (Kedderis, 1997). Hepatic CYP2E1 activity may be an important variable in determining the extent of any first-pass metabolism within an individual and therefore the extent of pre-systemic bioactivation following oral exposure. Hepatic first-pass metabolism could also determine the extent of systemic exposure and subsequent elimination in expired air. The absolute bioavailability of orally administered furan has not been studied.

2.1.2 Biotransformation

The finding that furan biotransformation kinetics determined with freshly isolated rat hepatocytes in vitro accurately predicted furan pharmacokinetics in vivo after inhalation exposure was used to develop a hepatocyte suspension/culture system with freshly isolated hepatocytes that utilized furan concentrations and incubation times similar to hepatic dosimetry in vivo. The biotransformation kinetics of furan by freshly isolated male F344 rat hepatocytes in suspension were determined in sealed flasks by measurement of changes in the furan headspace (HS) concentrations as a function of incubation time. The CYP inhibitor, 1-phenylimidazole, and the substrate, ethanol, were potent inhibitors of furan biotransformation. Furan biotransformation was also inhibited by pretreatment of the rats with aminobenzotriazole (ABT), a selective suicide inactivator of CYP. Pretreatment of the rats with phenobarbital to induce the CYP2B family had no effect on the rate of furan oxidation, whereas pretreatment with acetone to induce CYP2E1 increased the initial rate of furan oxidation approximately 5-fold. These results suggested that CYP2E1 is a major catalyst for furan oxidation (Kedderis et al., 1993).

Hepatocytes from male B6C3F1 mice and from three human donors (two males and one female) were also studied in the above suspension culture system. Hepatocytes from mice oxidized furan at a greater rate than found for rats in a previous study (Kedderis et al., 1993) or human hepatocytes. Hepatocytes from the three human donors oxidized furan at rates equal to or greater than those of rat hepatocytes. A greater than 2-fold variation in V_{max} was observed among the three preparations of human hepatocytes. The observation that two of the donors had died in automobile accidents following alcohol consumption was consistent with the interpretation that the higher V_{max} values in these samples relative to the other sample were due to the induction of CYP2E1 by ethanol (Kedderis & Held, 1996).

By analogy with the unsaturated aldehydes that have been identified as the microsomal metabolites of 2-methylfuran and 3-methylfuran (Ravindranath, Burka & Boyd, 1984), BDA was proposed as the reactive microsomal metabolite of furan. Liver microsomal fractions obtained from male F344 rats were incubated with concentrations of furan ranging from 0.025 to 20 mmol/l in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and semicarbazide. Given the reactive nature of the proposed metabolite, the assay required the presence of a trapping agent such as semicarbazide to prevent protein binding. HPLC analysis of the incubation mixtures revealed the formation of a metabolite that co-eluted with standards for the bis-semicarbazone adduct of BDA (Chen, Hecht & Peterson, 1995).

Incubation of furan (2 mmol/l) with F344 rat liver microsomes in the presence of cofactors and [glycine-2-^{3}H]glutathione (GSH) at 4 mmol/l led to the formation of radioactive peaks that co-eluted with synthetic standards for the bis-GSH conjugates (Chen, Hecht & Peterson, 1997). Using an HPLC with electron capture method for detection of GSH conjugates, the formation of bis-GSH conjugates in rat and human microsomes incubated with furan was confirmed, together with a previously uncharacterized type of reaction product, the mono-GSH conjugates (Peterson et al., 2005).

The urinary metabolites of furan from male F344 rats dosed intragastrically with 8 mg/kg bw of either [$^{12}C_4$]furan or [$^{13}C_4$]furan in corn oil were determined. Several metabolites resulting from bis- and mono-GSH conjugates of BDA were predicted, but the only metabolite detected in the 24 h urine was the mono-GSH conjugate. The finding of this metabolite in the urine of furan-dosed rats confirms that BDA is formed and conjugated with GSH in vivo. Although bis-GSH conjugates or their predicted metabolites were not detected in urine, the authors indicated that they might be formed and preferentially excreted via the bile and therefore might be detected in faeces. At least 18 other furan-derived urinary metabolites were detected; preliminary data indicated that they were conjugates and that the furan portion of the molecule derived from further metabolism of BDA (Peterson et al., 2006).

Metabolic profiling of products that had been excreted in the urine of rats during two 24 h periods after intragastric dosing with furan at 40 mg/kg bw in corn oil was conducted using liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis. Seven peaks that increased significantly following dosing were investigated as biomarkers of exposure to furan; the structures of five metabolites were elucidated. Of these, two were identified as being mercapturic acid derivatives (GSH/cysteine conjugates) of furan, one as a lysine adduct and two combining elements of both mercapturic acid derivatives and lysine adducts. While the two exclusively mercapturic acid derivatives were postulated to result from the reaction of BDA with GSH, the three metabolites containing lysine adducts were postulated to be derived from degraded protein adducts (Kellert et al., 2008a).

Further characterization of furan metabolites in urine of male F344 rats dosed intragastrically with 8 or 40 mg/kg bw [$^{12}C_4$]furan or [$^{13}C_4$]furan in corn oil and in freshly isolated hepatocytes from Sprague-Dawley or F344 rats incubated with furan at 100 µmol/l was conducted using LC-MS/MS analysis. It was concluded that the reaction of BDA with GSH does not completely deactivate BDA, as had been suggested previously (Chen, Hecht & Peterson, 1997). Chemical characterization of the urinary and hepatocyte metabolites of furan suggested that there are at least three different types of protein adducts generated by reactive furan metabolites: 1) BDA can react directly with protein lysine residues to generate pyrrolinone adducts; 2) BDA can react with protein thiol groups to generate a protein–thiol BDA intermediate that can then react with protein lysine groups to form a cysteine–BDA–lysine crosslink within a protein; and 3) a GSH–BDA–protein lysine crosslink can be formed (Lu et al., 2009).

In addition to the two urinary metabolites described by Lu et al. (2009), seven additional urinary metabolites have been characterized. All nine metabolites are derived from a common intermediate that results from the crosslinking of BDA to cysteine and lysine residues. Some of these metabolites are among the most abundant furan metabolites in urine, as judged by LC-MS/MS analysis. The authors concluded that the formation of cysteine–BDA–lysine crosslinks is an important component in the overall metabolism of furan in rats and indicated the strong possibility that these urinary metabolites are degraded protein adducts (Lu & Peterson, 2010).

2.1.3 Effects on enzymes and other biochemical parameters

A series of studies was carried out to investigate the covalent binding of furan with hepatic microsomal protein, to identify the CYP enzymes involved in metabolic activation and to determine possible mechanisms by which furan can interact with CYP. ^{14}C-labelled furan was found to be covalently bound to microsomal proteins following incubation with liver microsomes isolated from male F344 rats in the presence of NADPH. Microsomes isolated from phenobarbital- (PB), imidazole- or pyrazole (PY)-treated rats exhibited significantly enhanced covalent binding of furan to protein, indicating the increased formation of reactive species after these pretreatments. However, binding was reduced to almost half of control values when liver microsomes from β-naphthoflavone (β-NF)-treated animals were used. The greater degree of induction observed with imidazole and PY suggests that this metabolism-dependent covalent binding is preferentially catalysed by the CYP2E1 enzyme. To determine the nature of the functional groups involved in covalent binding of furan to microsomal proteins, binding was studied in the presence of compounds acting as nucleophiles: containing amine groups available for binding (semicarbazide); containing an amine group blocked for binding and a thiol group available for binding (*N*-acetylcysteine [NAC]); and containing both amine and thiol groups available for binding. GSH, with both free amine and thiol groups, provided maximum protection against binding for microsomes from control, PB-, imidazole- and β-NF-treated rats, reducing binding in control microsomes by 86%. Semicarbazide also provided significant protection against covalent binding (control, PB- and imidazole-treated), whereas NAC provided protection only in microsomes from imidazole-treated rats. Incubation of microsomes from rats dosed intragastrically with furan in corn oil at 0, 8 or 25 mg/kg bw resulted in a dose-related decrease in CYP concentration and in decreased activities of aniline hydroxylase (AH), ethoxyresorufin-*O*-deethylase (EROD) and 7-ethoxycoumarin-*O*-deethylase (ECOD) after 24 h, suggesting that the reactive species produced during CYP-catalysed metabolism reacts with nucleophilic groups located within the CYP itself. No significant inhibition of EROD or ECOD activity or significant change in the carbon monoxide binding spectrum of the CYP or in total haem content was noted in microsomes from untreated rats that were incubated for 5, 10 or 25 min in either the presence or absence of NADPH; an inhibition of AH activity (30%) was noted after incubation with furan for 5 min, with or without NADPH. When microsomes from rats treated with PB or PY were incubated with furan, no significant decrease in CYP concentration or in total haem content was noted compared with microsomes from untreated animals. In microsomes from PB-treated rats incubated with furan,

a significant increase in the activity of ECOD was noted, whereas the activity of EROD was unaffected and that of AH was significantly decreased. Incubation of microsomes from PY-treated rats with furan reduced the activities of EROD and AH, but did not affect the activity of ECOD, compared with controls. Studies conducted to investigate the covalent interaction with the haem and protein moieties of CYP found that furan-derived radioactivity was distributed almost equally to the haem and protein moieties. The authors were not able to characterize the chemical nature of the haem–furan adduct (Parmar & Burka, 1993).

An isolated male Fischer rat hepatocyte suspension system was used to show that furan-induced cytolethality can be produced in vitro via an apparent solvent effect at high (millimole per litre) concentrations or via a reactive metabolite at concentrations (micromole per litre) relevant to tissue doses in vivo. At conditions that more accurately reflect the dosimetry of hepatotoxic doses of furan in vivo, furan (2–100 µmol/l) produced a concentration- and time-dependent decrease in GSH concentrations and cell viability of hepatocytes. Addition of the CYP inhibitor, 1-phenylimidazole, prevented the furan-induced depletion of GSH and cytolethality. In contrast, pretreatment of the rats with the CYP2E1 inducer, acetone, increased GSH depletion and cytolethality at a concentration of 100 µmol/l, but not at 2 or 10 µmol/l. These data indicated that the oxidation of furan by CYP is required for GSH depletion and cytolethality in vitro at furan concentrations that are relevant in vivo (Carfagna, Held & Kedderis, 1993).

Incubation of isolated rat hepatocytes at concentrations (2–100 µmol/l) that had been found by Carfagna, Held & Kedderis (1993) to be relevant to in vivo exposure produced irreversible concentration- and time-dependent reductions in adenosine triphosphate (ATP) concentration that preceded cell death. In light of the fact that mitochondria produce about 95% of the total ATP needs of eukaryotic cells, the effect of furan on mitochondrial respiration in rat hepatocytes both in vivo and in vitro was investigated. Furan doses of 15 or 30 mg/kg bw administered intragastrically in corn oil to male F344 rats were found to greatly increase state 4 (succinate-stimulated) respiration in mitochondria of hepatocytes in a dose-related manner, without affecting state 3 (adenosine diphosphate [ADP]–stimulated) respiration. In addition, these doses of furan increased the activity of mitochondrial adenosine triphosphatase (ATPase) in hepatocytes of treated rats. Stimulation of oxygen uptake by mitochrondria in the absence of ADP and increased activity of the ATP-hydrolysing activity in mitochondria are characteristic of irreversible uncouplers of mitochondrial respiration. Similar results were found with the in vitro hepatocyte model. Pretreatment of rats or co-incubation of hepatocytes with 1-phenylimidazole prevented the effects of furan on mitochondrial respiration, indicating that biotransformation of furan was required for its cytotoxic activity. The authors concluded that furan-induced uncoupling of oxidative phosphorylation is an early, critical event in cytolethality both in vivo and in vitro (Mugford, Carfagna & Kedderis, 1997).

Blood was collected from F344 rats at all dose levels (0, 0.03, 0.12, 0.5, 2.0 and 8.0 mg/kg bw per day) in the Gill et al. (2009) 90-day intragastric study (see section 2.2.2) for analysis of oxidative stress and antioxidant defences. Furan did not cause significant changes in circulating 8-hydroxy-2′-deoxyguanosine, total

antioxidant capacity or oxidized low-density lipoprotein. Paraoxanase-1 was increased at 2 mg/kg bw in males and at 8 mg/kg bw in females (Jin, Kearns & Coughlan, 2009).

2.2 Toxicological studies

Searches for relevant publications were performed utilizing various search engines and databases, including, but not limited to, RefWorks, PubMed, Google, SpringerLink.com, Wiley Interscience and Elsevier. Methods involved keyword searches, journals, article titles, authors and previously cited references in related material.

2.2.1 Acute toxicity

(a) Mice

A single intraperitoneal dose of 300 mg/kg bw of furan in sesame oil was administered to 10 adult male Swiss albino mice; in addition, a group of 25 mice received piperonyl butoxide, an inhibitor of CYP, at 1360 mg/kg bw intraperitoneally 30 min prior to injection with furan. Control mice (number not indicated) received the vehicle. The animals were sacrificed 36 h after being dosed with furan. The livers and kidneys were removed and samples prepared for histopathological evaluation. At 300 mg/kg bw, furan produced a moderate to severe coagulative necrosis in both organs. In the liver, the lesions were concentrated in the centrilobular zone. Coagulative lesions of the kidney appeared to affect the proximal convoluted tubules of the outer cortex, without damaging glomerular or medullary cells. Very little tubular precipitate or tubular dilatation was present. Pretreatment with piperonyl butoxide resulted in eradication of kidney lesions and a reduction of severity of the liver lesions to mainly mild in nature (McMurtry & Mitchell, 1977).

The toxicity of single intraperitoneal doses of furan was assessed as part of a study of the hepatic and renal toxicity of a homologous series of 2- and 3-alkylfurans. Male ICR mice, 20–25 g, were initially administered a dose of 2.6 mmol/kg bw (180 mg/kg bw as furan) of each of the furans in sesame oil by intraperitoneal injection. Plasma urea nitrogen and plasma glutamate–pyruvate transaminase (GPT) (also known as alanine aminotransferase [ALT]) activity were determined 24 h later to assess the relative potency of renal and hepatic toxicity, respectively, for the compounds. This dose had been previously determined to be the maximum amount of the most potent member of the series that could be given without producing mortality during the course of the experiments. Furan dosing produced a non-statistically significant increase in plasma urea nitrogen that was about 2-fold greater than values for the controls. Of all the compounds tested, furan was the most potent in increasing serum GPT activity over a 24 h period, elevating activity by more than 2 orders of magnitude over control values. The same dose of furan (180 mg/kg bw) had no effect on urine-concentrating ability, compared with controls, in mice that had been deprived of food and water for 48 h. Histopathological evaluation of liver and kidney samples taken 24 h after a single intraperitoneal furan dose of 5.1 mmol/kg bw (350 mg/kg bw) revealed massive centrilobular necrosis and slight proximal tubular necrosis, respectively. HPLC analysis of heptane

extracts of liver and kidney tissue at 1, 2 and 5 h after administration of the test compound at 4.1 mmol/kg bw (280 mg/kg bw as furan) demonstrated that, in contrast to the other compounds shown to be hepatotoxic, unchanged furan was not found in high concentration in liver tissue over a 5 h period (Wiley et al., 1984).

The effects of a single intraperitoneal dose of furan (along with a series of 3-alkylfurans and 2-ethylfuran) in sesame oil on lung toxicity were assessed in male ICR mice. Twenty-four hours after mice were administered a furan dose of 5.15 mmol/kg bw, severe bronchiolar necrosis was observed in the lungs of the mice. Thymidine incorporation into the DNA of homogenized lungs of mice dosed intraperitoneally with furan at 1.56 mmol/kg bw (106 mg/kg bw) at 3 days after dosing was found to be 4 times higher in furan-dosed mice than in vehicle-treated controls. HPLC analysis of heptane extracts of lung tissue at 1, 2 and 5 h after test compound administration demonstrated that, in contrast to the other compounds, unchanged furan was not retained in lung tissue over a 5 h period (Gammal et al., 1984).

Male B6C3F1/CrlBR mice (five per group) were administered a single intragastric furan (>99% pure) dose of 50 mg/kg bw in corn oil. This dose was determined to be toxic to the liver without killing the animals during the period of the experiment. Two hours prior to sacrifice, the animals were injected intraperitoneally with methyl-[^{3}H]thymidine for incorporation into replicating cells. A blood sample was taken just prior to sacrifice for the determination of aspartate aminotransferase (AST), ALT and lactate dehydrogenase (LDH). The mice were sacrificed at 12 h, 24 h, 48 h, 4 days or 8 days following dosing with furan; corn oil–administered control animals were sacrificed 48 h after treatment. The livers were removed and prepared for histopathological evaluation in sections stained with haematoxylin and eosin (H&E) and determination of the labelling index (LI) by autoradiographic measurement of thymidine-labelled cells. A dose–response experiment for plasma enzymes was also conducted in which male mice were given a single gavage administration of furan at 0, 15, 28, 37 or 50 mg/kg bw in corn oil. Plasma samples were collected from all dose groups just prior to sacrifice, 24 h after dosing, and the activities of AST, ALT and LDH were determined.

Histopathological evaluation revealed moderate midzonal degeneration and necrosis as early as the first scheduled sacrifice after furan dosing (12 h), peaking at 24 h. Parenchymal cells typically had enlarged, pale-staining cytoplasms and condensed chromatin in the nuclei. Hepatocytes in the vicinity of the central vein were very eosinophilic, whereas those near the portal vein were vacuolated in some, but not all, livers. The subcapsular parenchyma of the visceral surface of the left and caudate hepatic lobes had necrosis with some inflammatory cell infiltrate, primarily at 12 h post-treatment. This was taken to suggest that some of the furan diffused through the stomach wall and into the liver subsequent to intragastric administration. By 48 h, there was extensive cell proliferation observed as cells in S-phase and by mitotic figures. Little or no necrosis was apparent at 48 h. Midzonal inflammation was observed in livers from mice sacrificed at either 48 h or 4 days following furan dosing, primarily in the form of infiltrating neutrophils and macrophages. By 8 days, livers had returned to near normal. A spike in LI of hepatocyte nuclei was noted 48 h after dosing, whereas the LI at other time points was not

significantly elevated above control values. Further evidence of the cytotoxicity of furan was large increases in AST, ALT and LDH that occurred as early as 12 h, reaching a peak at 24 h and declining at subsequent time points to reach normal values by 4 days. The dose–response study conducted for plasma enzymes revealed that at the lowest dose of furan tested (15 mg/kg bw), enzyme activity levels at 24 h were not significantly elevated from control values, but they increased markedly with the higher dose levels (Wilson et al., 1992).

(b) Rats

Male F344 rats/CrlBR rats (five per group) were administered a single intragastric dose of furan (>99% pure) at 30 mg/kg bw in corn oil. This dose was determined to be toxic to the liver without killing the animals during the period of the experiment. Two hours prior to sacrifice, the animals were injected intraperitoneally with methyl-[^{3}H]thymidine for incorporation into proliferating cells. A blood sample was taken just prior to sacrifice for the determination of AST, ALT and LDH. The rats were sacrificed at 12 h, 24 h, 48 h, 4 days or 8 days following dosing with furan; corn oil–administered control animals were sacrificed 48 h after dosing. The livers were removed and prepared for histopathological evaluation of H&E-stained sections and autoradiographic measurement of thymidine-labelled cells. A dose–response experiment for plasma enzymes was also conducted in which male rats were administered a single intragastric dose of furan in corn oil at 0, 8, 15, 22 or 30 mg/kg bw. Plasma samples were collected from all dose groups just prior to sacrifice, 24 h after dosing, and the plasma activities of AST, ALT and LDH were determined.

Histopathological evaluation revealed moderate midzonal degeneration and necrosis in rats receiving the single dose of furan as early as the first scheduled sacrifice after furan treatment (12 h). In rare cases, slight midzonal necrosis was observed that was most pronounced 24 h after treatment. Subcapsular necrosis and inflammatory cell infiltration were seen at 12 and 24 h and were more severe than those observed in the mouse. Necrosis began to subside by 48 h. Inflammation was observed at 24 and 48 h, with foci of inflammatory cell infiltrates still noted at 4 days. The livers had returned to near normal by 8 days. A spike in LI of hepatocyte nuclei was noted 48 h after a single furan dose of 30 mg/kg bw. Plasma enzymes were elevated at both 12 and 24 h following treatment, indicating marked cytotoxicity, returning to near-normal values after 4 days. In the dose–response study, plasma enzyme activities were not elevated above control values at the lowest dose (8 mg/kg bw), but they increased markedly with the higher dose levels (Wilson et al., 1992).

2.2.2 Short-term studies of toxicity

(a) Mice

Groups of six male B6C3F1 mice were administered furan intragastrically in corn oil at 15 mg/kg bw per day, 5 days/week, for 6 weeks. Six days prior to sacrifice, pumps containing [^{3}H]thymidine were implanted subcutaneously for incorporation of thymidine into replicating cells. Sections of liver were taken for histopathology

and autoradiographic measurement of thymidine-labelled cells. At the 1-week sacrifice, only an occasional necrotic cell or small cluster of inflammatory cells was noted. In some animals, the visceral surface of the liver was irregular with small foci of necrotic cells and early fibrosis. At the 3-week sacrifice, the subcapsular parenchyma of the visceral surface had necrosis and inflammation that were more extensive than at week 1. The liver was otherwise normal except for some perinuclear vacuolization of the cytoplasm. At the 6-week sacrifice, a centrilobular pattern of hepatocyte vacuolization was evident. No bile duct proliferation was noted. The hepatocyte LIs for furan-dosed mice were 25.1, 12.0 and 3.2 for weeks 1, 3 and 6, respectively, compared with LIs for controls of 0.41 and 0.89 for weeks 1 and 6, respectively (Wilson et al., 1992).

Groups of five male and five female B6C3F1 mice (51 days old) were dosed intragastrically with furan (>99% pure) in corn oil at 0, 10, 20, 40, 80 or 160 mg/kg bw per day for 12 days (days 1 through 5, 8 through 12 and at least 2 consecutive dosing days before terminal sacrifice). The mice were weighed at the start of the study and at days 8 and 16. Clinical observations were made twice daily. Complete necropsies were performed on all animals. Three of five male mice receiving 40 mg/kg bw per day and all male mice receiving 80 or 160 mg/kg bw per day died by day 6; four of five females that received 80 mg/kg bw per day and all females that received 160 mg/kg bw per day died by day 6. The body weights of male mice receiving 10 or 20 mg/kg bw per day were significantly increased compared with those of controls; in females from the 10, 20 and 40 mg/kg bw per day groups, body weights were similar to those of controls. Inactivity was the most commonly observed clinical finding in the treated mice. No observations at necropsy were clearly associated with furan treatment (NTP, 1993).

Groups of 10 male and 10 female B6C3F1 mice were dosed intragastrically with furan (>99% pure) in corn oil on 5 days/week for 13 weeks, starting when they were 51 days old. Males were dosed with 0, 2, 4, 8, 15 or 30 mg/kg bw per day; females were dosed with 0, 4, 8, 15, 30 or 60 mg/kg bw per day. The mice were observed twice daily, and clinical observations were recorded weekly. Body weights were taken at the start of the study and subsequently at weekly intervals. Surviving mice were killed at the end of the 13-week treatment period, and necropsies were performed on all animals. Weights of brain, heart, right kidney, liver, lungs and thymus were taken at necropsy. Complete histopathological examinations were made of all mice in the control and high-dose groups. Livers from all groups, with the exception of the lowest dose groups for each species (2 mg/kg bw per day and 4 mg/kg bw per day in rats and mice, respectively), were subjected to histopathological examination. Dosing had no effect on survival of the mice. The final mean body weights of male mice receiving 30 mg/kg bw per day were lower than those of controls; the final mean body weights of male mice in the other dose groups and of all dosed groups of female mice were similar to those of control mice. Clinical observations related to dosing with furan were observed only in the highest-dose females and consisted of reduced activity or inactivity. The absolute and relative liver weights at the two top dose levels (15 and 30 mg/kg bw per day in males and 30 and 60 mg/kg bw per day in females) were statistically increased compared with those of control mice; no other organ weight changes related to dosing were found.

A low incidence of liver lesions was noted, starting at a dose of 8 mg/kg bw per day in male mice and 15 mg/kg bw per day in female mice and increasing with dose, affecting nearly all the animals at doses of 30 and 60 mg/kg bw per day for most lesions (Table 1). The lesions included cytomegaly, degeneration and necrosis of hepatocytes, greenish-yellow to brown pigmentation of Kupffer cells (presumably bile), bile duct hyperplasia (most marked in highest-dose females) and cholangiofibrosis (females only). Hepatocyte lesions usually involved periportal hepatocytes, but extended to other areas of the lobules in more severe cases. Hepatocyte degeneration consisted of varying degrees of clear cytoplasmic vacuolization (NTP, 1993).

(b) Rats

Groups of six male and six female F344 rats were administered furan intragastrically in corn oil at 8 mg/kg bw per day, 5 days/week, for 6 weeks. Six days prior to sacrifice, pumps containing [^{3}H]thymidine were implanted subcutaneously for incorporation of thymidine into replicating cells. Sections of liver were taken for histopathology and autoradiographic measurement of the thymidine LI. Subcapsular focal areas of inflammatory cell infiltrate were observed on the visceral surface at the 1-week sacrifice. Several small foci of inflammatory cells were found scattered randomly in the hepatic parenchyma. Centrilobular hepatocytes were vacuolated, whereas the periportal cells were hypereosinophilic. At the 3- and 6-week sacrifices, a prominent pattern of visceral surface lesions and hypereosinophilic periportal hepatocytes was evident. Bile duct hyperplasia with attendant peribiliary fibrosis was observed in the liver parenchyma. In some cases, inflammatory cells from the subcapsular visceral surface lesion extended into the parenchyma, where they surrounded a small number of degenerated and/or necrotic hepatocytes. The subcapsular inflammation was often associated with a light brown pigment in macrophages within the inflammatory zone. Mild to moderate bile duct proliferation within this zone was evident in some sections. By 6 weeks of furan dosing, livers displayed cholangiofibrosis in subcapsular areas of the left or caudate lobes. Signs of metaplasia localized within these cholangiofibrotic areas were observed in two rats as ducts composed of columnar cells with basal nuclei. These cells resembled intestinal cells and have been referred to variously as intestinal cell metaplasia, bile duct hyperplasia and cholangiofibrosis. Evaluation of these ducts in liver sections that had been processed for autoradiography revealed that the nuclei of the cells that composed the ducts were often incorporating thymidine and were therefore actively proliferating. The hepatocyte LIs for furan-dosed male rats were 3.2, 9.2 and 6.5 for weeks 1, 3 and 6, respectively, compared with LIs for controls of 0.08 and 0.29 for weeks 1 and 6, respectively. The LIs for furan-dosed females were 11.7, 9.2 and 14.4 for weeks 1, 3 and 6, respectively, compared with LIs for controls of 0.77 and 0.75 for weeks 1 and 6, respectively (Wilson et al., 1992).

Table 1. Incidences of selected non-neoplastic hepatic lesions in mice in the 13-week NTP (1993) oral study

	Incidence of lesions											
	Males						Females					
	0 mg/kg bw	2 mg/kg bw	4 mg/kg bw	8 mg/kg bw	15 mg/kg bw	30 mg/kg bw	0 mg/kg bw	4 mg/kg bw	8 mg/kg bw	15 mg/kg bw	30 mg/kg bw	60 mg/kg bw
Biliary tract												
Cholangiofibrosis	—	—	—	—	—	—	0/10	—	0/10	0/10	4/10*	10/10**
Hyperplasia	0/10	—	0/10	0/10	0/10	2/10	0/10	—	0/10	0/10	8/10**	10/10**
Hepatocytes												
Cytomegaly	0/10	—	0/10	0/10	0/10	10/10**	0/10	—	0/10	0/10	10/10**	10/10**
Degeneration	0/10	—	0/10	0/10	1/10	10/10**	0/10	—	0/10	3/10	10/10**	10/10**
Necrosis	0/10	—	0/10	1/10	1/10	8/10**	0/10	—	0/10	0/10	9/10**	10/10**
Kupffer cells												
Pigmentation	0/10	—	0/10	0/10	0/10	3/10	0/10	—	0/10	0/10	9/10**	10/10**

* $P \leq 0.05$; ** $P \leq 0.01$; —, not examined

Groups of five male and five female F344 rats were dosed intragastrically with furan (>99% pure) in corn oil for 12 days (days 1 through 5, 8 through 12 and at least 2 consecutive dosing days before terminal sacrifice) at doses of 0, 5, 10, 20, 40 or 80 mg/kg bw per day (males) and 0, 10, 20, 40, 80 or 160 mg/kg bw per day (females) starting when they were 47 days old. The rats were weighed at the start of the study and at days 8 and 16. Clinical observations were made twice daily. Complete necropsies were performed on all animals. All rats receiving 80 mg/kg bw or higher died within the first 8 days of the study. There were no other deaths during the study. Body weights of male rats receiving 20 or 40 mg/kg bw per day and female rats receiving 40 mg/kg bw per day were lower than those of control rats at the end of the study. The only clinical findings consistently observed in furan-dosed groups were inactivity or reduced activity. At necropsy, mottled and often enlarged livers were observed in the 20, 40 and 80 mg/kg bw per day male dose groups and the 40, 80 and 160 mg/kg bw per day female dose groups (NTP, 1993).

Groups of 10 male and 10 female F344 rats were dosed intragastrically with furan (>99% pure) in corn oil at doses of 0, 4, 8, 15, 30 and 60 mg/kg bw per day, 5 days/week, for 13 weeks, starting when they were 51 days old. The rats were observed twice daily, and clinical observations were recorded weekly. Body weights were taken at the start of the study and subsequently at weekly intervals. Surviving animals were killed at the end of the 13-week dosing period, and necropsies were performed on all animals. Weights of brain, heart, right kidney, liver, lungs and thymus were taken at necropsy. Complete histopathological examinations were made of all rats in the control groups and those receiving 30 or 60 mg/kg bw per day. Livers from all surviving animals were subjected to histopathological examination.

Nine male rats and four female rats from the highest dose group (60 mg/kg bw per day) died before the end of the study. Reduced activity and occasional irregular breathing were noticed in both male and female rats of the highest dose group. The final mean body weights of rats in the 15 and 30 mg/kg bw per day groups and the remaining female rats in the 60 mg/kg bw per day group were lower than those of the respective controls. Both absolute and relative liver weights were increased in males receiving 30 mg/kg bw per day and in females receiving 15, 30 or 60 mg/kg bw per day, in addition to being increased at the next lower doses (15 mg/kg bw per day and 8 mg/kg bw per day for males and females, respectively), without statistical significance for both parameters. Relative and absolute weights of the right kidney were also increased in female rats receiving 15, 30 or 60 mg/kg bw per day. Decreased thymus weights were also noted in males receiving 30 mg/kg bw per day (both absolute and relative weights) and in females receiving 60 mg/kg bw per day (absolute weights only). Other differences in organ weight measurements were considered secondary to the lower body weights in the dosed rats compared with controls.

Lesions associated with the administration of furan were most pronounced in the liver (Table 2), but the kidney, thymus, testes and ovaries were also affected. The most prominent change was bile duct hyperplasia, which occurred in all furan-dosed groups; the incidence and severity of the lesions showed a dose-related pattern. Cholangiofibrosis was also noted together with the bile duct hyperplasia.

Table 2. Incidences of selected non-neoplastic hepatic lesions in rats in the 13-week NTP (1993) oral study

	Incidence of lesions											
	Males						Females					
	0 mg/kg bw	4 mg/kg bw	8 mg/kg bw	15 mg/kg bw	30 mg/kg bw	60 mg/kg bw	0 mg/kg bw	4 mg/kg bw	8 mg/kg bw	15 mg/kg bw	30 mg/kg bw	60 mg/kg bw
Biliary tract												
Cholangiofibrosis	0/10	4/10*	7/10**	10/10**	10/10**	10/10**	0/10	1/10	7/10**	10/10**	10/10**	9/10**
Hyperplasia	0/10	4/10*	9/10**	10/10**	10/10**	10/10**	0/10	7/10**	10/10**	10/10**	10/10**	9/10**
Hepatocytes												
Cytomegaly	0/10	0/10	0/10	8/10**	10/10**	10/10**	0/10	0/10	0/10	10/10**	10/10**	9/10**
Degeneration	0/10	0/10	7/10**	9/10**	10/10**	10/10**	0/10	0/10	1/10	10/10**	10/10**	10/10**
Necrosis	0/10	0/10	0/10	9/10**	10/10**	10/10**	0/10	0/10	0/10	8/10**	10/10**	10/10**
Hyperplasia, nodular	0/10	0/10	0/10	0/10	10/10**	10/10**	0/10	0/10	0/10	0/10	8/10**	9/10**
Kupffer cells												
Pigmentation	0/10	4/10*	6/10**	10/10**	10/10**	9/10**	0/10	2/10	8/10**	10/10**	10/10**	9/10**

* $P \leq 0.05$; ** $P \leq 0.01$

Lesions of hepatocytes included cytomegaly, degeneration, necrosis and nodular hyperplasia. In addition, greenish-brown pigmentation of Kupffer cells (presumably bile) was noted. The kidneys of nearly all the highest-dose (60 mg/kg bw per day) males and females and two of the 30 mg/kg bw per day females had dilated renal tubules up to approximately twice the normal diameter. Epithelial necrosis of the tubular epithelium was also present in most of the animals of the highest dose group. Atrophy of the testes, ovaries or thymus was observed in many of the male and female rats receiving 60 mg/kg bw per day. These changes were considered in the study report to be a result of general debilitation associated with furan toxicity (NTP, 1993).

A 90-day repeated-dose study was conducted on groups of 12 male and 12 female F344 rats, 5–6 weeks of age, dosed intragastrically with furan (99.9% purity) in corn oil at 0, 0.03, 0.12, 0.5, 2.0 and 8.0 mg/kg bw per day, 5 days/week, starting after a 12-day acclimatization period. Animals were observed for clinical signs twice daily on weekdays and once daily on weekends and holidays. The rats were weighed daily prior to dosing. Food consumption was measured on a weekly basis. At the end of the study, the animals were sacrificed and blood samples were collected from the abdominal aorta for measurement of haematology and clinical biochemistry parameters, including serum testosterone (reported in section 2.2.6) and thyroid hormone analysis. Necropsies were performed, and liver, kidneys, thymus, uterus, ovaries, ventral prostate, seminal vesicles and testes were weighed. Suspensions of thymus cells were prepared for lymphocyte phenotyping. In addition, furan residue analysis was conducted on samples of the median liver lobe. Samples of 26 organs and tissues were preserved for histopathological examination, including, for the liver, samples of the left lateral, median, right lateral and caudate lobes.

Dosing with furan over this dose range had no effect on body weights or food consumption. A dose-related increase in platelet count was noted in both male and female rats, which was statistically significant at 8.0 mg/kg bw per day in males and at 0.5 mg/kg bw per day and higher in females. Slight, dose-related, numerically lower red blood cell counts and haematocrits were noted in the male rats, without statistical significance, which were within the historical control range. Thymic lymphocyte populations shifted towards a decline in $CD4^+/CD8^+$ cells, with a corresponding increase in all the other populations of lymphocytes ($CD4^-/CD8^-$, $CD4^+/CD8^-$, $CD4^-/CD8^+$). Although these changes reflect altered thymocyte maturation, there were no accompanying changes in thymus weight or histology. Dose-related changes in clinical chemistry that achieved statistical significance were noted for numerous parameters related to liver function and included the following: decreases in ALT and AST activities (statistically significant for ALT in both sexes and for AST in females); slightly increased alkaline phosphatase (AP) activity in males only; decreased serum amylase (males, significant linear trend in females); increased serum albumin concentration (both sexes), decreased serum globulin concentration (males), increased albumin/globulin ratio (males) and increased total protein (females); slightly increased total cholesterol (males) and decreased serum triglycerides (both sexes); increased serum thyroxine (males) and triiodothyronine (significant linear trend in both sexes); and increased serum inorganic phosphorus, without notable effects on other electrolytes (both sexes). The concentration of unchanged furan in the liver increased 2-fold, 4-fold and 6-fold at doses of 0.5, 2.0 and 8.0 mg/kg bw per day, respectively.

Absolute liver weights were increased at the highest dose tested, 8.0 mg/kg bw per day, in both sexes. Macroscopic changes were observed in all of the livers of both male and female rats in this group. The caudate and left lateral lobes were primarily affected. The changes consisted of single or multiple small, firm, white and/or yellow nodules on the margins of these lobes; in some cases, the entire caudate lobe was affected, giving it an irregular surface. A greenish-yellowish discoloration of the surface and curling of the margin of the affected areas were also observed. Histological lesions of the liver were observed in both male and female rats (Table 3), starting at a dose of 0.12 mg/kg bw per day.

The severity and number of lesions increased with increasing dose of furan. The caudate lobe of the liver was most severely affected, followed by the left lateral lobe. At 0.12 mg/kg bw per day, rare to occasional apoptosis of hepatocytes, Kupffer cells filled with yellow pigment and microfoci of inflammatory cells were observed in the immediate subcapsular region, which were restricted to the dorsal margin and visceral surface (i.e. closest to the stomach) of the left lateral and caudate lobes. In the next higher dose group, 0.5 mg/kg bw per day, these lesions were noted at increased incidence, with the addition of a layer 1–2 cells thick interior to the subcapsular lesions of hepatocytes with cytomegaly, karyomegaly and dark, homogeneous, slightly basophilic cytoplasm. At 2.0 mg/kg bw per day, the layers of affected hepatocytes were increased up to five cells thick, with increased vacuolation of zone 3 (centrilobular) hepatocytes. In the more severely affected animals of this group, more extensive subcapsular and/or periportal proliferation of oval cells, bile duct hyperplasia, inflammatory cell infiltrate and mild fibrosis were observed. In the 8.0 mg/kg bw per day group, the lesions were similar to those at 2.0 mg/kg bw per day, but were more severe in nature and more extensive in distribution. Of note were the greater oval cell and bile duct epithelial cell hyperplasia, bile duct metaplasia and interstitial fibrosis, which formed the macroscopic raised lesions and replaced areas of normal hepatic parenchyma. The hyperplastic ductular structures varied in appearance from normal bile ducts, intestinal gland-like structures with flattened to tall columnar epithelium and interspersed mucus-producing cells, to cystic structures of variable size and shape, containing eosinophilic material and cell debris. These areas with their variable fibrotic and glandular components were classified by Elmore & Sirica (1993) (section 2.2.6) as primary hepatic intestinal-type adenocarcinomas (or intestinal-type cholangiocarcinomas) rather than hepatic cholangiocarcinomas. The lesions were focal (subcapsular and periportal), bridging portal tracts, in this way forming islands of normal hepatic parenchyma or completely replacing hepatic parenchyma. The caudate lobe was the most severely affected, but variable and considerable areas of the left lateral lobe were also affected, and the lesions were also evident in the margin of the right lateral lobe in a few animals (Gill et al., 2009).

In a separate report, immunohistochemical investigation of the livers from the above Gill et al. (2009) study revealed increased staining for proliferating cell nuclear antigen-positive nuclei in males at 0.12 mg/kg bw per day and in females at 0.5 mg/kg bw per day. Immunohistochemical-positive glutathione *S*-transferase-pi (GST-P) foci were found in males at 0.12 mg/kg bw per day and in females at 2.0 mg/kg bw per day (Curran et al., 2009).

Table 3. Incidences of selected non-neoplastic hepatic lesions in rats in the 13-week Gill et al. (2009) oral study

	Incidence of lesions											
	Males						Females					
	0 mg/kg bw	0.03 mg/kg bw	0.12 mg/kg bw	0.5 mg/kg bw	2.0 mg/kg bw	8.0 mg/kg bw	0 mg/kg bw	0.03 mg/kg bw	0.12 mg/kg bw	0.5 mg/kg bw	2.0 mg/kg bw	8.0 mg/kg bw
Biliary tract												
Cholangiofibrosis	0/12	0/12	0/12	0/12	2/12	12/12	0/12	0/12	0/12	0/12	0/12	11/12
Hyperplasia	0/12	0/12	0/12	0/12	6/12	12/12	0/12	0/12	0/12	0/12	1/12	12/12
Hepatocytes												
Apoptosis	3/12	4/12	6/12	12/12	12/12	12/12	2/12	0/12	1/12	10/12	12/12	12/12
Cytoplasmic basophilia	0/12	0/12	0/12	8/12	12/12	12/12	0/12	0/12	0/12	0/12	12/12	11/12
Anisokaryosis	0/12	0/12	0/12	0/12	10/12	12/12	0/12	0/12	0/12	0/12	12/12	10/12
Kupffer cells												
Pigmentation	1/12	1/12	5/12	10/12	12/12	12/12	2/12	0/12	3/12	12/12	12/12	12/12

2.2.3 Long-term studies of toxicity and carcinogenicity

(a) Mice

(i) Oral route

Groups of 50 male and 50 female B6C3F1 mice that were on average 58 days of age at the start of the study were administered intragastric doses of furan in corn oil at 0, 8 or 15 mg/kg bw per day, 5 days/week, for 2 years. The mean body weights of both male and female high-dose mice were lower than those of the controls. From week 80 to the end of the study, survival of the male mice in both dose groups and the female mice of the high dose group were lower than in the control group due to moribund condition associated with liver neoplasms.

The strongest treatment-related effects were observed in the liver. The incidences of hepatocellular adenomas and carcinomas were significantly increased in mice dosed with furan (Table 4).

Table 4. Incidences of neoplastic liver lesions in mice in the 2-year NTP (1993) study

	Incidence of lesions		
	0 mg/kg bw	8 mg/kg bw	15 mg/kg bw
Male			
2-year survival	33/50	17/50	16/50
Hepatocellular adenoma			
Overall rates[a]	20/50	33/50	42/50
Terminal rates[b]	15/33	12/17	16/16
Hepatocellular carcinoma			
Overall rates	7/50	32/50	34/50
Terminal rates	0/7	7/17	7/16
Hepatocellular adenoma, hepatocellular carcinoma or hepatocellular adenoma and hepatocellular carcinoma[c]			
Overall rates	26/50	44/50	50/50
Terminal rates	15/33	16/17	16/16
Female			
2-year survival	29/50	25/50	2/50
Hepatocellular adenoma			
Overall rates	5/50	31/50	48/50
Terminal rates	5/29	17/25	2/2

Table 4 (contd)

	Incidence of lesions		
	0 mg/kg bw	8 mg/kg bw	15 mg/kg bw
Hepatocellular carcinoma			
Overall rates	2/50	7/50	27/50
Terminal rates	1/29	3/25	1/2
Hepatocellular adenoma, hepatocellular carcinoma or hepatocellular adenoma and hepatocellular carcinoma[d]			
Overall rates	7/50	34/50	50/50
Terminal rates	6/29	18/25	2/2

[a] Number of tumour-bearing animals/number of animals.
[b] Observed incidence at terminal necropsy.
[c] Historical incidence in 2-year NTP corn oil gavage studies for vehicle control groups (mean ± standard deviation): 210/599 (35.1% ± 11.0%), range 14–52%.
[d] Historical incidence: 60/597 (10.1% ± 4.3%), range 2–16%.

The incidences of numerous non-neoplastic hepatocellular lesions were also increased in dosed mice (Table 5). These lesions included hepatocyte cytomegaly, degeneration and necrosis; multifocal hyperplasia; cytoplasmic vacuolization; and biliary tract dilatation, fibrosis, hyperplasia and inflammation (NTP, 1993).

Table 5. Incidences of non-neoplastic liver lesions in mice in the 2-year NTP (1993) study

	Incidence of lesions		
	0 mg/kg bw	8 mg/kg bw	15 mg/kg bw
Male			
Cytoplasmic vacuolization	8/50	24/50	36/50
Focal hyperplasia	1/50	44/50	49/50
Mixed cell cellular infiltration	2/50	23/50	29/50
Bile duct dilatation	0/50	0/50	6/50
Biliary tract			
Chronic inflammation	0/50	44/50	49/50
Fibrosis	0/50	45/50	49/50
Hyperplasia	0/51	46/50	49/50

Table 5 (contd)

	Incidence of lesions		
	0 mg/kg bw	8 mg/kg bw	15 mg/kg bw
Hepatocyte			
Cytomegaly	8/50	45/50	50/50
Degeneration	0/50	43/50	43/50
Necrosis	2/50	39/50	41/50
Kupffer cell pigmentation	2/50	43/50	50/50
Parenchymal focal atrophy	1/50	45/50	50/50
Female			
Cytoplasmic vacuolization	6/50	29/50	36/50
Focal hyperplasia	0/50	48/50	48/50
Lymphoid hyperplasia	27/50	33/50	42/50
Mixed cell cellular infiltration	8/50	23/50	32/50
Bile duct dilatation	0/50	1/50	11/50
Biliary tract			
Chronic inflammation	2/50	48/50	50/50
Fibrosis	0/50	47/50	50/50
Hyperplasia	0/50	47/50	50/50
Hepatocyte			
Cytomegaly	0/50	48/50	50/50
Degeneration	0/50	47/50	48/50
Necrosis	0/50	44/50	47/50
Kupffer cell pigmentation	5/50	48/50	50/50
Parenchymal focal atrophy	0/50	48/50	50/50

Male and female high-dose mice also had increased incidences of haematopoietic cell proliferation in the spleen, possibly secondary to hepatic inflammation (NTP, 1993).

Groups of female B6C3F1 mice (numbers in parentheses) 5–6 weeks of age were dosed intragastrically with furan (>99% pure) in corn oil (5 ml/kg bw) at 0 (50), 0.5 (100), 1.0 (100), 2.0 (50), 4.0 (50) and 8.0 (50) mg/kg bw per day, 5 days/week, for 2 years. At study start, group mean body weight was similar in all groups (10.1–19.6 g). Up to week 89, body weight was comparable in control animals and mice dosed with furan. At weeks 89 and 101, there was a significant decrease in mean group body weight in mice dosed with 8.0 mg/kg bw per day compared with

the mean body weight of control mice. At the terminal necropsy, mean group body weight was 42.5 g in control mice and 29.8, 39.7, 38.5, 42.4 and 38.6 g in mice dosed with 0.5, 1.0, 2.0, 4.0 and 8.0 mg/kg bw per day, respectively. In mice dosed with 2.0 and 8.0 mg/kg bw per day, there were significant decreases in body weight gain from study initiation to the terminal necropsy; the decrease in body weight gain was not dose dependent (data not shown). There were no differences in the percentage of mice that survived to the scheduled terminal necropsy in any group dosed with furan relative to control mice. At the terminal necropsy, the per cent mortality in control mice was 34% compared with 31%, 40%, 19%, 36% and 46% in mice exposed to 0.5, 1.0, 2.0, 4.0 and 8.0 mg/kg bw per day, respectively.

Complete macroscopic examinations were performed at necropsy. Livers were removed and weighed. Representative sections from the left, median, right posterior, right anterior and caudate lobes of the liver and sections of macrosopic lesions greater than 0.5 cm in size along with accompanying normal tissue were fixed in 10% neutral buffered formalin. At the terminal necropsy, mean group absolute and relative liver weights of mice dosed with 0.5, 1.0 and 2.0 mg/kg bw per day were comparable with those of control mice. In mice dosed with 4.0 and 8.0 mg/kg bw per day, absolute and relative liver weights were increased ($P = 0.007$ and 0.004, respectively) (data not shown).

The incidence of liver nodules at the terminal necropsy was significantly increased to 60% (15/25) and 100% (22/22) in mice exposed to 4.0 and 8.0 mg/kg bw per day ($P < 0.001$), respectively. The incidence of macroscopic liver nodules was 8% (2/25) in control mice and 17–20% in remaining groups of dosed mice. In animals that were necropsied before the terminal necropsy date, a significant increase in the incidence of visible liver nodules was found in mice exposed to 4.0 mg/kg bw per day (54%; 6/11) and 8.0 mg/kg bw per day (71%; 12/17) ($P = 0.032$ and 0.002, respectively). The incidence of gross liver nodules in control mice was 9% (1/11). Macroscopic nodules at the terminal necropsy were grouped by size. The largest nodules (≥0.5 mm) were present in mice exposed to 8.0 mg/kg bw per day (data not shown).

There was a dose-dependent trend in the incidence of foci of altered hepatocytes, adenomas, carcinomas, and adenomas or carcinomas ($P < 0.001$ for all) (Table 6). Increased incidences of hepatocellular foci developed in mice dosed with furan at 4.0 and 8.0 mg/kg bw per day ($P = 0.030$ and <0.001, respectively) compared with control mice, but there was no difference in the percentage of foci that were classified as basophilic, eosinophilic or clear-cell foci. Significant increases were found in the incidences of hepatocellular adenomas in groups dosed with 4.0 and 8.0 mg/kg bw per day ($P = 0.018$ and <0.001, respectively), carcinomas in mice dosed with 8.0 mg/kg bw per day ($P < 0.001$), and adenomas or carcinomas in those dosed with 4.0 and 8.0 mg/kg bw per day ($P = 0.015$ and <0.001, respectively) (Table 6). Only animals exposed to furan at 4.0 and 8.0 mg/kg bw per day had both adenomas and carcinomas (1/3 [3%] and 7/39 [18%], $P = 0.007$, respectively). There was a significant increase in the mean number of adenomas per animal in mice administered furan at dose levels of 4.0 and 8.0 mg/kg bw per day ($P = 0.015$ and <0.001, respectively) and adenomas or carcinomas at 8.0 mg/kg bw per day ($P = 0.002$) (data not shown). In general, latency period or time to first tumour was decreased with increasing dose of furan.

Table 6. Preneoplastic and neoplastic liver lesions in female mice exposed to furan in the 2-year Moser et al. (2009) study

	Incidence of lesions					
	Control	0.5 mg/kg bw	1.0 mg/kg bw	2.0 mg/kg bw	4.0 mg/kg bw	8.0 mg/kg bw
	n = 36	*n* = 72	*n* = 53	*n* = 41	*n* = 36	*n* = 39
Number of altered hepatocytes						
Overall rate[a]	3/36 (8%)	5/72 (7%)	4/53 (8%)	5/41 (12%)	10/36 (28%)	19/39 (49%)
Adjusted rate[b] (%)	10	7	9	14	32	55
Terminal rate[c]	3/25 (12%)	5/55 (9%)	4/36 (11%)	5/31 (16%)	10/25 (49%)	13/22 (59%)
Latency	725	725	725	725	725	590
Poly-3 test[d]		$P = 0.511$	$P = 0.620$	$P = 0.445$	$P = 0.030$[f]	$P < 0.001$[f]
Dose trend[e]	$P < 0.001$					
Hepatocellular adenoma						
Overall rate	3/36 (8%)	4/72 (6%)	4/53 (8%)	4/41 (10%)	11/36 (31%)	25/39 (64%)
Adjusted rate (%)	10	6	9	11	34	72
Terminal rate	3/25 (12%)	3/55 (5%)	4/36 (11%)	3/31 (10%)	8/25 (32%)	18/22 (82%)
Latency	725	713	725	525	511	489
Poly-3 test		$P = 0.407$	$P = 0.620$	$P = 0.596$	$P = 0.018$[f]	$P < 0.001$[f]
Dose trend	$P < 0.001$					

Table 6 (contd)

	Incidence of lesions					
	Control	0.5 mg/kg bw	1.0 mg/kg bw	2.0 mg/kg bw	4.0 mg/kg bw	8.0 mg/kg bw
	n = 36	*n* = 72	*n* = 53	*n* = 41	*n* = 36	*n* = 39
Hepatocellular carcinoma						
Overall rate	0/36 (0%)	4/72 (6%)	2/53 (4%)	1/41 (2%)	2/36 (6%)	11/39 (28%)
Adjusted rate (%)	0	6	4	3	6	33
Terminal rate	0/25 (0%)	2/55 (4%)	1/36 (3%)	1/31 (3%)	1/25 (4%)	6/22 (27%)
Latency	NA[g]	439	566	726	511	652
Poly-3 test		*P* = 0.208	*P* = 0.322	*P* = 0.532	*P* = 0.244	*P* < 0.001[f]
Dose trend	*P* < 0.001					
Hepatocellular adenoma or carcinoma						
Overall rate	3/36 (8%)	8/72 (11%)	6/53 (11%)	5/41 (12%)	12/36 (33%)	29/39 (74%)
Adjusted rate (%)	10	12	13	14	35	63
Terminal rate	3/25 (12%)	5/55 (9%)	5/36 (14%)	4/31 (13%)	9/25 (36%)	14/22 (64%)
Latency	725	439	566	525	511	489
Poly-3 test		*P* = 0.516	*P* = 0.449	*P* = 0.455	*P* = 0.015[f]	*P* < 0.001[f]
Dose trend	*P* < 0.001					

Table 6 (contd)

n, number examined microscopically

[a] Number of tumour-bearing animals/total number of animals evaluated microscopically.

[b] Number of tumour-bearing animals/effective number of animals, i.e. number of animals alive at first occurrence of this tumour type in any of the groups.

[c] Observed incidence at terminal kill.

[d] Beneath the control incidence are the *P*-values associated with the trend test. Beneath the dosed group incidence are the *P*-values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard tumours in animals dying prior to terminal kill as non-fatal.

[e] Dose-related trend as calculated by a modified Cochran-Armitage linear trend test.

[f] Significant increase compared with control mice ($P < 0.05$).

[g] Not applicable; no tumours in animal group.

Among mice examined at the terminal necropsy at 24 months, the incidence of foci, adenomas, carcinomas, and adenomas or carcinomas was similar in controls and animals exposed to furan at 0.5, 1.0 and 2.0 mg/kg bw per day. Increases at 4.0 mg/kg bw per day in the prevalence of foci ($P = 0.030$), adenomas ($P = 0.018$), and adenomas or carcinomas ($P = 0.015$) and at 8.0 mg/kg bw per day in the prevalence of foci, adenomas, carcinomas, and adenomas or carcinomas ($P < 0.001$ for all) were observed (Table 6) (Moser et al., 2009).

(ii) Intraperitoneal route

Groups of B6C3F1 infant mice were dosed intraperitoneally with furan in tricaprylin by two different regimens: either 400 mg/kg bw (the lethal dose for 10% of mice, or LD_{10}) was administered once on postnatal day 15 to a group of 215 mice, or 200 mg/kg bw (the lethal dose for 5% of mice, or LD_5) was administered 6 times, once each on postnatal days 3, 6, 9, 12, 15 and 18, to a group of 78 mice. Corresponding control groups, 52 and 79 mice, respectively, received tricaprylin only. Mice were randomly selected and killed at 16 time points between 28 and 95 weeks after dosing for collection of benign and malignant liver tumours. The total number of mice killed was 215 in the 400 mg/kg bw furan group, 52 in the single-dose vehicle (tricaprylin) group, 78 in the 6 × 200 mg/kg bw furan group and 79 in the 6 × vehicle (tricaprylin) group.

Body weights were consistently slightly greater, starting at week 14, in mice administered furan at 200 mg/kg bw 6 times than in mice in the other three groups (data not shown). At necropsy, livers were excised, weighed and examined for visible lesions. For any tumour larger than 0.5 cm in diameter, a representative section was submitted for histological analysis. There was no statistically significant increased incidence in hepatocellular tumours in mice given a single preweaning dose of 400 mg/kg bw over the single-dose vehicle controls during the study (Table 7). The overall frequency of hepatocytic neoplasia was 27% in the 400 mg/kg bw furan group compared with 15% in the single-dose controls, and the overall liver tumour multiplicity was increased. In mice given six neonatal furan doses of 200 mg/kg bw, there was an increased incidence of hepatocellular neoplasms compared with the multiple-dose vehicle control group as well as the 400 mg/kg bw furan group. The tumour multiplicity was 1.1 in the 6 × 200 mg/kg bw furan group and 0.41 in the six-dose vehicle control group (Johansson et al., 1997).

(b) Rats

(i) Oral route

Groups of 70 male and 70 female F344 rats that were on average 51 days of age at the start of the study were administered intragastric doses of furan in corn oil at 0, 2, 4 or 8 mg/kg bw per day, 5 days/week, for 2 years. After dosing for 9 and 15 months, 10 rats from each group were necropsied. Mean body weights of male rats that received 8 mg/kg bw per day were lower than those of controls from approximately week 73 to the end of the study. Survival of male and female rats that received 8 mg/kg bw per day was lower than that of controls from approximately week 85 to the end of the study as a result of moribund condition associated with liver and biliary tract neoplasms and mononuclear cell leukaemia.

Table 7. Liver tumour incidence and multiplicity in the Johansson et al. (1997) study

Treatment	Overall incidence			Mean (± SE) liver tumour (adenomas and carcinomas) multiplicity	
	Adenomas	Carcinomas	Adenomas and carcinomas	All mice	Tumour-bearing mice
Vehicle control	5/52	3/52	8/52	0.17 (± 0.06)	1.12 (± 0.12)
(1 × tricaprylin)	(9.6%)	(5.8%)	(15.4%)		
Furan—1 dose	47/215	16/215	58/215	0.40 (± 0.05)	1.50 (± 0.10)
(1 × 400 mg/kg bw)	(21.9%)	(7.4%)	(27.0%)		
Vehicle control	14/79	8/79	21/79	0.41 (± 0.10)	1.52 (± 0.22)
(6 × tricaprylin)	(17.7%)	(10.1%)	(26.6%)		
Furan—6 doses	31/78*	14/78**	40/78*	1.09 (± 0.18)***	2.12 (±0.25)†
(6 × 200 mg/kg bw)	(39.7%)	(17.9%)	(51.3%)		

SE, standard error

* $P = 0.001$ compared with 6 × tricaprylin control and 1 × 400 mg/kg bw furan group. The statistical analysis was based on logistic regression.

** $P = 0.001$ compared with 1 × 400 mg/kg bw furan group. The statistical analysis was based on logistic regression.

*** $P < 0.001$ compared with 6 × tricaprylin control and 1 × 400 mg/kg bw furan group. The statistical analysis was based on Poisson regression of tumour counts on days on study.

† $P = 0.013$ compared with 6 × tricaprylin control and $P < 0.001$ compared with 1 × 400 mg/kg bw furan group. The statistical analysis was based on Poisson regression of tumour counts on days on study.

The most marked dosing-related effects were observed in the liver (Table 8). In all dosed groups, cholangiocarcinoma of the liver was observed in many rats of each sex at the 9- and 15-month interim evaluations. At 2 years, incidences of cholangiocarcinoma were 86%, 96% and 98% in furan groups. Incidences of hepatocellular adenomas or carcinomas (combined) were increased in both male and female rats after 2 years of furan exposure, and incidences of hepatocellular adenomas were significantly increased in female rats. The incidences of mononuclear cell leukaemia were increased in male and female rats that received 4 or 8 mg/kg bw per day, and the incidence in the 8 mg/kg bw per day groups of each sex exceeded the historical control ranges for corn oil gavage studies (NTP, 1993).

Non-neoplastic liver lesions, including biliary tract fibrosis, hyperplasia and chronic inflammation and hepatocyte cytomegaly, cytoplasmic vacuolization, degeneration, nodular hyperplasia and necrosis were abundant in all rats administered furan (Table 9). Also, at 9 months, male rats at all doses showed an

Table 8. Incidences of neoplastic lesions in F344/N rats in the 2-year NTP (1993) study

	Incidence of lesions			
	Vehicle control	2 mg/kg bw	4 mg/kg bw	8 mg/kg bw
Male				
2-year survival	33/50	28/50	26/50	16/50
Cholangiocarcinoma				
Overall rates[a]	0/50	43/50	48/50	49/50
Terminal rates[b]	0/33	28/28	26/26	16/16
Hepatocellular adenoma				
Overall rates	1/50	4/50	18/50	27/50
Terminal rates	0/33	4/28	13/26	8/16
Hepatocellular carcinoma				
Overall rates	0/50	1/50	6/50	18/50
Terminal rates	0/33	1/28	5/26	5/16
Hepatocellular adenoma, hepatocellular carcinoma or hepatocellular adenoma and hepatocellular carcinoma[c]				
Overall rates	1/50	5/50	22/50	35/50
Terminal rates	0/33	5/28	16/26	10/16
Mononuclear cell leukaemia[d]				
Overall rates	8/50	11/50	17/50	25/50
Terminal rates	4/33	6/28	8/26	8/16
Female				
2-year survival	34/50	32/50	28/50	19/50
Cholangiocarcinoma				
Overall rates	0/50	49/50	50/50	48/50
Terminal rates	0/34	32/32	28/28	19/19
Hepatocellular adenoma				
Overall rates	0/50	2/50	4/50	7/50
Terminal rates	0/34	1/32	3/28	6/19
Hepatocellular carcinoma				
Overall rates	0/50	0/50	0/50	1/50
Terminal rates[e]	—	—	—	—

Table 8 (contd)

	Incidence of lesions			
	Vehicle control	2 mg/kg bw	4 mg/kg bw	8 mg/kg bw
Hepatocellular adenoma, hepatocellular carcinoma or hepatocellular adenoma and hepatocellular carcinoma[f]				
Overall rates	0/50	2/50	4/50	8/50
Terminal rates	0/34	1/32	3/28	6/19
Mononuclear cell leukaemia[g]				
Overall rates	8/50	9/50	17/50	21/50
Terminal rates	1/34	5/32	9/28	7/19

[a] Number of tumour-bearing animals/number of animals necropsied.
[b] Observed incidence at terminal kill.
[c] Historical incidence for 2-year NTP corn oil gavage studies for vehicle control groups (mean ± standard deviation): 19/770 (2.5% ± 2.8%), range 0–10%.
[d] Historical incidence: 164/770 (21.3% ± 8.9%), range 4–38%.
[e] Data not provided.
[f] Historical incidence: 9/770 (1.2% ± 2.7%), range 0–10%.
[g] Historical incidence: 206/770 (26.8% ± 7.0%), range 16–38%.

increased incidence of bone marrow hyperplasia and congestion/haematopoietic cell proliferation of the spleen, along with lower red blood cell count, haemoglobin level and haematocrit, at the high dose. Females showed bone marrow hyperplasia at all doses and splenic haematopoietic cell proliferation in the spleen at the intermediate and high doses. Haematology was not altered.

A 2-year stop-exposure study was conducted in male F344/N rats 47–61 days of age at the start of the study. Groups of 50 rats were dosed intragastrically either with corn oil (vehicle control) or with furan in corn oil at 30 mg/kg bw per day, 5 days/week, for 13 weeks and then maintained for up to 90 additional weeks without further furan administration. Groups of 10 animals were necropsied at the end of the 13-week dosing phase, after 9 months and after 15 months. Further, 6 animals that died in a moribund condition between the 9- and 15-month terminations and 14 animals that died between the 15-month termination and the end of the 2-year study were evaluated. Histopathological evaluation was performed on the livers from all 13-week interim rats; on livers and kidneys from all 9-month interim rats; and on livers, kidneys and lungs from all 15-month interim rats.

The incidences of neoplastic and non-neoplastic liver lesions are presented in Table 10. No liver lesions were found in the vehicle control animals that were examined at the 9- and 15-month interim evaluations; a single control male had cytoplasmic vacuolization of hepatocytes. In dosed rats, no liver neoplasms were

Table 9. Incidences of non-neoplastic liver lesions in F344/N rats in the 2-year NTP (1993) study

	Incidence of lesions			
	Vehicle control	2 mg/kg bw	4 mg/kg bw	8 mg/kg bw
Male				
Multilocular cyst	0/50	1/50	17/50	24/50
Biliary tract				
Chronic focal inflammation	0/50	44/50	48/50	49/50
Cyst	0/50	44/50	47/50	49/50
Focal fibrosis	0/50	44/50	48/50	49/50
Focal hyperplasia	0/50	44/50	48/50	49/50
Metaplasia	0/50	44/50	48/50	49/50
Hepatocyte				
Cytomegaly	0/50	35/50	46/50	49/50
Cytoplasmic vacuolization	1/50	39/50	45/50	49/50
Focal degeneration	0/50	33/50	46/50	49/50
Focal hyperplasia	0/50	30/50	46/50	49/50
Focal necrosis	0/50	32/50	46/50	49/50
Kupffer cell focal pigmentation	0/50	44/50	48/50	49/50
Female				
Multilocular cyst	0/50	6/50	2/50	12/50
Biliary tract				
Chronic focal inflammation	0/50	49/50	50/50	49/50
Cyst	0/50	49/50	50/50	46/50
Focal fibrosis	0/50	49/50	50/50	49/50
Focal hyperplasia	0/50	49/50	50/50	49/50
Metaplasia	0/50	49/50	50/50	49/50
Hepatocyte				
Cytomegaly	0/50	44/50	50/50	49/50
Cytoplasmic vacuolization	0/50	43/50	49/50	47/50
Focal degeneration	0/50	35/50	49/50	47/50
Focal hyperplasia	0/50	32/50	47/50	46/50
Focal necrosis	0/50	18/50	46/50	47/50
Kupffer cell focal pigmentation	0/50	49/50	50/50	48/50

Table 10. Neoplastic and non-neoplastic liver lesions in male rats in the NTP (1993) stop-exposure study

	Incidence of lesions				
	13 weeks	9 months	9–15 months	15 months	After 15 months
Number of animals examined	10	10	6[a]	10	14[a]
Neoplasms					
Cholangiocarcinoma	0/10	10/10	6/6	10/10	14/14
Hepatocellular carcinoma	0/10	0/10	0/6	2/10	4/14
Non-neoplastic lesions					
Biliary tract					
Fibrosis, multifocal	10/10	10/10	6/6	10/10	14/14
Hyperplasia, multifocal	10/10	10/10	6/6	10/10	14/14
Inflammation, chronic, multifocal	0/10	10/10	6/6	10/10	14/14
Cysts, multiple	0/10	10/10	4/6	10/10	14/14
Hepatocytes					
Cytomegaly	10/10	10/10	6/6	10/10	14/14
Degeneration, multifocal	10/10	10/10	6/6	10/10	14/14
Hyperplasia, nodular, multifocal	10/10	10/10	6/6	10/10	14/14
Necrosis, multifocal	10/10	10/10	6/6	10/10	14/14
Vacuolization, cytoplasmic	10/10	10/10	6/6	10/10	14/14
Kupffer cells					
Pigmentation, multifocal	10/10	10/10	6/6	10/10	14/14

[a] Animals died in a moribund condition between the interim evaluations.

present at the end of dosing, but by 9 months, all of the rats (40/40) that survived had cholangiocarcinomas; and in 15% (6/40) of the rats, hepatocellular carcinomas developed, of which the first two were observed at the 15-month evaluation (NTP, 1993).

Two separate experiments were performed in young adult male F344 rats (160–190 g). In the first experiment, 12 rats were dosed intragastrically with furan (>99% pure) in corn oil at 30 mg/kg bw per day, 5 days/week, for 13 weeks. In the second experiment, three groups of 10 rats each were dosed with furan at 30 mg/kg bw per day as above, for 6, 9 or 12 weeks. In both experiments, the rats

were sacrificed at 16 months after initiation of the furan dosing. A complete necropsy was performed on each rat. Samples of all resulting hepatic and non-hepatic tumours, as well as random non-tumorous portions of each liver lobe, were processed for histopathology. The furan-dosed rats in both experiments exhibited very high incidences of hepatic adenocarcinoma at 16 months. In the first experiment, this incidence was 90%, and in the second experiment, it was 75% and 71% for rats that received furan for 9 and 12 weeks, respectively. Even when furan was administered for only 6 weeks, 44% of the dosed rats were found to have a hepatic adenocarcinoma at the end of the 16-month experimental period (Elmore & Sirica, 1993) (see section 2.2.3 for more details).

2.2.4 Genotoxicity

The results of genotoxicity studies with furan are summarized in Table 11, and those with its reactive metabolite BDA (see section 2.1.2) in Table 12. In the in vitro testing, an S9 fraction was used for bioactivation in many studies. The S9 was usually obtained from Aroclor 1254–induced rodent liver. Aroclor 1254 has been reported to be a poor inducer of CYP2E1 (Borlak & Thum, 2001; Escobar-Garcia et al., 2001; Mori et al., 2001), the CYP that is mainly responsible for the metabolism of furan to BDA. Thus, negative results could be due to deficient metabolism.

Table 11. Results of furan genotoxicity testing

End-point	Test system	Concentration/dose	Result	Reference
In vitro DNA alteration				
Strand breaks	Mouse L5178Y $tk^{+/-}$ lymphoma cells	0.225–3.1 mmol/l, −S9 activation	Negative	Kellert et al. (2008b)
DNA repair	Primary rat hepatocytes	Up to 681 µg/ml, −S9 activation	Negative	Wilson et al. (1992)
DNA repair	Primary mouse hepatocytes	Up to 681 µg/ml, −S9 activation	Negative	Wilson et al. (1992)
Bacterial gene mutation				
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537	33–10 000 µg/plate, +S9 activation	Negative	Mortelmans et al. (1986); NTP (1993)
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33–10 000 µg/plate, −S9 activation	Negative	Mortelmans et al. (1986); NTP (1993)

Table 11 (contd)

End-point	Test system	Concentration/ dose	Result	Reference
In vitro mammalian gene mutation assays				
Thymidine kinase gene mutation	Mouse L5178Y lymphoma cells	125–3800 µg/ml, −S9 activation	Positive	McGregor et al. (1988); NTP (1993)
Thymidine kinase gene mutation	Mouse L5178Y $tk^{+/-}$ lymphoma cells	0.225–3.1 mmol/l, −S9 activation	Negative	Kellert et al. (2008b)
In vitro chromosomal alterations in mammalian cells				
SCEs	CHO cells	Up to 13 614 µg/ml, −S9 activation	Negative	Stich et al. (1981)
SCEs	CHO cells	Up to 13 614 µg/ml, +S9 activation	Positive	Stich et al. (1981)
SCEs	CHO cells	1.6–160 µg/ml, −S9 activation	Positive	NTP (1993)
SCEs	CHO cells	16–500 µg/ml, +S9 activation	Positive	NTP (1993)
Chromosomal aberrations	CHO cells	Up to 13 614 µg/ml, −S9 activation	Negative	Stich et al. (1981)
Chromosomal aberrations	CHO cells	Up to 13 614 µg/ml, +S9 activation	Positive	Stich et al. (1981)
Chromosomal aberrations	CHO cells	100–500 µg/ml, −S9 activation	Positive	NTP (1993)
Chromosomal aberrations	CHO cells	160–1000 µg/ml, +S9 activation	Positive	NTP (1993)
Micronucleus induction	Human lymphocyte cultures	136–1361 µg/ml, −S9 activation	Negative	Durling, Svensson & Abramsson-Zetterberg (2007)
Micronucleus induction	Human lymphocyte cultures	136–1361 µg/ml, +S9 activation	Negative	Durling, Svensson & Abramsson-Zetterberg (2007)

Table 11 (contd)

End-point	Test system	Concentration/ dose	Result	Reference
Micronucleus induction	Mouse L5178Y $tk^{+/-}$ lymphoma cells	0.225–3.1 mmol/l, −S9 activation	Negative	Kellert et al. (2008b)
In vivo DNA alteration				
DNA repair	Mouse hepatocytes	10–200 mg/kg bw intragastrically for 2 h; 10–100 mg/kg intragastrically for 12 h	Negative	Wilson et al. (1992)
DNA repair	Rat hepatocytes	5–100 mg/kg bw intragastrically for 2 h and 12 h	Negative	Wilson et al. (1992)
Strand breaks	B6C3F1 mouse splenocytes	2–15 mg/kg bw intragastrically for 4 weeks, 24 h after last dosing; 15–250 mg/kg bw intragastrically, single dose for 3 h	Negative	Leopardi et al. (2010)
In vivo chromosomal alterations				
SCEs	B6C3F1 mouse bone marrow	87.5–350 mg/kg bw for 17 h; 62.5–250 mg/kg bw for 36 h	Positive in 250 mg/kg bw for 36 h	NTP (1993)
SCEs	F344 rat bone marrow	0.1–2.0 mg/kg bw for 5 days or 4 weeks	Negative	Mosesso (2009)
Chromosomal aberrations	B6C3F1 mouse bone marrow	87.5–350 mg/kg bw for 23 h; 25–100 mg/kg bw for 48 h	Negative	NTP (1993)
Chromosomal aberrations	F344 rat bone marrow	0.1–2.0 mg/kg bw for 5 days or 4 weeks	Negative	Mosesso (2009)
Micronuclei	B6C3F1 mouse splenocytes	2–15 mg/kg bw for 4 weeks, 24 h after last dosing	Positive	Leopardi et al. (2010)
Micronuclei	B6C3F1 mouse splenocytes	15–250 mg/kg bw single dose for 3 h	Statistically insignificant trend	Leopardi et al. (2010)

Table 11 (contd)

End-point	Test system	Concentration/dose	Result	Reference
Micronuclei	Balb/C mouse erythrocytes	50–300 mg/kg bw intraperitoneally; 150 and 225 mg/kg bw subcutaneously	Negative	Durling, Svensson & Abramsson-Zetterberg (2007)
Micronuclei	CBA mouse erythrocytes	225 mg/kg bw intraperitoneally	Negative	Durling, Svensson & Abramsson-Zetterberg (2007)
In vivo mutagenicity				
Sex-linked recessive lethal mutations	*Drosophila melanogaster*	10 000 ppm feed	Negative	NTP (1993); Foureman et al. (1994)
		25 000 ppm injection	Negative	

CHO, Chinese hamster ovary; ppm, parts per million; SCE, sister chromatid exchange

Table 12. Results of BDA genotoxicity testing

End-point	Test system	Concentration	Result	Reference
In vitro DNA alteration				
Strand breaks	CHO-K1 cells	0.17–1.5 mmol/l	Positive	Marinari, Ferro & Sciaba (1984)
Strand breaks	Mouse L5178Y $tk^{+/-}$ lymphoma cells	6.3–50 µmol/l	Positive	Kellert et al. (2008b)
Bacterial gene mutation				
Reverse mutation	*Salmonella typhimurium* TA97, TA98, TA100, TA102, TA104	1.4–4.3 µmol/plate (95.3–292.7 µg/plate)	Positive in TA104, negative in the other strains	Peterson, Naruko & Predecki (2000)

Table 12 (contd)

End-point	Test system	Concentration	Result	Reference
Gene mutation in mammalian cells				
Thymidine kinase gene mutation	Mouse L5178Y $tk^{+/-}$ lymphoma cells	6.3–50 µmol/l	Positive	Kellert et al. (2008b)
Chromosomal alterations in mammalian cells				
Micronucleus	Mouse L5178Y $tk^{+/-}$ lymphoma cells	6.3–50 µmol/l	Negative	Kellert et al. (2008b)

(a) In vitro assays

(i) Furan

Furan was tested without addition of S9 in vitro in L5178Y $tk^{+/-}$ mouse lymphoma cells for the induction of strand breaks using the alkaline comet assay at the nominal concentrations of 0, 0.25, 0.5, 1, 2 and 4 mmol/l. The "effective" concentrations of furan (i.e. corrected for losses through evaporation and diffusion into the dish material) were estimated to have been 0, 0.225, 0.45, 0.9, 1.6 and 3.1 mmol/l, respectively. Furan did not affect DNA migration under the test conditions. Furan was non-toxic to the cells (Kellert et al., 2008b).

Unscheduled DNA synthesis (UDS) as an indicator for DNA repair was measured in vitro in primary rat and mouse hepatocytes after exposure to furan concentrations up to 10 mmol/l (681 µg/ml). No increased UDS was found in these experiments (data not shown); however, the authors ascribe only limited importance to these findings because of the rapid loss of furan (due to its high vapour pressure) from the medium under these conditions (Wilson et al., 1992).

In mutagenicity assays using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537, furan was found not to induce reverse mutations with and without preincubation with Aroclor 1254–induced rat or hamster liver S9 at concentrations of 33, 100, 333, 1000, 3333 and 10 000 µg/plate (Mortelmans et al., 1986; NTP, 1993).

In mouse L5178Y lymphoma cells, furan induced trifluorothymidine resistance at concentrations of 1139 µg/ml (2600 µg/ml in a batch of less respondent cells) and above without S9 activation; it was not tested with S9 (McGregor et al., 1988; NTP, 1993).

Furan was tested in the L5178Y $tk^{+/-}$ mouse lymphoma gene mutation assay at concentrations of 0, 0.25, 0.5, 1, 2 and 4 mmol/l (0, 0.225, 0.45, 0.9, 1.6 and 3.1 mmol/l effective concentrations, respectively) without addition of S9. Furan gave negative results for the induction of mutations under the test conditions (Kellert et al., 2008b).

The induction of sister chromatid exchanges (SCEs) of furan at concentrations of up to 200 mmol/l (13 614 µg/ml) was measured in Chinese hamster ovary (CHO) cells with and without the addition of S9 mixture derived from Aroclor 1254–induced rat liver. Furan induced SCEs only in the presence of S9. The data are presented in a graph only and do not allow the quantification of the response; no lowest effective dose is indicated. However, a dose-dependent response was observed (Stich et al., 1981). Interestingly, some of the concentrations applied are far above the concentrations reported in other studies as being too cytotoxic to evaluate.

In CHO cells, furan was tested for the induction of SCEs at concentrations of 1.6, 5, 16, 50 and 160 µg/ml without activation with Aroclor 1254–induced rat liver S9 and at concentrations of 16, 50, 160 and 500 µg/ml with S9 activation. With S9 activation, SCEs were induced only at the highest concentration of 500 µg/ml (NTP, 1993). Without S9 activation, in one trial, a significant induction of SCEs was measured over the whole range of concentrations, whereas in another trial in the same study, in a batch of less respondent cells also without S9 activation, furan induced a significant amount of SCEs only at the concentration of 160 µg/ml and not at the lower concentrations tested (see above).

The induction of chromosomal aberrations at furan concentrations of up to 200 mmol/l (13 614 µg/ml) was measured in CHO cells with and without the addition of S9 mixture derived from Aroclor 1254–induced rat liver. Furan induced chromosomal aberrations only in the presence of S9. The data are presented in a graph only and do not allow the quantification of the response; no lowest effective dose is indicated. However, a dose-dependent response was observed (Stich et al., 1981).

Furan induced chromosomal aberrations at concentrations of 100, 160, 300 and 500 µg/ml without S9 activation. With S9 activation, furan was tested at the concentrations of 160, 300, 500 and 1000 µg/ml; furan significantly increased chromosomal aberrations at the high concentrations of 500 and 1000 µg/ml (NTP, 1993). These results differ from the results of the aforementioned study by Stich et al. (1981) in which furan was found to have clastogenic effects in CHO cells only in the presence of S9.

In the NTP (1993) report, it is mentioned that the evaluation of the high doses tested in the CHO assays was limited by toxicity. It has also been suggested that the SCEs and chromatid aberrations could be due to lysosome breakdown secondary to cytotoxicity and not from direct chemical action on DNA (NTP, 1993).

The formation of micronuclei was tested in human lymphocyte cultures with furan at concentrations up to 100 mmol/l (6.807 mg/ml). Micronuclei were not formed to a statistically significant extent in the presence or absence of S9 mixture. Concentrations of 25 mmol/l (1702 µg/ml) and above could not be evaluated due to furan's cytotoxicity (Durling, Svensson & Abramsson-Zetterberg, 2007).

Furan was tested without addition of S9 in vitro in L5178Y $tk^{+/-}$ mouse lymphoma cells for the induction of micronuclei at concentrations of 0, 0.25, 0.5, 1, 2 and 4 mmol/l (0, 0.225, 0.45, 0.9, 1.6 and 3.1 mmol/l effective concentrations, respectively). It gave negative results under the test conditions over the whole dose range tested (Kellert et al., 2008b).

(ii) BDA

BDA, also referred to as malealdehyde, was tested for induction of single-strand breaks in CHO-K1 cells. Concentrations of 0.17, 0.5 and 1.5 mmol/l were applied for 90 min, and breaks were assessed by alkaline elution. BDA increased elution at 0.5 and 1.5 mmol/l in a dose-related manner (Marinari, Ferro & Sciaba, 1984).

BDA was tested in the comet assay in L5178Y $tk^{+/-}$ mouse lymphoma cells for the induction of DNA damage at concentrations of 0, 6.3, 12.5, 25 and 50 µmol/l. BDA slightly increased (1.6-fold) the DNA migration at 25 µmol/l. At 50 µmol/l, BDA was toxic (viability was reduced to below 50%) (Kellert et al., 2008b).

BDA was tested in several *Salmonella typhimurium* strains for the induction of reverse mutation. BDA tested positive in strain TA104 (which is sensitive to aldehydes) and negative in strains TA97, TA98, TA100 and TA102. The induction of reverse mutations was dose dependent (1000 ± 180 revertants/µmol per plate). In strain TA104, the concentrations 1.4, 1.7, 2.1, 2.9 and 4.3 µmol/plate were tested, of which 2.9 and 4.3 µmol/plate were toxic. Incubation of BDA with GSH prior to the addition of the cells prevented the toxic and genotoxic activity of BDA. BDA was not mutagenic in the other strains at non-toxic concentrations (Peterson, Naruko & Predecki, 2000).

BDA was tested in the mouse lymphoma thymidine kinase gene mutation assay at concentrations of 0, 6.3, 12.5, 25 and 50 µmol/l. BDA produced a dose-dependent induction of thymidine kinase gene mutations (Kellert et al., 2008b).

BDA was tested for the induction of micronuclei in mouse L5178Y lymphoma cells at concentrations of 0, 6.3, 12.5, 25 and 50 µmol/l. It did not induce micronucleus formation under the conditions tested (Kellert et al., 2008b).

(b) In vivo assays

DNA repair activity in liver after furan dosing of male B6C3F1/CrlBR mice and male F344/CrlBR rats was determined by measurement of UDS in isolated hepatocytes. Mice were dosed intragastrically with furan at 10, 50 or 200 mg/kg bw 2 h before hepatocyte isolation or with 10, 50 or 100 mg/kg bw 12 h before hepatocyte isolation. Rats were dosed intragastrically with 5, 30 or 100 mg/kg bw 2 and 12 h before hepatocyte isolation. No increase in UDS in cultured hepatocytes was observed under any of the tested conditions (Wilson et al., 1992).

To investigate the genotoxicity of furan in vivo, splenocytes of male B6C3F1 mice after acute and repeated dosing were evaluated for DNA damage using the alkaline comet assay and immunofluorescence detection of foci of phosphorylated histone H2AX. The spleen was selected as target tissue due to the capability of quiescent splenocytes to accumulate DNA damage induced by repeated-dose exposure. Animals in the repeated-dose exposure groups were dosed with furan in corn oil at 2, 4, 8 or 15 mg/kg bw per day by intragastric administration for 4 weeks; animals in the acute studies received furan in corn oil at 15, 100 or 250 mg/kg bw per day by intragastric administration. The rats were sacrificed 24 h after the last dosing in the repeated-dose groups and 3 h after dosing in the acute groups. To

increase the sensitivity of the assay, splenocytes of furan-dosed mice in the repeated-dose exposure study were additionally incubated with a DNA repair inhibitor. Furan at 8 and 15 mg/kg bw increased γ-H2Ax foci, but did not produce DNA damage (Leopardi et al., 2010).

To assess the frequency of SCEs in bone marrow after furan dosing, male B6C3F1 mice (five animals per dose group) were injected intraperitoneally with 87.5, 175 and 350 mg/kg bw furan in corn oil (0.4 ml) 23 h before harvest and 25, 50 and 100 mg/kg bw 48 h before sacrifice and harvest. Solvent control mice received equivalent injections of corn oil only; the mice in the positive control study received injections of dimethylbenzanthracene at 100 mg/kg bw. No increase of SCEs was observed in the bone marrow of mice dosed with furan (NTP, 1993).

The induction of SCEs was studied in rat bone marrow of male F344/N rats dosed orally with furan for 5 and 28 days and 28 days followed by 2-week recovery at doses of 0.1, 0.5 and 2.0 mg/kg bw. A slight reduction in mitotic index was observed at the highest dose level after 5 days and 28 days plus recovery. No effect on SCEs was found (Mosesso, 2009).

The frequency of chromosomal aberrations in bone marrow of male B6C3F1 mice (10 per group) after furan dosing was assessed in the NTP (1993) bioassay. The mice were dosed intraperitoneally with furan at 87.5, 175 and 350 mg/kg bw 17 h before sacrifice and harvest and at 62.5, 125 and 250 mg/kg bw 36 h before sacrifice and harvest. The 36 h dosing experiments were repeated. Only at the highest dose tested (i.e. 250 mg/kg bw) in the 36 h experiments was an increase in aberrations observed (similar to the positive control response) (NTP, 1993).

The induction of chromosomal aberrations was studied in rat bone marrow of male F344/N rats dosed orally with furan for 5 and 28 days and 28 days followed by a 2-week recovery at doses of 0.1, 0.5 and 2.0 mg/kg bw. A slight reduction in mitotic index was observed at the highest dose level after 5 days and 28 days plus recovery. No effect on chromosomal aberrations was found (Mosesso, 2009).

The induction of micronuclei was also evaluated after both repeated and single dosing. Animals in the repeated-dose exposure groups were dosed with furan in corn oil at 2, 4, 8 or 15 mg/kg bw per day by intragastric administration for 4 weeks; animals in the acute studies received furan in corn oil at 15, 100 or 250 mg/kg bw per day by intragastric administration. The rats were sacrificed 24 h after the last dosing in the repeated-dose groups and 3 h after dosing in the acute dose groups. In the repeated-dose exposure studies, furan significantly induced the formation of micronuclei at doses of 4 mg/kg bw per day ($P < 0.005$) and above ($P < 0.001$). In the acute study, a statistically insignificant dose-related trend of increased micronuclei induction was observed (Leopardi et al., 2010).

The induction of micronuclei in erythrocytes of male Balb/C and CBA mice after intraperitoneal and subcutaneous administration was investigated. Furan was administered by intraperitoneal injection to Balb/C mice at 0, 50, 75, 90, 110, 125, 150, 175, 200, 250 and 300 mg/kg bw and to CBA mice at 225 mg/kg bw. Further Balb/C mice were dosed with furan at 0, 150 and 275 mg/kg by subcutaneous injection. No increased level of micronucleated erythrocytes was detected in any of the in vivo experiments (Durling, Svensson & Abramsson-Zetterberg, 2007).

Furan was tested for mutagenicity in the *Drosophila melanogaster* sex-linked recessive lethal assay. When fed at 10 000 parts per million (ppm) or injected at 25 000 ppm, it produced 21% and 28% mortality, respectively, but no mutagenicity (NTP, 1993; Foureman et al., 1994).

2.2.5 Reproductive and developmental toxicity

No reproductive or developmental toxicity studies were found. Hormonal effects are reported in section 2.2.6.

2.2.6 Special studies

(a) Covalent binding

(i) Furan

Intragastric administration to male F344 rats of a single 8 mg/kg bw dose of diluted [2,5-^{14}C]furan, representing 370 kBq/kg bw, resulted in binding to protein in liver and to lesser degrees in kidney and blood. The level of binding increased with repeated 8 mg/kg bw doses over 8 days. In this study, DNA binding was not found in liver at 24 h after intragastric dosing of [2,5-^{14}C]furan at 8 mg/kg bw, either diluted (370 kBq/kg bw) or undiluted (1.95 GBq/kg bw) (Burka, Washburn & Irwin, 1991).

In vitro incubation of rat liver microsomes with [^{14}C]furan in the presence of NADPH resulted in the covalent binding of furan-derived radioactivity to microsomal protein. Compared with microsomes from untreated rats, a 2- to 3-fold increase in binding was observed with microsomes from PB-treated rats, and a 4- to 5-fold increase was observed with microsomes from rats pretreated with imidazole or PY. Covalent binding was reduced with microsomes from rats pretreated with β-NF (Parmar & Burka, 1993).

In an in vitro study, an oligonucleotide synthesized with nucleosides linked via the 2′ amino group on the ribose to furan formed interchain crosslinks upon chemical oxidation (Halila et al., 2005).

(ii) BDA

In order to characterize the chemistry by which the reactive microsomal metabolite of furan, BDA, could alkylate protein, the products formed upon reaction of this unsaturated dialdehyde with *N*-acetyl-L-lysine, *N*-acetyl-L-cysteine and GSH were investigated. BDA reacted rapidly and completely with amino acid residues to form pyrrole and pyrrolin-2-one derivatives. The authors speculated that the ease with which BDA crosslinks amino acids suggests that pyrrole–thiol crosslinks may be involved in the toxicity observed following furan exposure (Chen, Hecht & Peterson, 1997).

BDA reacts in vitro with 2′-deoxynucleosides (Gingipalli & Dedon, 2001; Byrns, Predecki & Peterson, 2002). Reaction of BDA with 2′-deoxycytidine (dCyd) was greater than that with 2′-deoxyadenosine (dAdo) or 2′-deoxyguanosine (dGuo). The reaction with dCyd formed a stable oxadiazabicycloctaimine adduct

(Gingipalli & Dedon, 2001). BDA was reacted with nucleosides dCyd, dGuo and dAdo, and the reaction products that formed across a pH range of 6.0–8.0 at 37 °C were characterized. All of the nucleosides formed reasonably stable adducts with BDA. Thymidine did not react with BDA (Byrns, Predecki & Peterson, 2002).

It was found subsequently that although the adducts formed with dCyd were relatively stable, the initial adducts formed with dGuo and dAdo readily undergo dehydration to form substituted etheno adducts. This rearrangement exposes a reactive aldehyde, which has significant implications for the toxicological properties of furan, such as induction of point mutations, as has been observed with other etheno-dAdo and etheno-dGuo adducts; and the generation of DNA–DNA and/or DNA–protein crosslinks, as has been observed with a number of α,β-unsaturated aldehydes and dialdehydes (Byrns, Vu & Peterson, 2004). These investigators then developed an assay for the detection of BDA-derived DNA adducts. The dCyd and dAdo adducts were detected in digests of DNA treated with nanomole per litre concentrations of BDA as well as in DNA isolated from *Salmonella typhimurium* strain TA104 that had been treated with mutagenic concentrations of BDA used in a mutagenicity assay. There was a dose-dependent increase in the concentration of dCyd and dAdo adducts that roughly corresponded to the previously published mutagenicity results (see Peterson, Naruko & Predecki, 2000 in section 2.2.4) (Byrns et al., 2006).

To investigate the capability of BDA to induce DNA crosslinking, the alkaline elution assay for DNA strand breaks was performed. CHO cells that were labelled for 24 h with 0.9 mBq/ml [2-^{14}C]thymidine were lysed, and elution from cellulose ester filters was measured. The average retention of DNA from control cells was 0.96 ± 0.02. Cells were incubated for 90 min with BDA at 0.17, 0.5 and 1.5 mmol/l, with and without methyl methanesulfonate (MMS) at 1 mmol/l for 60 min prior to lysis to induce single-strand DNA breaks, which increase the kinetics of DNA elution. MMS reduced retention to 0.55 ± 10. BDA, like several other aldehydes, was found to reduce the effect of MMS on the kinetics of DNA elution in a dose-dependent manner, which indicates that it induced either interstrand or DNA–protein crosslinks (Marinari, Ferro & Sciaba, 1984).

Induction of crosslinking was assessed in L5178Y tk$^{+/-}$ mouse lymphoma cells using the alkaline comet assay. Gamma irradiation–induced DNA migration was not inhibited by concentrations of BDA up to 100 µmol/l, in contrast to glutaraldehyde, which produced inhibition at greater than 10 µmol/l (Kellert et al., 2008b).

In splenocytes isolated from B6C3F1 mice 5–6 weeks of age, furan administered intragastrically at 8 and 15 mg/kg bw per day, 5 days/week, for 4 weeks did not result in DNA crosslinks measured in a comet assay in which slides were irradiated with gamma rays prior to cell lysis to induce DNA breaks (data not shown) (Leopardi et al., 2010).

(b) Effects on gene expression

Male Sprague-Dawley rats were administered furan (>99% pure) intragastrically at 4 or 40 mg/kg bw in corn oil for 1, 3, 7 or 14 days. Liver was collected and frozen in liquid nitrogen, and slices were taken for histopathology. Total ribonucleic acid (RNA) was isolated, and from it, complementary DNA (cDNA)

targets were prepared by oligo(dT)-primed polymerization. Fluorescently labelled targets were hybridized to cDNA chips on which sequence-verified rat clone cDNAs were applied. Hepatocellular degeneration was observed as early as the first day in all high-dose rats and was present in all animals at this dose at the 3- and 7-day time points and in one animal at the 14-day time point. Inflammation occurred in both dose groups at all time points. Biliary hyperplasia and cholangiofibrosis appeared by day 7 in the high dose group.

Principal component analysis of gene expression was suggestive of dose- and time-dependent responses in alterations. Focus was placed on genes altered at later time points when cholangiofibrosis was present. Mild to subtle alterations were studied, as it was considered that a subset of these could represent diluted gene expression originating in minority cell types such as bile duct cells. Among such slightly elevated gene expressions were those of *O*-acetyl disialoganglioside synthase and stellate cell activation-associated protein, whose products could contribute to fibrosis. The gene expression changes were assumed to be indicative of alterations in transcription, but changes in cell types present could contribute. Similar gene expression changes were found in different lobes of the liver, but the magnitude of induction/repression was higher in the right lobe, followed by the caudate (Hamadeh et al., 2004).

Liver was obtained from the F344 rats at all dose levels (0, 0.03, 0.12, 0.5, 2.0 and 8.0 mgkg bw per day) in the Gill et al. (2009) 90-day intragastric study (section 2.2.2) for study of gene expression. Total RNA was isolated, and cDNA was generated. In high-dose males, CYP1A1 and GST-P transcript levels were increased, whereas other CYPs were reduced. Females at this dose had increased expression of CYP3A11, GST-P, cyclin D1, p21 and cOX2, whereas some CYPs were reduced. Proliferating cell nuclear antigen expression was increased in males at 0.03 mg/kg bw and in females at 0.12 mgkg bw, and p21 was increased in both at the low dose. Thus, a no-observed-effect level (NOEL) was not established (Curran et al., 2009).

(c) Gene mutations in induced neoplasms

Liver tumours induced in infant male B6C3F1 mice by preweaning administration of furan, either as a single dose of 400 mg/kg bw or as six doses of 200 mg/kg bw, were collected for analysis of Ha-*ras*-1 (*Hras1*) mutations. After polymerase chain reaction (PCR) amplification of isolated DNA, slot-blot oligonucleotide hybridization was used to screen for mutations at known mutational hot-spots in the first three exons of *Hras1*. The relative frequency of *Hras1* activation was found in 82% of the 28 tumours analysed from the single-dose group and in 32% of the 28 tumours analysed from the multiple-dose group (Johansson et al., 1997).

(d) Hepatotoxicity

(i) Mice

Female B6C3F1 mice were dosed for 3 weeks under the NTP (1993) bioassay conditions (i.e. intragastric administration for 5 days/week) with either vehicle (corn oil) or furan (containing 0.025% butylated hydroxytoluene [BHT] as a

polymerization inhibitor) at doses of 4, 8 or 15 mg/kg bw per day. In a second experiment, female mice were given corn oil, furan at 15 mg/kg bw per day or furan at 15 mg/kg bw per day plus intraperitoneal injections of ABT, an inhibitor of CYP, at 10 mg/kg bw per day, 7 days/week, starting 5 days prior to the start of treatment with furan. Control and furan-treated groups consisted of 6–11 animals. Seven days prior to necropsy, mice were implanted with mini-osmotic pumps to dispense 5-bromo-2′-deoxyuridine (BrdU) for incorporation into replicating cells. Immediately prior to sacrifice, blood samples were collected for determination of ALT, sorbitol dehydrogenase (SDH) and total bile acids. At necropsy, the liver was excised, weighed and examined for visible lesions. Representative liver samples were taken from each liver lobe and prepared for histopathological evaluation, measurement of apoptosis and immunohistochemical measurement for determination of BrdU LI. As no significant differences were observed between the lobes of control and furan-dosed mice, measurements of labelling and apoptotic indices were based on the median lobe.

There was no evidence of clinical signs, mortality or changes in body weight related to dosing of furan. Whereas liver weight was not affected by furan dosing in the first experiment, in the second experiment, liver weight was slightly increased (by about 1.2-fold) compared with controls in mice dosed with furan at 15 mg/kg bw per day, both with and without ABT. The serum activities of both ALT and SDH were increased by furan dosing at 8 and 15 mg/kg bw per day, and concentrations of serum total bile acids were statistically significantly increased at 15 mg/kg bw per day. The only pathological finding in liver associated with furan dosing was a dose-related increase in minor subcapsular inflammation with minimal histological evidence of occasional necrosis in mice receiving 8 and 15 mg/kg bw per day. Co-treatment with ABT counteracted the observed furan-induced inflammation and necrosis as well as the elevation in serum ALT, SDH and total bile acids. Mice exposed to ABT displayed minimal to mild hepatocyte vacuolization, which was compatible with lipid accumulation; this observation was absent from control mice or mice that received furan alone. The apoptotic index determined from morphological evaluation of apoptotic cells and bodies in H&E-stained liver sections from the first experiment was statistically significantly increased above control values in the 8 and 15 mg/kg bw per day groups from the first experiment (6- and 15-fold, respectively) and in the 15 mg/kg bw per day group from the second experiment (approximately 8-fold). A significant dose-dependent increase in the hepatocyte LI relative to control values was noted at all dose levels of furan in the first experiment (1.3-, 1.6- and 1.7-fold at doses of 4, 8 and 15 mg/kg bw per day, respectively) and in the mice dosed with furan alone at 15 mg/kg bw per day in the second experiment (1.4-fold). Concurrent exposure to ABT statistically significantly reduced the effects of furan at 15 mg/kg bw per day on labelling and apoptotic indices—in the case of LI, significantly below control values (Fransson-Steen et al., 1997).

Groups of 15 female B6C3F1 mice were dosed intragastrically with furan (>99% pure) in corn oil at doses of 0, 0.5, 1.0, 2.0, 4.0 and 8.0 mg/kg bw per day, 5 days/week, for 3 weeks. Blood samples were collected at sacrifice for determination of ALT activity. For quantification of LI of hepatocytes in the left lobe

of the liver, 10 mice at each dose level received BrdU in the drinking-water for 5 days prior to sacrifice. Complete macroscopic examinations were conducted at necropsy. The liver was removed for organ weight determination, and representative sections of the left, median, right posterior, right anterior and caudate lobes of the liver and sections of macroscopic lesions were prepared using H&E stain and evaluated histologically for the presence of liver cytotoxicity. For LI analysis, a section of liver was processed for immunohistochemical assessment of BrdU incorporation. All animals survived to terminal sacrifice, and no dosing-related clinical observations relevant to liver were noted. Dosing with furan for 3 weeks had no effect on body weight or body weight gain. Doses of 1.0 mg/kg bw per day and higher resulted in a dose-related increase in ALT activity. Relative liver weights and LI were increased at the highest dose, 8.0 mg/kg bw per day, compared with untreated controls. An increased incidence of hepatic cytotoxicity, characterized by hepatic parenchymal degeneration and inflammation and/or subcapsular hepatocyte necrosis and inflammation localized on visceral surfaces of the liver in contact with the forestomach, was noted at doses of 1.0 mg/kg bw per day and higher. A histopathological qualitative assessment of hepatocyte proliferation was performed based on increases in mitotic figures, which were noted in 1/10, 3/10, 4/10 and 5/10 mice in 1.0, 2.0, 4.0 and 8.0 mg/kg bw per day dosed groups, respectively (Moser et al., 2009).

Male B6C3F1/CrlBR mice, six per group, were dosed intragastrically 5 days/week with furan in corn oil (purity >99%) at 15 mg/kg bw per day, the highest dose used in the NTP (1993) 2-year assay. The animals were sacrificed after 1, 3 or 6 weeks of dosing with furan or after 1 or 6 weeks receiving the corn oil vehicle alone. Six days prior to sacrifice, osmotic pumps containing [^{3}H]thymidine were implanted subcutaneously for determination of LI. At sacrifice, the livers were collected and sections prepared for histoautoradiographic determination of LI in hepatocyte nuclei and histopathological evaluation. After 1 week of administration of a dose of 15 mg/kg bw per day, 5 days/week, only an occasional necrotic cell or small cluster of inflammatory cells was noted. In some animals, the visceral surface of the liver was irregular, with small foci of necrotic cells and early fibrosis. At the 3-week sacrifice, the subcapsular parenchyma of the visceral surface had necrosis and inflammation that was more extensive than at 1 week. Otherwise, the liver was normal with the exception of some perinuclear vacuolization of the cytoplasm. At the 6-week sacrifice, a centrilobular pattern of hepatocyte vacuolization was evident. No bile duct proliferation was noted. A marked increase in hepatocyte LI was noted at week 1, which decreased linearly through 6 weeks (LIs of 25.1, 12.0 and 3.2 for weeks 1, 3 and 6, respectively, compared with 0.41 and 0.89 for controls at 1 and 6 weeks, respectively). Although the proliferative response decreased over the 6 weeks, it was still significantly elevated over control values (Wilson et al., 1992).

(ii) Rats

To investigate the early cellular changes in liver associated with furan-induced cholangiocarcinogenesis, young adult male F344 rats were dosed intragastrically with furan (>99% pure) in corn oil once a day, 5 days/week, for 2–3 weeks at doses of 0, 15, 30, 45 and 60 mg/kg bw per day. During the course of the

dosing period, rats receiving furan exhibited a dose-dependent decrease in their average body weight gain. Survival was decreased at 45 mg/kg bw per day, and all rats dosed with 60 mg/kg bw per day were dead by the end of the second week. The most conspicuous feature observed in the livers of animals dosed with 30 mg/kg bw per day and greater was a cholangiofibrosis characterized by the presence of bile ductular hyperplasia, intestinal metaplasia and fibrosis. This lesion was found to be almost exclusively localized to the caudate liver lobe, whereas the other lobes primarily showed cirrhosis. Both the hyperplastic bile ductular epithelial cells and the intestinal-like epithelial cells in the cholangiofibrotic areas selectively exhibited a strongly positive immunohistochemical staining for cytokeratin 19, normally found in intrahepatic biliary epithelial cells. However, in contrast to the hyperplastic bile ductules, electron microscopy of the metaplastic intestinal glands revealed them to be composed mostly of columnar epithelial cells. A precursor relationship between the bile ductular structures and the metaplastic cholangiofibrotic areas was proposed (Elmore & Sirica, 1991).

Male and female F344/CrIBR rats, six per group, were dosed intragastrically 5 days/week with furan (purity >99%) in corn oil at 8 mg/kg bw per day, the highest dose used in the NTP (1993) assay. The animals were sacrificed after 1, 3 or 6 weeks of dosing with furan or after 1 or 6 weeks receiving the corn oil vehicle alone. Six days prior to sacrifice, osmotic pumps containing [^{3}H]thymidine were implanted subcutaneously for determination of LI. Immediately prior to sacrifice, a blood sample was collected for determination of AST, ALT and LDH. At sacrifice, the livers were collected and sections prepared for histoautoradiographic determination of LI in hepatocyte nuclei and histopathological evaluation. After 1 week of administration of furan at 8 mg/kg bw per day, 5 days/week, subcapsular focal areas of inflammatory cell infiltrate were observed on the visceral surface of the liver. Several small foci of inflammatory cells were found scattered randomly in the hepatic parenchyma. While centrilobular hepatocytes were vacuolated, the periportal cells were hypereosinophilic. At the 3- and 6-week sacrifices, a prominent pattern of visceral surface lesions and hypereosinophilic periportal hepatocytes was evident. Bile duct hyperplasia accompanied by peribiliary fibrosis was observed in the liver parenchyma. In some cases, inflammatory cells from the subcapsular visceral surface lesion extended into the parenchyma, where they surrounded a small number of degenerated and/or necrotic hepatocytes. The subcapsular inflammation was often associated with a light brown pigment in macrophages within the inflammatory zone. Mild to moderate bile duct proliferation within this zone was evident in some sections. By 6 weeks of furan dosing, cholangiofibrosis was detected in subcapsular areas of the left or caudate lobes of the liver. Signs of metaplasia localized within these cholangiofibrotic areas were observed in two rats as ducts composed of columnar cells with basal nuclei. These cells resembled intestinal cells and have been referred to variously as intestinal cell metaplasia, bile duct hyperplasia and cholangiofibrosis (Elmore & Sirica, 1991) (see section 2.2.2). Evaluation of these ducts in liver sections that had been processed for autoradiography revealed that often the nuclei of ductal cells incorporated thymidine and were therefore actively dividing. This surface lesion was qualitatively similar to, but less severe and less advanced than, the cholangiohepatitis described in a 90-day gavage study with 30 mg/kg bw per day in male F344 rats (NTP, 1993).

Hepatocyte proliferation in the livers of male and female rats was significantly elevated above control values at all time points examined. In furan-dosed males, the LI was 3.2, 9.2 and 6.5 at weeks 1, 3 and 6, respectively, compared with control values of 0.08 and 0.29 at weeks 1 and 6, respectively. In the dosed females, the LI was 11.7, 9.2 and 14.4 at weeks 1, 3 and 6, respectively, compared with control values of 0.77 and 0.75 at weeks 1 and 6, respectively. No significant increases in plasma enzyme levels were observed (Wilson et al., 1992).

To determine if the hepatic tumours induced within rat liver after long-term furan dosing could be correlated in terms of both their cellular composition and their liver lobe sites of origin with the small-intestinal metaplasia and cholangiofibrosis that occurs early and essentially within the right and caudate liver lobes of rats following short-term chronic exposures to furan (Elmore & Sirica, 1991), two separate experiments were performed in young adult male F344 rats weighing 160–190 g. In the first experiment, 12 rats were dosed intragastrically with furan (>99% pure) in corn oil at 30 mg/kg bw per day, 5 days/week, for 13 weeks. In the second experiment, three groups of 10 rats each were dosed with furan at 30 mg/kg bw per day, as above, for 6, 9 or 12 weeks. In both experiments, the rats were sacrificed at 16 months after initiation of the furan dosing. Complete necropsies were performed on each rat, with particular attention being paid to the liver lobe distribution of the hepatic tumours. Samples of all resulting hepatic and non-hepatic tumours as well as random non-tumorous portions of each liver lobe were processed for histopathology.

The furan-dosed rats in both experiments exhibited very high incidences of hepatic adenocarcinoma at 16 months. In the first experiment, this incidence was 90%, and in the second experiment, it was 75% and 71% for rats that received furan for 9 and 12 weeks, respectively. Even when furan was administered for only 6 weeks, 44% of the dosed rats were found to have a hepatic adenocarcinoma at the end of the 16-month experimental period. The hepatic adenocarcinomas that developed in the furan-dosed rats were preferentially localized to the right/caudate liver lobes. The epithelial glandular structures within the adenocarcinomas were characterized by their abundant mucin production. These glandular structures were also found to exhibit a homogeneous pattern of staining for cytokeratin 19, similar to the early developing cholangiofibrosis (Elmore & Sirica, 1991). Twenty-six of the twenty-seven hepatic adenocarcinomas exhibited evidence of small-intestinal differentiation, as reflected by the presence of goblet cells, Paneth cells and serotonin-positive neuroendocrine cells. The authors concluded that the small-intestinal metaplasia and subsequent cholangiofibrosis developing early in the right/caudate liver lobes of furan-dosed rats do not simply reflect reactive changes, but strongly correlate with the high incidences of intestinal-type primary hepatic adenocarcinoma that occurs in the right/caudate liver lobes of rats after long-term dosing with furan (Elmore & Sirica, 1993).

Male Sprague-Dawley rats 6–8 weeks of age were dosed intragastrically with furan (>99% pure) at 30 mg/kg bw per day, 5 days/week, for periods up to 3 months. Animals ($n = 5$) were removed at periodic intervals beginning 8 h after the first furan dose was administered, with the caudate, left, right and median liver lobes processed for histopathological analysis. In addition, livers from at least three

animals per time point were assessed by immunohistochemistry for a variety of markers related to cellular proliferation, apoptosis, cell communication and DNA oxidative damage. Tissue samples in which cholangiofibrosis was evident (caudate and right liver lobes) were also processed for gene expression analysis (messenger ribonucleic acid [mRNA] quantification) by real-time PCR.

Within 8 h after the initial furan dosing, hepatocyte necrosis and apoptosis were evident in most liver lobes, whereas after 3 days of furan dosing, hepatocytes within the parenchymal region were mostly restored by compensatory hepatocyte proliferation. By 7 days of furan exposure, focal proliferation of biliary ducts into areas of hepatocellular damage, with no or limited compensatory repair, was evident, which correlated with the subsequent incidence and location of cholangiofibrosis (by day 12). Hepatic areas of significant CYP2E1 expression (centrilobular), as determined by immunostaining, also correlated with furan-induced cellular necrosis and apoptosis. Furan dosing also caused a significant increase in 8-oxo-2′-deoxyguanine in necrotic centilobular and subcapsular liver areas. At the end of the dosing period or after 1 month of recovery, increased staining for 8-oxo-2′-deoxyguanine was still evident in cholangiofibrotic lesions as well as in hepatocytes near focal areas of inflammation. Gene expression was assessed in the cholangiofibrotic lesions that persisted 1 month after termination of dosing. In total, 135 genes were determined to be upregulated (greater than 2-fold increase compared with controls), including a number of genes associated with cellular/oxidative stress, transformation, growth arrest, DNA damage and proliferation. Cholangiofibrotic lesions, associated with a marked inflammatory reaction, were found to persist and continue to expand following 1 month after termination of furan dosing. The authors suggested that chronic indirect DNA damage from oxygen radicals, as evidenced by DNA oxidation and the continued expression of genes associated with oxidative stress in areas of cholangiofibrosis, ultimately contributes to the progression and development of cholangiocarcinomas (Hickling et al., 2010a).

Additional analysis of liver and cholangiofibrotic areas was conducted at the same time intervals as previously described (Hickling et al., 2010a) for identification of fibroblast populations (hepatic, vascular and biliary) and the determination of various growth factors and cell-specific markers (i.e. epithelia, dendritic cells, leukocytes). Following furan-induced centrilobular parenchymal damage and subsequent compensatory repair, proliferation of ductular biliary cells was also evident, extending beyond the portal tracts into the liver parenchyma. Altered differentiation of the biliary epithelium into cells expressing a hepatocyte phenotype was also observed. In areas of more extensive damage, however, cells with an intestinal metaplastic phenotype also developed following 12 days of furan dosing. These cells exhibited increased expression of the mesenchymal-epithelial transition growth factor and proliferating cell nuclear antigen, markers for active cellular proliferation and invasive growth. Reconstruction by serial sectioning of cholangio-fibrotic areas showed that these proliferating cells with an intestinal metaplasia phenotype were an integral part of the biliary ductal system. The authors proposed that this process of abnormal hepatocyte differentiation during the repair process following high-dose furan administration leads eventually to aberrant biliary duct proliferation and, eventually, cholangiofibrosis (Hickling et al., 2010b).

(e) Hormonal activity/effects

Serum and testes were obtained from the F344 rats at all dose levels (0, 0.03, 0.12, 0.5, 2.0 and 8.0 mg/kg bw per day) in the Gill et al. (2009) 90-day intragastric study (section 2.2.2) for analysis of the following parameters: serum testosterone levels, intratesticular testosterone levels, serum luteinizing hormone (LH), mRNA expression of the testicular LH receptor, testicular cholesterol side-chain cleavage enzyme, testicular benzodiazepine receptor and testicular steroidogenic acute regulatory (StAR) protein levels. Serum testosterone levels increased with dose, achieving statistical significance at 2.0 and 8.0 mg/kg bw per day (elevations of 2.5- and 3-fold, respectively, from control). All doses of furan resulted in significantly increased intratesticular testosterone levels. Significantly decreased LH levels were observed in rats dosed with furan at 2.0 and 8.0 mg/kg bw per day. The mRNA expression levels for the LH receptor, cholesterol side-chain cleavage enzyme and peripheral dibenzodiazepine receptor in rat testes preparations were not significantly affected by furan dosing. In contrast, the mRNA expression levels of StAR protein were significantly increased following furan dosing, with all doses resulting in significant increases. This study indicates that furan does not disrupt testosterone synthesis by affecting the rate of its formation from cholesterol or by altering sensitivity of the testicular LH receptor. As the functional capacity of Leydig cells to produce testosterone was not compromised, the biological significance of the changes observed remains uncertain (Cooke, 2009).

2.3 Observations in humans

A search for pertinent publications on clinical observations and epidemiological studies of the potential health effects of exposure to furan was conducted using various search engines to query multiple databases, including PubMed, Google, Wiley Interscience, Elsevier and SpringerLink.com. No relevant publications were identified, consistent with the observation of Heppner & Schlatter (2007), who noted, in their review of furan, that “there are no data from human studies.”

2.3.1 Biomarker studies

Plasma and urine samples were obtained from 100 healthy volunteers (49 men and 51 women) between the ages of 30 and 70 following a 12 h fast. The participants consumed a normal Korean diet and did not regularly consume large amounts of furan-containing food items, listed by the authors as smoked salmon, powdered milk, apple juice, spaghetti, potatoes, peas, coffee or canned or jarred food. The length and timing of the collection period for urine were not indicated. Blood samples were analysed for concentrations of glucose, total, high-density and low-density lipoprotein cholesterol, triglycerides and enzyme activities of ALT, AST and gamma-glutamyl transpeptidase (GGT). Unmetabolized furan was detected in urine samples from 56 of the participants (31 males, 25 females). Of these, only 15 samples exceeded the limit of quantification (LOQ) of 1.0 µg/l, up to the highest value of 3.14 µg/l. The authors did not indicate how the data from samples that were at the LOQ were handled. Unchanged furan was not detected in blood samples.

The investigators found a strong positive correlation ($r = 0.56$, $P < 0.0001$) between the activity of GGT and urinary furan concentration in individuals with detectable urinary furan (Jun et al., 2008).

3. ANALYTICAL METHODS

3.1 Chemistry

Furan (C_4H_4O) (CAS No. 110-00-9) is a colourless, flammable liquid with an ethereal odour, having a low molecular weight of 68.08, a high volatility, with a boiling point of 31.36 °C, and a specific gravity of 0.9371 at 101.1 kPa. Furan is insoluble in water but is soluble in alcohol, ether and most common organic solvents. Chemically, furan is classified as a cyclic, dienic ether (NTP, 1993).

All ring atoms of furan lie in a plane and form a slightly distorted pentagon. The small dipole moment of furan (0.71 D) confirms that one electron pair of the oxygen atom is included in the conjugated system and therefore delocalized. Data obtained by ultraviolet (UV) and nuclear magnetic resonance (NMR) indicate that a diamagnetic ring current is induced in the furan molecule, which fulfils an important experimental criterion for aromaticity. Furan undergoes reactions with electrophilic reagents, often with substitution. However, it can also react by addition and/or ring opening, depending on reagents and reaction conditions (Eicher, Hauptmann & Speicher, 2003). Furan is stable to alkalis but forms resins on evaporation or in contact with mineral acids. Upon standing, furan turns brown; however, the colour change can be retarded with the addition of a small amount of water. Unless stabilized, furan will react slowly with air to form an unstable, explosion-prone peroxide (HSDB, 2001).

3.2 Description of analytical methods

3.2.1 Introduction

Analytical methods for the determination of furan in foods have been reviewed by some authors (Crews & Castle, 2007a,b; Wenzl, Lachenmeier & Gökmen, 2007; Wenzl, 2008; Vranová & Ciesarová, 2009). The extraction strategy and the measurement techniques have been defined taking into account the high volatility and the low molecular weight of the analyte.

3.2.2 Quantitative methods

(a) Sample preparation

To avoid furan loss, food samples and standards need to be chilled and handled quickly (Crews & Castle, 2007a). Temperatures between 0 °C and 10 °C have been applied, but most laboratories store samples at 4 °C for at least 4 h before homogenization. In the case of frozen samples, the thawing should be performed under controlled conditions at low temperature (e.g. in an ice bath), and increased attention has to be paid to sample homogeneity after thawing (Wenzl, 2008).

Puréed, liquid samples or reconstituted powdered samples can be transferred directly to HS vials, whereas solid samples (non-homogeneous and with variable amounts of fat) should be homogenized prior to extraction (Wenzl, Lachenmeier & Gökmen, 2007). Homogenization of solid samples has to be done as quickly as possible; depending on the physical state of the sample and other specific properties, such as the fat content, different strategies for sample homogenization can be applied (USFDA, 2004; Nyman et al., 2008). Samples that formed a starch gel can be treated in the sealed HS vial with amylase at room temperature overnight for about 14 h (Zoller, Sager & Reinhard, 2007). During homogenization and subsampling, it is recommended that the samples be kept in an ice bath or that dry ice be added in order to avoid furan losses. For homogenization, blenders and Ultra-Turrax are most frequently employed. In general, test portion size varies from 0.05 to 10 g, depending on the physical state of the samples and on the furan content.

(b) Addition of water or saline solutions

Water is usually added to the samples in order to reduce the viscosity, which favours equilibration and changes the ratio between the gas phase and sample phase (Wenzl, 2008). The amount of added water varies from 0.5 to 15 ml. In the procedure described by the United States Food and Drug Administration (USFDA), water was replaced by a saturated sodium chloride solution (USFDA, 2004), whereas in other methods, the salt is added directly into the vial (Becalski et al., 2005; Goldmann et al., 2005; Altaki, Santos & Galceran, 2007; Yoshida et al., 2007; Zoller, Sager & Reinhard, 2007; Altaki, Santos & Galceran, 2009; Bononi & Tateo, 2009; Jestoi et al., 2009). The use of salt (sodium chloride, sodium sulfate or ammonium sulfate) might reduce the solubility of furan in the aqueous phase and hence lead to more intensive signals. However, the relative signal intensities of furan and internal standard did not significantly change (Crews et al., 2007; Wenzl, 2008).

(c) Addition of internal standard

All published methods use an internal standard in furan analysis. Most laboratories use deuterium-labelled furan ([$^2H_4$]furan), d_4-furan, which is normally added to the homogenized sample before the extraction. Only one method describes the addition of d_4-furan at the homogenization step (Becalski et al., 2005). However, the authors noted a significant variation in the recovery of the samples, which may impart errors of a 10–20% magnitude in the determination of furan. It is recommended that the content of d_4-furan be adjusted to the content of native furan in the test sample. Senyuva & Gökmen (2005) reported the only method in which d_6-acetone was used as internal standard. According to the authors, complete resolution of the peaks of d_4-furan and furan could not be achieved under the gas chromatographic (GC) separation conditions. Instead, d_6-acetone could be completely resolved from the native furan and was found to be more suitable than d_4-furan as an internal standard.

(d) Headspace (HS) extraction

The first quantitative method for furan analysis in different matrices employed HS extraction (USFDA, 2004), and several procedures described in the literature are based on this approach, with or without modifications. Both static and dynamic HS extractions have been used (Hoenicke et al., 2004; Becalski & Seaman, 2005; Becalski et al., 2005; Kuballa, Stefan & Nina, 2005; Senyuva & Gökmen, 2005; Hasnip, Crews & Castle, 2006; Nyman et al., 2006; Crews et al., 2007; Senyuva & Gökmen, 2007; Vranová, Bednáiková & Ciesarová, 2007; Yoshida et al., 2007; Zoller, Sager & Reinhard, 2007; Morehouse et al., 2008; Nyman et al., 2008; Roberts et al., 2008; Lachenmeier, Reusch & Kuballa, 2009).

Static HS extraction is a relatively simple and well-proven methodology very suitable to volatile compounds. In this technique, a food sample in liquid or slurry form is heated in a sealed vial to achieve equilibrium partitioning between the liquid phase and the gaseous HS. The HS gas is sampled and the vapour injected into a GC (Vranová & Ciesarová, 2009). In dynamic HS extraction (purge and trap), the volatiles are removed by bubbling a purge gas through the sample matrix and subsequently collected on an analytical trap. After the purging is complete, the trap is heated, and the volatiles are released and delivered to a GC for separation and detection (Slack, Snow & Kou, 2003). The direct and accurate analysis of volatiles in foods by HS extraction requires careful standardization of parameters such as equilibration time and temperature. According to published methods, the equilibration temperature in HS analysis varies between 30 °C and 80 °C, and the equilibration time varies between 10 and 60 min, but most methods use 30 min. Prolonged equilibration time in static HS extraction can be reduced by using automated vial shaking, as suggested by Hasnip, Crews & Castle (2006).

(i) Trueness of the HS extraction methods

Partitioning of analytes for HS sampling can be increased by raising the incubation temperature; for many analytes, it is typical to use a temperature of about 80 °C (Crews & Castle, 2007b). However, some authors have noted that furan can be formed in certain matrices when the equilibration temperature is above 60 °C, especially in green coffee (Senyuva & Gökmen, 2005; Hasnip, Crews & Castle, 2006; Nyman et al., 2008), although others have observed furan formation only above 80 °C (Nyman et al., 2006; Yoshida et al., 2007; Morehouse et al., 2008). The method initially described by the USFDA employed an equilibration temperature of 80 °C, which was changed to 60 °C after these observations (USFDA, 2004). The reduction in the incubation temperature raised questions regarding the accuracy of previously reported data for canned and jarred foods that were determined using 80 °C and posted on the USFDA web site (Nyman et al., 2008). Therefore, additional HS studies were conducted to address this concern by analysing selected foods containing low levels of furan and coffee by the old and new methods. The furan level found in green beans increased by 3 ng/g (24%) between 60 °C and 80 °C (not significantly different at the 5% level), whereas in other foods, no significant increase was noted, which indicates that the data previously published are valid (Nyman et al., 2008). According to Senyuva & Gökmen (2005), a matrix-matched calibration for each particular food matrix is necessary to compensate for furan formation during HS sampling and thus to quantify furan more accurately.

(e) Headspace solid-phase microextraction (HS-SPME)

Several methods described in the literature have used the headspace solid-phase microextraction (HS-SPME) technique for furan determination in different matrices (Fan, 2005a,b; Goldmann et al., 2005; Ho, Yoo & Tefera, 2005; Bianchi et al., 2006; Fan & Mastovska, 2006; Fan & Sommers, 2006; Altaki, Santor & Galceran, 2007; Fan & Geveke, 2007; Limacher et al., 2007; Fan & Sokorai, 2008; Fan, Huang & Sokorai, 2008; Limacher et al., 2008; Altaki, Santos & Galceran, 2009; Bononi & Tateo, 2009; Jestoi et al., 2009; Kim et al., 2009a,b; La Pera et al., 2009). SPME is an alternative solvent-free sampling technique widely used for the analysis of volatile compounds (Wenzl, Lachenmeier & Gökmen, 2007). In SPME, a fibre coated with a polymeric material is first exposed to the HS vapours of the vial to absorb volatiles. Then, the fibre is thermally desorbed in the injection port of the GC to drive off the volatiles onto the GC column (Crews & Castle, 2007a). Proper selection of the SPME fibre is important to increase the extraction yield (Wenzl, Lachenmeier & Gökmen, 2007). According to published methods, the most used fibre for furan analysis is the 75 μm carboxen–polydimethylsiloxane. The bipolar phase has unique characteristics, such as pore size, distribution, volume, shape and particle size, which are ideal for small analytes (Shirey, 1999). Few methods have reported the use of other fibres, such as 85 μm carboxen–polydimethylsiloxane (Fan & Sommers, 2006; Fan & Geveke, 2007; Fan, Huang & Sokorai, 2008) and 50/30 μm divinylbenzene–carboxen–polydimethylsiloxane (Bononi & Tateo, 2009).

Extraction temperatures reported in the literature usually vary between 25 °C and 50 °C. Higher temperatures have been associated with less intense signals due to the desorption of furan from the fibre and the increase in the distribution constant of furan between the HS and the fibre coating (Bianchi et al., 2006; Altaki, Santos & Galceran, 2007). Extraction times between 10 and 30 min as well as magnetic stirring have normally been employed. Desorption has been carried out from 220 °C for 1 min up to 300 °C for 10 min.

(f) HS versus HS-SPME

In 2008, the first proficiency test on the determination of furan in baby food consisting of carrot and potato purée was conducted (Kubiak, Karasek & Wenzl, 2008). From 22 laboratory participants, about 70% applied HS coupled with gas chromatography–mass spectrometry (GC-MS), and 30% used HS-SPME with GC-MS. The results indicated that 16 out of 22 laboratories were capable of analysing furan in food matrices, reporting results that were satisfactory according to international guidelines. In relation to the performance of analytical methods, both techniques (HS and HS-SPME) were suitable for furan analysis in foods. More recently, several food samples, such as apple juice, honey, coffee, chicken pap baby food and cooked chickpeas, were analysed by automated HS and HS-SPME, both coupled to GC-MS (Altaki, Santos & Galceran, 2009). Both methods gave similar results for furan determination in selected food samples, although slightly worse precision (relative standard deviation 9–12%) and higher limits of detection (LODs) (5–20 times higher) were obtained by the HS method.

(g) Other extraction techniques used for furan analysis

(i) Solid-phase dynamic extraction (SPDE/GC-MS)

Ridgway, Lalljie & Smith (2006) used a commercial in-tube sorptive extraction device, known as solid-phase dynamic extraction (SPDE), for the extraction of furan from aqueous solutions in both HS and liquid injection modes. This technology allows the dynamic extraction of samples due to the fact that it uses significantly high amounts of sorbent material (4.5 µl) (Chromtech, 2006). The authors reported an LOD of 1.5 ng/g and observed no improvement in sensitivity compared with static HS extraction.

(ii) Microdistillation-GC-MS

Kuballa & Ruge (2005) published a comparison of the USFDA method (HS-GC-MS) and microdistillation-GC-MS. By this method, six HS vials were distilled using an automated microdistiller into prepared vials with a cooled solution. An aliquot from each solution was sampled and injected into a GC-MS system. The authors declared that this method can shorten the total time of analysis (in comparison with the USFDA method) by parallel distillation of six samples. Furthermore, the analysis of complex matrices (e.g. coffee) is easier.

(h) Determination by GC-MS

(i) Chromatographic performance

Chromatographic separation of furan from co-extractives has been mostly performed on porous layer open tubular capillary columns under a variety of instrument parameters (Crews & Castle, 2007a; Wenzl, Lachenmeier & Gökmen, 2007; Wenzl, 2008). Porous layer open tubular columns have a bonded porous polymer based on polystyrene and divinylbenzene, which is relatively inert and stable to water (Crews & Castle, 2007b). Other stationary phases have also been described for furan analysis, such as wax-based columns (polyethylene glycol) (Bianchi et al., 2006; Limacher et al., 2007; Yoshida et al., 2007; Bononi & Tateo, 2009), (5% phenyl)-methylpolysiloxane (Fan, 2005a,b; Fan & Mastovska, 2006; Fan & Sommers, 2006; Fan & Geveke, 2007; Fan & Sokorai, 2008; Fan, Huang & Sokorai, 2008) and cyanopropylphenyl polysilphenylene-siloxane (Altaki, Santos & Galceran, 2007; La Pera et al., 2009). Splitless injection, with or without cryogenically refocusing the injected HS gas, is the natural choice to obtain sufficient sensitivity of the method (Wenzl, Lachenmeier & Gökmen, 2007).

(ii) Detection

Quadrupole mass spectrometers are used in most cases to detect furan and d_4-furan in samples. Few procedures have described the use of ion trap analysers (Altaki, Santos & Galceran, 2007, 2009; Zoller, Sager & Reinhard, 2007). The positive electron ionization mode is usually applied, with 70 eV of electron energy, and the mass spectrometers are commonly operated in selected ion monitoring mode.

(iii) Confidence

The identification of furan is assured by checking for the correct retention time and for the presence and relative abundance of characteristic ions. The mass spectrum of furan has a relatively intense molecular ion with mass-to-charge ratio (*m/z*) of 68, which is used as quantifier ion, and a fragment ion *m/z* of 39 with sufficient abundance for confirmatory purposes (qualifier ion). Goldmann et al. (2005) used two characteristic ions as qualifiers, *m/z* 39 and *m/z* 69. For d_4-furan, *m/z* 72 (quantifier ion) and *m/z* 42 (qualifier ion) are usually monitored. The relative abundance between *m/z* 68 and *m/z* 39 for the test portions should agree with the average of the response ratios for the calibration standards by ± 10%, and the retention time for the test portions should agree with the average retention times for the calibration standards by ± 2% (USFDA, 2004).

(iv) Quantitative aspects

Quantification of furan in samples has been based on standard additions or external calibration graphs, both incorporating an internal standard. Crews et al. (2007) applied both approaches to samples having low (tomato sauce), medium (tomato soup) and high (coffee powder) levels of furan. The coffee powder sample contained 59 ng/g of furan when measured by external calibration and 62 ng/g of furan by standard additions. The tomato soup sample contained 24 ng/g of furan by external calibration and 28 ng/g of furan by standard additions. The tomato sauce sample contained 3 ng/g of furan by external calibration and 2 ng/g of furan by standard additions. These results were considered essentially identical, providing further evidence that both methods of quantification appear to be equally suitable. The external calibration procedure is especially useful when a large number of food samples are to be analysed, as it reduces the need for duplication of sample preparation (Crews et al., 2007).

Some published methods based on HS extraction have been demonstrated to be linear over concentration ranges up to 0–1000 ng/ml (Becalski et al., 2005; Yoshida et al., 2007). LODs and LOQs vary from 0.1 to 4.85 ng/g and from 0.44 to 13 ng/g, respectively. Recoveries of between 83% and 122% have been reported. In methods using HS-SPME, linearity over a concentration range up to 0–100 ng/g has already been reported in the literature (Bianchi et al., 2006). As SPME allows sample concentration and affords higher sensitivity (Crews & Castle, 2007b), LODs and LOQs have been reported from 0.001 to 1.9 ng/g and from 0.006 to 4 ng/g, respectively. Recoveries varied between 87% and 116%.

3.2.3 Summary of analytical methods

GC-MS has been shown to be the most suitable technique for the reliable detection of low levels of furan in foods. GC-MS is usually preceded by HS extraction or HS-SPME. Owing to the high volatility of furan, food samples and standards need to be chilled and handled quickly. Puréed, liquid samples or reconstituted powdered samples can be transferred directly to HS vials, whereas solid samples have to be homogenized. LODs and LOQs from 0.1 to 5 ng/g and from 0.4 to 13 ng/g, respectively, have been reported for methods based on HS extraction. Lower LODs and LOQs are reported for methods using HS-SPME. There is currently no certified reference material available, and few authors have reported the measurement

uncertainty of the methods, which varied between 9.5% and 29% (Goldmann et al., 2005; Jestoi et al., 2009). It is highly recommended that laboratories implement a well-documented internal quality control system and participate in an appropriate proficiency testing programme or interlaboratory comparison to authenticate the accuracy and reliability of data produced.

4. FORMATION, EFFECTS OF PROCESSING AND FATE IN FOOD

The formation of furan in foods has been discussed and reviewed by several authors (Yaylayan, 2006; Crews & Castle, 2007a; Vranová & Ciesarová, 2009). It has been demonstrated that furan can be formed from different precursors, either by thermal or by non-thermal processing.

4.1 Thermal-induced formation of furan in foods

Although the exact mechanism is not completely understood, data available in the literature indicate multiple routes of furan formation under heat treatment, such as thermal degradation or Maillard reaction of reducing sugars, alone or in the presence of amino acids, thermal degradation of certain amino acids and thermal oxidation of ascorbic acid, polyunsaturated fatty acids and carotenoids (Perez Locas & Yaylayan, 2004; Becalski & Seaman, 2005; Fan, 2005b; Märk et al., 2006). The proposed pathways for thermal-induced formation of furan are summarized in Figure 1.

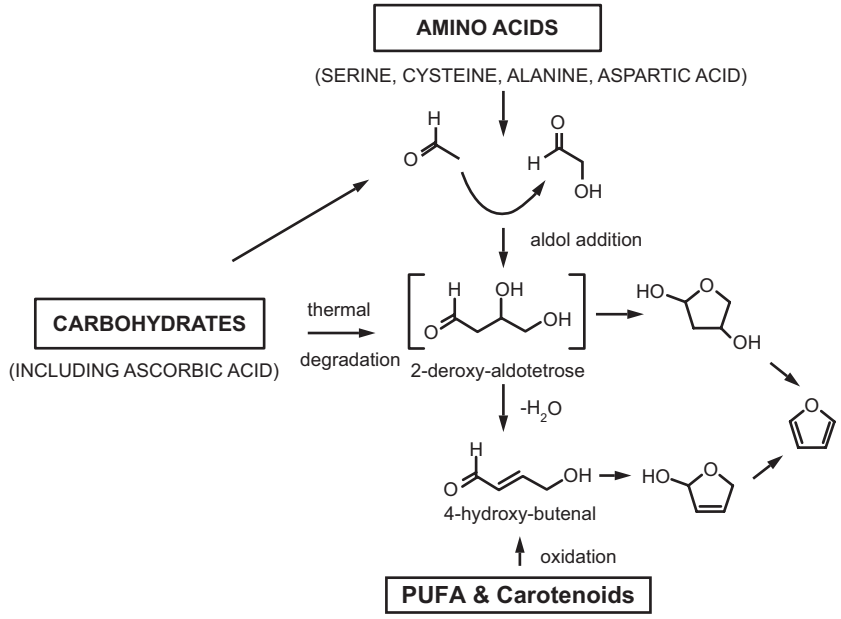
Ascorbic acid was first reported as the major precursor of furan in simple model systems (Perez Locas & Yaylayan, 2004; Märk et al., 2006). However, experiments conducted with pumpkin purée, apple cider, and carrot and orange juices have demonstrated that ascorbic acid and sugars are only minor precursors of furan in such foods (Limacher et al., 2007, 2008; Fan, Huang & Sokorai, 2008). Therefore, the formation of high amounts of furan may be associated with the presence of fatty acids or a combination of fatty acids with other compounds (Fan, Huang & Sokorai, 2008).

4.1.1 Chemical mechanisms

(a) Formation from carbohydrates and amino acids

The pathway of furan formation from carbohydrate degradation basically encompasses the production of aldotetrose derivatives that can undergo eventual cyclization to form furan. Reducing sugars undergo Maillard reactions in the presence of amino acids and generate reactive intermediates such as 1-deoxyosone and 3-deoxyosone. To form furan, the 1-deoxyosone has to undergo α-dicarbonyl cleavage to produce aldotetrose, followed by dehydration reactions to generate 3-furanone, which produces furan after reduction and dehydration reactions. To a lesser extent, the 3-deoxyosone undergoes α-dicarbonyl cleavage, followed by oxidation and decarboxylation to generate 2-deoxyaldotetrose, a direct precursor of furan (Perez Locas & Yaylayan, 2004).

Figure 1. Proposed pathways and precursors of furan



PUFA, polyunsaturated fatty acids

Source: Yaylayan (2006); based on Perez Locas & Yaylayan (2004) and Becalski & Seaman (2005)

(b) Formation from carbohydrates

Furan can also be formed from carbohydrates in the absence of amino acids. Hexose sugars, such as glucose, lactose and fructose, can undergo retro-aldol cleavage to generate aldotetrose, followed by dehydration to produce 3-furanone. To a lesser extent, furan can be formed from glucose after a dehydration reaction followed by a retro-aldol cleavage to form 2-deoxy-3-ketoaldotetrose. The latter intermediate can cyclize after a dehydration step to produce 3-furanone (Perez Locas & Yaylayan, 2004). Limacher et al. (2007) described a potential pathway from glucose and fructose via 4-deoxyhexo-2,3-diulose and observed that the formation mechanisms depend greatly on the reaction conditions applied, such as roasting and pressure cooking.

(c) Formation from amino acids

It has been demonstrated that amino acids capable of being metabolized to acetaldehyde and glycolaldehyde, such as serine or cysteine, can undergo thermal degradation to produce furan without the need for any other source. Acetaldehyde and glycolaldehyde react by aldol condensation, producing aldotetrose derivatives and, eventually, furan. In contrast, alanine, threonine and aspartic acid alone do not

produce furan, as these amino acids generate only acetaldehyde; therefore, the presence of reducing sugars, serine or cysteine is required to produce glycolaldehyde (Perez Locas & Yaylayan, 2004; Vranová & Ciesarová, 2009).

(d) Formation from ascorbic acid

In browning reactions, ascorbic acid behaves in a similar manner to reducing sugars (Crews & Castle, 2007a). A proposed pathway describes the oxidation of ascorbic acid to dehydroascorbic acid in aerobic conditions in food systems, followed by hydrolysis to 2,3-diketogulonic acid, which is converted to aldotetrose and later to furan. However, under anaerobic conditions, ascorbic acid cannot undergo oxidation to produce 2,3-diketogulonic acid. Instead, it can hydrolyse and undergo β-elimination followed by decarboxylation to produce 3-deoxypentosulose, which can generate 2-deoxyaldotetrose, a direct precursor of furan (Perez Locas & Yaylayan, 2004). Becalski & Seaman (2005) proposed that the formation of furan from ascorbic acid derivatives could proceed via decomposition of 2-furoic acid, a degradation product of ascorbic acid, as large amounts of furan were found in a model system formed from this compound. According to Limacher et al. (2007), furan may also be generated directly from 2-furaldehyde, which is also a degradation product of ascorbic acid, by electrophilic aromatic substitution-type reaction.

(e) Formation from polyunsaturated fatty acids

Lipid hydroperoxides can be formed non-enzymatically by reactive oxygen species or enzymatically by lipoxygenases. Subsequent homolytic cleavages of polyunsaturated fatty acid hydroperoxides, catalysed by transition metal ions, result in the formation of 2-alkenals, 4-oxo-2-alkenals and 4-hydroxy-2-alkenals. On this basis, it was proposed that the parent furan could be formed from corresponding 4-hydroxy-2-butenal through cyclization and formation of 2,5-dihydro-2-furanol and subsequent dehydration (Perez Locas & Yaylayan, 2004).

4.1.2 Formation factors

(a) Heat treatment

Several studies have demonstrated that temperature and cooking conditions are important parameters on the formation of furan. Treating sugars and organic acids in boiling water for 5 min did not result in measurable levels of furan (Fan, 2005b). In toasted bread, temperatures of about 140–160 °C resulted in a furan concentration of 3 ng/g, whereas temperatures of about 170– 200 °C resulted in furan concentrations of 12–129 ng/g (Hasnip, Crews & Castle, 2006). Higher amounts of furan are normally formed under roasting conditions (dry heating, 200 °C, 10 min) compared with pressure-cooking conditions (sterilization, 121 °C, 25 min) (Limacher et al., 2007, 2008). The link between final temperature and furan level is not clear, but generally higher temperatures produced higher levels of furan, especially above 120 °C (Hasnip, Crews & Castle, 2006; Senyuva & Gökmen, 2007). Furan can also be formed during reheating of processed foods in closed jars (Lachenmeier, Reusch & Kuballa, 2009).

(b) Precursors

(i) Carbohydrates

Studies carried out to compare the relative efficiency of different sugars to generate furan by using pyrolysis/GC-MS in conjunction with ^{13}C-labelling experiments have shown the following order of reactivity: D-erythrose > D-ribose > D-sucrose > D-glucose = D-fructose (Perez Locas & Yaylayan, 2004). Under roasting conditions, arabinose was the most efficient precursor of furan, followed by fructose, glucose and erythrose (Limacher et al., 2008).

(ii) Ascorbic acid and derivatives

The ability of ascorbic acid and its derivatives to form furan has been shown in experiments using model systems heated at 118 °C for 30 min under aqueous pressure-cooking conditions. Dehydroascorbic and isoascorbic acids generated approximately 10-fold more furan than ascorbic acid. Sodium ascorbate and isoascorbate generated less furan compared with their free acid counterparts (Becalski & Seaman, 2005). The addition of iron(III) chloride did not influence the amounts of furan formed from free acids, but significantly increased furan formation from their corresponding sodium salts (Becalski & Seaman, 2005). The furan yields from ascorbic acid were lowered in an oxygen-free atmosphere (30%) or in the presence of reducing agents (e.g. sulfite, 60%), indicating the important role of oxidation steps in the furan formation pathway (Märk et al., 2006). It has also been demonstrated that when ascorbic acid is mixed in model systems with single amino acids, sugar or unsaturated fatty acid, the mixtures produced far less furan on heating than did ascorbic acid alone (Märk et al., 2006; Limacher et al., 2007).

(iii) Polyunsaturated fatty acids

Studies performed using model systems heated at 118 °C for 30 min have indicated that only polyunsaturated fatty acids such as linoleic and linolenic acids can generate furan upon heating. It has been observed that lipids having three double bonds, such as linolenic acid, formed more furan than lipids containing two double bonds, such as linoleic acid (Becalski & Seaman, 2005; Märk et al., 2006). The influence of antioxidants (e.g. tocopherol acetate, butylated hydroxyanisole) and iron(III) chloride on furan formation from polyunsaturated fatty acids has been investigated by some authors, but no consistent results have been obtained (Becalski & Seaman, 2005; Märk et al., 2006).

(c) pH

Several studies have demonstrated that pH plays a complex role in the mechanism of furan formation (Fan, 2005b; Limacher et al., 2007, 2008; Fan, Huang & Sokorai, 2008). For ascorbic acid and sucrose solutions, an increase in thermally induced furan was observed at a lower pH, whereas in glucose solution and linoleic acid emulsion, the formation of furan was favoured at neutral pH. The presence of phosphate may also play a significant role in determining the level of furan, and its effect is pH dependent (Fan, Huang & Sokorai, 2008).

4.2 Non-thermal-induced formation of furan in foods

It has been reported that ionizing radiation induced the formation of furan in orange and apple juices, in grape and pineapple and in simple model systems (solutions of glucose, fructose, sucrose, ascorbic acid) (Fan, 2005a,b; Fan & Sokorai, 2008). Furan levels increased linearly as the radiation dose increased from 0 to 5 kGy and can continue to increase in the first 3 days of storage after the treatment, probably due to the residual effect of irradiation (Fan, 2005a). Compared with the thermal treatment (sterilization), an irradiation dose of 5 kGy in sugars and ascorbic acid solutions produced similar amounts of furan (Fan, 2005b). Irradiation exerts its effect in aqueous solutions through generation of free radicals from radiolysis of water. These free radicals (hydrated electrons, hydrogen atoms and hydroxyl radicals) react with the food components, such as ascorbic acid and sugars, which results in the formation of furan (Fan & Sokorai, 2008).

4.3 Effects of processing and fate in food

Limited data are available on the stability of furan during cooking, storing and reheating of meals. For coffee, the amount of furan formed in beans varies according to the level of roasting. Grinding may reduce furan levels by 10–60%, and further decreases occur in the production of instant coffee powder and in brewing (Hasnip, Crews & Castle, 2006; Zoller, Sager & Reinhard, 2007; EFSA, 2009). Losses of furan up to 85% were reported during heating of opened baby food jars over a period of 5.5 h in boiling water, whereas a reduction of ~50% was observed if the jar was opened but not heated (Goldmann et al., 2005). In vegetable purées heated in a microwave oven by different warming procedures, a decrease of 29–55% furan has been reported (Zoller, Sager & Reinhard, 2007). Reduction in the furan content of canned meats during preparation before consumption has also been reported (Kim et al., 2009a; La Pera et al., 2009).

According to some researchers, furan persists during normal warming procedures that precede consumption (Hasnip, Crews & Castle, 2006; Lachenmeier, Reusch & Kuballa, 2009). As furan appears to be well dissolved within the matrix, opening the jars exposes only a relatively small surface area. Therefore, despite furan's volatility, its evaporation is apparently hindered by its slow diffusion inside the food matrix (Lachenmeier, Reusch & Kuballa, 2009). However, if canned foods are heated in a saucepan under stirring, larger declines in furan content can be observed (Roberts et al., 2008). Losses of furan in heated foods left for cooling seem to be insignificant. No experimental data are available concerning chemical mechanisms for elimination of furan in foods.

4.4 Summary of formation and fate

Furan can be formed from different precursors either by thermal or non-thermal processing (ionizing radiation). Although the exact mechanism is not completely understood, data available in the literature indicate multiple routes of furan formation, such as thermal degradation or Maillard reaction of reducing sugars, alone or in the presence of amino acids, thermal degradation of certain amino acids, thermal oxidation of ascorbic acid, polyunsaturated fatty acids and

carotenoids and free radical reactions during irradiation (Perez Locas & Yaylayan, 2004; Becalski & Seaman, 2005; Fan, 2005b; Märk et al., 2006). It has been demonstrated that ascorbic acid and sugars are only minor precursors of furan in foods, whereas the formation of high amounts of furan may be associated with the presence of fatty acids or a combination of fatty acids with other compounds (Limacher et al., 2007, 2008; Fan, Huang & Sokorai, 2008). Higher amounts of furan are normally formed under roasting conditions (dry heating, 200 °C, 10 min) compared with pressure-cooking conditions (sterilization, 121 °C, 25 min), and pH plays a complex role in the mechanism of furan formation. Studies on the stability of the contaminant in foods during normal warming procedures have shown conflicting results. Some authors reported furan losses of 29–85% during warming under different times (Goldmann et al., 2005; Zoller, Sager & Reinhard, 2007), whereas others have found that furan persists during normal heating practices (Hasnip, Crews & Castle, 2006; Lachenmeier, Reusch & Kuballa, 2009).

5. LEVELS AND PATTERNS OF CONTAMINATION OF FOOD COMMODITIES

5.1 National occurrence

Furan concentration data were made available to the Committee through submissions from Australia, Brazil, Canada, the European Union (EU), Japan, the Republic of Korea, Switzerland and the USA, representing 21 countries. The total number of analytical results (single or composite samples) evaluated at the present meeting was 5662, with 58.8% from Europe, 16.7% from North America, 22.8% from Asia, 1.0% from Latin America and 0.7% from the Pacific region. The occurrence of furan has been mainly investigated in thermally processed foods, such as coffee, canned and jarred foods, including baby foods, soups and sauces. Table 13 shows the summary for individual occurrence data, including the percentage of data below the reporting limit, collected from 21 countries from 2004 to 2009.

5.1.1 Australia

Australia submitted the results of furan occurrence in different kinds of brewed coffee (FSANZ, 2009). Furan concentrations were obtained from 41 composite samples (4 samples per composite). Analyses were performed using the GC-MS technique, and the practical quantification limit (PQL) was 0.1 µg/l. All analysed samples showed furan levels above the PQL. The mean concentrations were as follows: cappuccino, 32.3 µg/l; latte, 22.9 µg/l; flat white, 33.2 µg/l; long black, 42.0 µg/l; short black, 112.5 µg/l; mocha, 23.5 µg/l; instant black, 2.0 µg/l; instant white, 2.5 µg/l; and ground coffee, 23.0 µg/l.

5.1.2 Brazil

Brazil submitted the results of furan occurrence in foods to the Committee (Arisseto, Vicente & Toledo, 2009) using the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) format. Furan concentrations were obtained from 55 individual food samples

Table 13. Summary of furan occurrence data from various countries from 2004 to 2009

Country	Number of analytical results	%	% of values below LOR[a]
Australia	41	0.7	0.0
Brazil	55	1.0	45.5
Canada	176	3.1	0.0
European Union[b]	2908	51.4	32.1
Japan	886	15.6	15.0
Republic of Korea	407	7.2	8.6
Switzerland	418	7.4	17.2
USA	771	13.6	19.7

[a] Limit of reporting (detection limit, quantification limit and practical quantification limit).
[b] Data submitted by the following European Union member states: Austria, Belgium, Cyprus, Finland, Germany, Greece, Ireland, Italy, Lithuania, the Netherlands, Poland, Slovakia, Slovenia and the United Kingdom.

purchased in 2009 from different supermarkets. Analyses were performed in samples "as bought" using the GC-MS technique. The limits of the method were, respectively, 0.7 and 2.4 µg/kg for detection and quantification. The highest average (or upper-bound mean) levels of contamination were found in roasted coffee (powder) (2998.1 µg/kg), soya sauce (25.7 µg/kg) and baby food (18.6 µg/kg).

5.1.3 Canada

Canada submitted the results of furan occurrence in foods from a recent evaluation (Becalski et al., 2009). Furan concentrations were obtained from 176 individual food samples purchased in 2006 from various retail outlets (154 canned or jarred products, including 3 coffee products, 5 packaged meat pâtés and 17 baby foods). Analyses were performed using the GC-MS technique. The LOD was 0.05 µg/kg, but only concentrations higher than 1 µg/kg were reported. The highest average levels of contamination were found in roasted coffee (powder) (4590 µg/kg), baked beans (580.6 µg/kg), instant coffee (powder) (413 µg/kg), pasta (396.4 µg/kg) and chili con carne (386.4 µg/kg).

5.1.4 European Union

The European Food Safety Authority (EFSA) submitted the results of furan occurrence to the Committee from the monitoring database of furan levels in food (EFSA, 2009), which includes data from 14 member states. Furan concentrations were obtained from 2908 samples analysed from 2004 to 2009 (124 from Austria, 229 from Belgium, 30 from Cyprus, 65 from Finland, 1746 from Germany, 48 from Greece, 264 from Ireland, 58 from Italy, 6 from Lithuania, 88 from the Netherlands,

100 from Poland, 70 from Slovakia, 35 from Slovenia and 45 from the United Kingdom). The samples were analysed without any preparation of the purchased foodstuff (e.g. coffee powder, juices, jars and cans not heated before consumption), as well as after further preparation as if consumed (e.g. brewed coffee, canned and jarred products heated before consumption). Analyses were performed using the GC-MS technique. The minimum and maximum values reported for LOD and LOQ ranged from 0.1 to 40 µg/kg and from 0.2 to 100 µg/kg, respectively. The highest average levels (upper-bound mean) of contamination were found in roasted beans coffee (2272 µg/kg), roasted ground coffee (powder) (1114 µg/kg), instant coffee (powder) (589 µg/kg), baked beans (27 µg/kg), baby food (25 µg/kg) and soya sauce (25 µg/kg).

5.1.5 Japan

Japan submitted the results of furan occurrence in foods for the current meeting of the Committee from different surveys (Yoshida et al., 2007; MAFF, 2009a,b). Furan concentrations were obtained from 886 individual food samples. Analyses were performed using the GC-MS technique. The minimum and maximum values reported for LOD and LOQ ranged from 0.2 to 0.5 µg/kg and from 0.4 to 2.0 µg/kg, respectively. The highest average levels of contamination were found in fermented soya bean pastes made with soya bean kouji (250 µg/kg), sauces (73 µg/kg) and canned coffee (65 µg/kg).

5.1.6 Republic of Korea

The Republic of Korea submitted the results of furan occurrence in foods from a recent publication (Kim et al., 2009b). Furan concentrations were obtained from 407 individual food samples purchased from the local market. Analyses were performed using the GC-MS technique. The limits of the method were 0.4 and 1.4 µg/kg for detection and quantification, respectively. The highest average levels of contamination were found in ground roasted coffee (814.1 µg/kg), canned oyster (181.6 µg/kg), instant coffee (90.1 µg/kg), Korean seasoned pork (63.3 µg/kg) and canned fish (66.7 µg/kg).

5.1.7 Switzerland

Switzerland submitted the results of furan occurrence in foods for the current meeting of the Committee (Kantonales Laboratorium Basel, 2004; Reinhard et al., 2004; SFOPH, 2004). Furan concentrations were obtained from 418 individual food samples purchased in local retail stores. Analyses were performed using the GC-MS technique. The LOQs ranged from 1 to 10 µg/kg. The highest average levels of contamination were found in roasted coffee (powder) (1979 µg/kg), roasted flour/starch (1932.8 µg/kg), instant coffee (powder) (783.3 µg/kg) and caramel (312 µg/kg).

5.1.8 United States of America

The USA submitted the results of furan occurrence in foods from the 2004–2008 Exploratory Data on Furan in Food: Individual Food Products (USFDA, 2009).

Furan concentrations were obtained from 771 individual food samples. Analyses were performed using the GC-MS technique. The minimum and maximum values reported for LOD and LOQ ranged from 0.2 to 3.2 µg/kg and from 0.6 to 9.6 µg/kg, respectively. The highest average levels of contamination were found in baked beans (60.4 µg/kg), soya sauce (52.1 µg/kg), gravies (47.8 µg/kg) and brewed roasted coffee (46.7 µg/kg).

5.1.9 Summary of national occurrence data

The range of national mean levels of furan for foods with the highest contamination levels were as follows: roasted coffee (powder) (814–4590 µg/kg), instant coffee (powder) (90–783 µg/kg), brewed roasted coffee (34–113 µg/kg), baby food (19–96 µg/kg), soya sauce (16–52 µg/kg), canned fish (6–76 µg/kg) and baked beans (27–581 µg/kg). Detailed data are presented in Appendix 1.

6. FOOD CONSUMPTION AND DIETARY EXPOSURE ESTIMATES

Although the presence of furan as a flavouring component in food was reported in 1979 (Maga, 1979), the first national dietary exposure assessment for furan was not undertaken until 2004, when data on furan concentrations in a variety of foods in the USA became available (USFDA, 2009).

At the present meeting, the Committee considered dietary exposure estimates submitted by the USA (DiNovi & Mihalov, 2007; USFDA, 2009), the EU (EFSA, 2009; Fromberg, Fagt & Granby, 2009) and Brazil (Arisetto, Vicente & Toledo, 2009).

6.1 Furan concentrations used in dietary exposure estimates

Occurrence data are discussed more fully in section 5; however, details relevant to the dietary exposure estimates for furans are discussed below.

For the USA, furan levels were measured in foods prepared as for consumption, and only these foods were included in the dietary exposure assessments for the population in the USA (DiNovi & Mihalov, 2007; USFDA, 2009).

For the European estimates for 14 countries, mean furan levels for broad food groups were derived from the 2004–2009 results for individual foods, and these were then grouped according to the wider food groups described in the EFSA Concise European Food Consumption Database (EFSA, 2008) for use in the dietary exposure assessment (EFSA, 2009). The majority of analyses were for foods as purchased and not necessarily in the form as consumed; for example, most coffee was analysed as instant powder, beans or ground coffee, so a weighted mean furan level for coffee was derived and a dilution factor of 18 applied to convert all coffee as analysed to coffee as drunk. This furan level was then assigned to the wider food group, “coffee, tea and cocoa”.

The level of furan in coffee is of particular interest, as it tends to be higher than levels in most other foods, and therefore the method of deriving the value selected for use in dietary exposure assessments to assign to different types of

coffee can have an impact on estimated dietary exposures. An average level of 42–52 µg/kg was used in the USA estimates for brewed coffee, including decaffeinated coffee, and a higher value of 80 µg/kg was used in the EFSA dietary exposure assessment for all coffee, tea and cocoa (EFSA, 2009).

Different coffees may have different amounts of furans to start with. For example, EFSA (2009) and Crews (2009) reported that green coffee beans contain only traces of furans, whereas high levels of furans are found in full roasted beans (average 3400 µg/kg); grinding coffee beans reduces furan levels by between 10% and 60% (average 1114 µg/kg), and the production of instant coffee powder further reduces furan levels (average 589 µg/kg). These reported levels from European countries were consistent with those reported by Kuballa (2007), Hasnip, Crews & Castle (2006) and Zoller, Sager & Reinhard (2007). The high furan levels in roasted coffee are likely due to the very high temperatures used, which exceed those used in other food processing procedures (Crews & Castle, 2007a). The proportion of furan lost in the making of the coffee from beans varies according to the level of roasting, methods of brewing and equipment used. The furan content of coffee made with closed automatic machines was reported to be higher, at 57–115 µg/kg, than with other systems of brewing: home machine, 9–33 µg/kg; manual machine, 17–24 µg/kg; and French press cafetière, 33–66 µg/kg (Crews & Castle, 2007a; Crews, 2009). The Crews (2009) report also indicates that furan can be inhaled from coffee preparation or cooking and that furan is exhaled at elevated levels after coffee drinking, but there were insufficient data for reliable estimates of intake from air samples.

Recent information from Australia for coffee as drunk supports other values reported in the literature. Furan levels were the lowest for instant coffee (made up), at 2–3 µg/l, and highest for short black coffee, at 210 µg/l, whereas furan levels for other brewed coffees, such as lattes, flat whites and cappuccinos, were reported to be between these values, ranging from 7 to 77 µg/l (FSANZ, 2009). These values were also consistent with the USFDA mean furan values reported for coffee of 1–7 µg/kg for instant coffee (made up) and 34–84 µg/kg for brewed coffee (Morehouse et al., 2008).

Furan levels used in reported dietary exposure assessments for adults in 14 European countries and the USA are given in Table 14.

Table 14. Mean furan content of foods used in dietary exposure assessments

Food group	Europe[a]		USA[b]	
	N	Mean concentration (µg/kg)	*N*	Mean concentration (µg/kg)
Cereal & cereal products[c]	99	10 LB 14 UB		
Bread			7	0.2
Macaroni & cheese			2	15.9

Table 14 (contd)

Food group	Europe[a]		USA[b]	
	N	Mean concentration (μg/kg)	*N*	Mean concentration (μg/kg)
Vegetable soups[c]	198	23 LB 24 UB		
Chicken broth			8	11.6
Vegetable beef soup			6	88.0
Vegetables, nuts, pulses, other[c]	344	9 LB 14 UB		
Vegetables[d]	81 14	11.1 (2007–2009) 20.0 (2004–2006)		
Baked beans[d]	18 24	24.1 (2007–2009) 28.3 (2004–2006)	11	84.2
Corn			2 2	1.3 (kernel[e]) 23.0 (creamed)
Green beans[e]			4	5.2
Peas, sweet[e]			3	12.4
Peanut butter			4	6.5
Fruits[c]	84	2 LB 7 UB		
Pears			4	4.9
Pineapples			4	1.9
Fruit cocktail			5	5.9
Peaches			6	5.1
Apple sauce			7	1.9
Fruit & vegetable juices[c]	248	4 LB 7 UB		
Fruit juice[d]	137 66	2.9 (2007–2009) 13.6 (2004–2006)		
Vegetable juice				
Apple juice			7	1.7
Grape juice			6	1.8
Cranberry juice			4	1.3
Tomato juice			4	4.6
Vegetable juice			3	6.3

Table 14 (contd)

Food group	Europe[a]		USA[b]	
	N	Mean concentration (μg/kg)	*N*	Mean concentration (μg/kg)
Coffee, tea, cocoa as liquid[c]	398	82 LB 82 UB		
Coffee instant (made up)[f]			4 4	1.5 4.5 (decaffeinated)
Coffee brewed[f]			3 3	51.7 41.8 (decaffeinated)
Coffee instant, powder	33 15	437 (2007–2009) 925 (2004–2006)		
Coffee roast bean	9	2272		
Coffee roast ground	66	1114		
Coffee ns	152 123	2384 (2007–2009) 836 (2004–2006)		
Beer & substitutes[c]	86	4 LB 6 UB		
Beer[d]	19 67	8.3 (2007–2009) 4.0 (2004–2006)	8	1.0
Meat & meat products[c]	65	19 LB 22 UB		
Luncheon meat			3	0.9
Canned meat products[e]			5	17.4
Fish & seafood[c]	9	8 LB 10 UB		
Tuna in water[e]			6	5.0
Milk- & dairy-based drinks[c]	20	13 LB 15 UB		
Evaporated milk			3	12.5
Dairy-based products[c]	20	13 LB 15 UB		
Miscellaneous[c]	337	22 LB 23 UB		
Sauces[d]	94 113	7.8 (2007–2009) 16.0 (2004–2006)		
Hot dog chili sauce			2	40.5
Pasta sauce			9	8.7

Table 14 (contd)

Food group	Europe[a]		USA[b]	
	N	Mean concentration (μg/kg)	*N*	Mean concentration (μg/kg)
Pasta & sauce products			7	39.4
Soya sauce[d]	49	25.0 (2007–2009) 29.6 (2004–2006)	6	51.1
Caramel topping			2	3.2
Nutritional diet drinks			20	29.1
Pudding snacks			6	6.4
Gel snacks			3	3.1
Jellies, jams, preserves			30	4.4
Fruit butter spreads			8	18.6
Syrup			7	19.7

LB, lower bound; ns, not specified; UB, upper bound

[a] EFSA (2009). HS-GC-MS and HS-SPME/GC-MS methods of analysis. Years data were collected are given in parentheses.

[b] Morehouse et al. (2008). Furan found in prepackaged, processed adult foods, concentration of 0 μg/kg assigned to non-detect values, HS-GC-MS method of analysis.

[c] Mean furan content according to broad food categories in the EFSA Concise European Food Consumption Database (upper-bound values, non-detect values assigned the LOQ), standard dilution factor of 18 applied to convert coffee beans, grounds, powder to coffee as drunk.

[d] Reported in EFSA (2009). Records used to derive the mean furan levels for broad food groups.

[e] Solid portion, without liquid added by manufacturer.

[f] After preparation, following label instructions.

As the data indicated that heat-processed foods tend to have higher furan levels than do foods prepared by other processes, concern has been expressed about potentially high levels in foods that may be the sole or major source of nutrition for young children, such as infant formula and jarred or canned infant foods (EFSA, 2004, 2009; Morehouse et al., 2008; Arisseto, Vicente & Toledo, 2010). Reported levels of furans in foods sold for young children are summarized in Table 15.

Table 15. Mean furan content of infant foods used in dietary exposure assessments

Food group	Europe[a]		USA[b]		Brazil[c]	
	N	Mean concentration (µg/kg)	*N*	Mean concentration (µg/kg)	*N*	Mean concentration (µg/kg)
Baby food	447 538	24.2 (2007–2009) 26.4 (2004–2006)			31	1.7–31.8
Apple juice			5	4.0		
Apple sauce			9	4.3		
Sweet potatoes			18	80.3		
Carrots			7	37.6		
Green beans			6	48.2		
Squash			6	49.3		
Garden vegetables			7	75.5		
Dinners with chicken			7	32.9		
Pears			4	5.3		
Bananas			5	21.2		
Infant formula	28 7	21.2 (2007–2009) 9.5 (2004–2006)	4 11 15	0.9 (made up) 7.6 (concentrate) 7.2 (ready to eat)		

[a] EFSA (2009). HS-GC-MS and HS-SPME/GC-MS methods of analysis. Years data were collected are given in parentheses.
[b] Morehouse et al. (2008). Furan found in infant foods, concentration of 0 µg/kg assigned to non-detect values, HS-GC-MS method of analysis.
[c] Arisseto, Vicente & Toledo (2010).

6.2 Estimates of dietary exposure

6.2.1 International estimates of dietary exposure

As furan occurs primarily in heat-processed foods, the Committee noted that international estimates using the GEMS/Food consumption cluster diets could not be generated, as appropriate food consumption data were not available for heat-processed foods across all diets.

6.2.2 National estimates of dietary exposure

Estimates of potential dietary exposure to furans were first reported for Europe in 2004, using the USFDA mean furan concentration data and other available data sources for specific food groups combined with mean food consumption data for the European population as a whole taken from the GEMS/Food diet for Europe (EFSA, 2004; Heppner & Schlatter, 2007; Crews & Castle, 2007a).

Predicted dietary exposure to furan for individual foods from these estimates ranged from 0.04 to 1.93 µg/kg bw per day for coffee, from 0.018 to 0.0378 µg/kg bw per day for vegetables (canned or jarred), from 0.015 to 0.142 µg/kg bw per day for meat products, from 0.007 to 0.009 µg/kg bw per day for bread, from 0.021 to 0.057 µg/kg bw per day for beer, from 0.002 to 0.007 µg/kg bw per day for fruit juices, from 0.003 to 0.007 µg/kg bw per day for fish, from 0.003 to 0.005 µg/kg bw per day for milk and from 0.000 05 to 0.0002 µg/kg bw per day for honey, assuming an average body weight of 60 kg (EFSA, 2004; Crews & Castle, 2007a; Heppner & Schlatter, 2007).

More recent comprehensive reports of dietary exposure at a national level for adults were submitted by the EU to the present meeting for 14 European countries (EFSA, 2009). All these assessments were based on individual dietary records from national nutrition surveys and data collected from 2004 to 2009. Other countries were excluded, as food consumption data were not available for all the relevant food categories. For results expressed per kilogram body weight, individual body weights were used to adjust individual dietary exposure estimates before population statistics were derived. The additional estimate reported for Denmark used the Danish national nutrition survey data combined with new furan data on a number of heat-processed foods from a recent study (Fromberg, Fagt & Granby, 2009), with EFSA data used for other foods (EFSA, 2009).

The dietary exposure estimates submitted by the USA were based on 2004 and 2007 furan data. In the first estimate, the furan concentrations reported in 2004 for foods as consumed were combined with relevant food consumption amounts from 2-day individual records of 24 h recall from the 1994–1998 Continuing Survey of Food Intakes by Individuals (CSFII) surveys (Morehouse et al., 2008). These were updated in the second estimates using more recent furan data collected in 2007 (DiNovi & Mihalov, 2007).

Dietary exposure estimates for furan for adults in 14 European countries and for the whole population and children in Denmark and the USA are summarized in Table 16.

For adults, the 2009 estimates of mean dietary exposure to furan for 14 European countries ranged from 0.29 to 1.17 µg/kg bw per day. Ninety-fifth-percentile dietary exposures to furan ranged from 0.60 to 2.22 µg/kg bw per day (EFSA, 2009).

A more accurate dietary exposure estimate for Danish adults indicated mean dietary exposures to furan for adults of 27 µg/day (median 33.5 µg/day) or 0.39 µg/kg bw per day (median 0.48 µg/kg bw per day), assuming a 70 kg body weight for adults (Fromberg, Fagt & Granby, 2009). In this study, the 95th-percentile dietary exposure to furan was estimated for mean and high furan levels for coffee

Table 16. Estimates of dietary exposure to furan

Country/region	Data source	Mean exposure (μg/kg bw per day)	95th-percentile exposure (μg/kg bw per day)	Comments
Europe (summary)[a]	Concise European Food Consumption Database (adults)	0.29 min LB 0.72 median LB 1.17 max LB	0.60 min LB 1.68 median LB 2.22 max LB	Broad food categories (foods assigned analytical values to represent all foods in category, weighted means used for some food groups) Individual dietary and body weight records
	Furan levels collated from 2004–2009 records	0.29 min UB 0.72 median UB 1.17 max UB	0.69 min UB 1.75 median UB 2.27 max UB	
Austria	EFSA (2009)	0.73 LB 0.80 UB	1.77 LB 1.83 UB	24 h recall, 2005–2006, adults
Belgium	EFSA (2009)	0.67 LB 0.72 UB	1.56 LB 1.69 UB	2 × 24 h recall, 2004–2005, adults
Bulgaria	EFSA (2009)	0.29 LB 0.34 UB	0.65 LB 0.73 UB	24 h recall, 2004, adults
Czech Republic	EFSA (2009)	0.79 LB 0.85 UB	1.54 LB 1.60 UB	2 × 24 h recall, 2003–2004, adults
Denmark	EFSA (2009)	0.95 LB 1.02 UB	2.10 LB 2.19 UB	EFSA (2009): adults Fromberg, Fagt & Granby (2009): 7 day, pre-coded diary with open fields, 2000–2004, 70 kg bw for adults, assumed 20 kg bw for children aged 4–6 years
	Fromberg, Fagt & Granby (2009)	0.39 (0.48 median) adults	0.97–1.82	
	Fromberg, Fagt & Granby (2009)	0.08 (0.06 median) children 4–6 years		

Table 16 (contd)

Country/region	Data source	Mean exposure (μg/kg bw per day)	95th-percentile exposure (μg/kg bw per day)	Comments
France	EFSA (2009)	0.57 LB 0.63 UB	1.23 LB 1.30 UB	7-day dietary record, 2006–2007, adults
Great Britain	EFSA (2009)	0.98 LB 1.04 UB	2.00 LB 2.05 UB	7-day dietary record, 2000–2001, adults
Hungary	EFSA (2009)	0.38 LB 0.44 UB	0.86 LB 0.92 UB	3-day dietary record, 2003–2004, adults
Iceland	EFSA (2009)	0.63 LB 0.68 UB	1.61 LB 1.67 UB	Adults
Ireland	EFSA (2009)	0.99 LB 1.06 UB	1.85 LB 1.93 UB	7-day dietary record, 1997–1999, adults
Italy	EFSA (2009)	0.32 LB 0.39 UB	0.60 LB 0.69 UB	3-day dietary record, 2005–2006, adults
Netherlands	EFSA (2009)	1.17 LB 1.23 UB	2.22 LB 2.27 UB	2 × 24 h recall, 2003, adults
Poland	EFSA (2009)	1.01 LB 1.08 UB	1.85 LB 1.93 UB	24 h recall, 2000, adults
Slovakia	EFSA (2009)	0.70 LB 0.75 UB	1.75 LB 1.81 UB	24 h recall, 2006, adults
USA[b]	CSFII 1994–1998, whole population over 2 years, two 24 h recalls (>5000 subjects) 2004 USFDA furan levels (USFDA, 2009)	0.25 mean 2+ years 0.23 mean 2–5 years 0.24 mean 15–45 years		Individual dietary records and body weights, only foods with analytical values assigned a furan level Analytical values for foods as consumed

Table 16 (contd)

Country/region	Data source	Mean exposure (μg/kg bw per day)	95th-percentile exposure (μg/kg bw per day)	Comments
USA[c]	CSFII 1994–1998, whole population over 2 years, two 24 h recalls (>5000 subjects) 2007 USFDA furan levels (DiNovi & Mihalov, 2007)	0.26 mean 2+ years 0.41 mean 0–1 years	0.61 90th percentile 2+ years 0.99 90th percentile 0–1 years	Individual dietary records and body weights, only foods with analytical values assigned a furan level Analytical values for foods as consumed

LB, lower bound; max, maximum; min, minimum; UB, upper bound

[a] EFSA (2009). LB values assume that a concentration of 0 μg/kg is assigned to non-detects, whereas UB values assume that the LOQ is assigned to non-detects.

[b] Morehouse et al. (2008).

[c] DiNovi & Mihalov (2007).

and ranged from 0.97 µg/kg bw per day (mean furan level for coffee of 38 µg/kg) to 1.82 µg/kg bw per day (high furan level for coffee of 72 µg/kg), assuming a 70 kg body weight for adults. For Danish children aged 4–6 years, estimated mean dietary exposures were 1.5 µg/day (median 1.1 µg/day); body weights were not reported for this age group. If an average 20 kg body weight is assumed, then estimated mean dietary exposure would be 0.08 µg/kg bw per day (median 0.06 µg/kg bw per day).

Mean dietary exposure to furan for the whole population in the USA from the 2004 study was 0.25 µg/kg bw per day, and for adults 15–45 years of age, 0.24 µg/kg bw per day. The estimated mean dietary exposure to furan for children aged 2–5 years from this study was also very similar, at 0.23 µg/kg bw per day (Morehouse et al., 2008). The updated 2007 USA estimate of mean dietary exposure of 0.26 µg/kg bw per day for the whole population was consistent with the earlier findings (DiNovi & Mihalov, 2007). Additional results were also presented in the second study, with a 90th-percentile dietary exposure estimate of 0.61 µg/kg bw per day for the whole population; for infants aged 0–1 years, estimated mean dietary exposure was 0.41 µg/kg bw per day, and the 90th-percentile exposure was 0.99 µg/kg bw per day.

Estimates of dietary exposure to furan for infants in Europe and Brazil assumed that all food consumed by the infants other than infant formula had been in jars or cans; results are summarized in Table 17. These estimates were considered appropriate for infants fed solely on these products, but would be overestimates of dietary exposure for the whole infant population.

Predicted dietary exposures for European infants and children aged 3–12 months were modelled and reported by EFSA (2004, 2009), with 95th-percentile consumption results for 2009 given for two different scenarios for infants aged 6 and 9 months.

In 2004, predicted furan dietary exposures for a 6-month-old child, assuming consumption of 273 g jarred food (Kersting et al., 1998) and 870 g infant formula (EC, 2003), ranged from <0.03 to 3.5 µg/kg bw per day, using minimum and maximum furan levels reported by the USFDA in 2004 (USFDA, 2009).

In 2009, EFSA predicted dietary furan exposures for infants aged 3–12 months based on food consumption amounts for infant formula and jarred foods reported by Kersting et al. (1998) and furan levels for European countries collated from 2004–2009 records. Mean dietary exposures were 0.27 µg/kg bw per day for a 3-month-old infant, 0.93 µg/kg bw per day for a 6-month-old infant, 1.01 µg/kg bw per day for a 9-month-old infant and 0.69 µg/kg bw per day for a 12-month-old infant. Ninety-fifth-percentile dietary exposures to furan were predicted for 6- and 9-month-old infants by assuming either 95th-percentile consumption of infant formula and mean consumption of jarred foods or vice versa. For 6-month-old infants, predicted 95th-percentile exposures ranged from 1.14 to 1.34 µg/kg bw per day, and for 9-month-old infants, from 1.15 to 1.26 µg/kg bw per day; in both cases, dietary exposure was higher in the scenario where jarred foods were consumed at the 95th-percentile amount. Estimated dietary furan exposures for infants aged 6–11 months in Brazil were similar to those reported for European countries, ranging

Table 17. Predicted upper-bound dietary exposure to furans for infants

Country/region	Data source	Mean exposure (µg/kg bw per day)	95th-percentile exposure (µg/kg bw per day)[a]	Comments
Europe[b]	Kersting et al. (1998), consumption of infant formula and jarred food Furan levels collated from 2004–2009 records	0.27 3 months 0.93 6 months 1.01 9 months 0.69 12 months	— 1.14 6 months scenario 1 1.34 6 months scenario 2 1.15 9 months scenario 3 1.26 9 months scenario 4 —	Higher body weights for boys were used[c]
Europe[d]	Kersting et al. (1998), consumption of jarred food, infant formula amount from EC (2003) 2004 USFDA furan levels (USFDA, 2009)	<0.03 minimum to 3.5 maximum 6 months		Range reflects maximum and minimum furan levels reported Body weight of 7.5 kg for 6-month-old child
Brazil (Arisseto, Vicente & Toledo, 2010)	Analysed jarred baby food, recommended and actual food consumption	0.46–0.82	1.34–2.40 (99th percentile)	Range based on actual food consumption (lower estimate) and recommended consumption of jarred food (upper estimate) Body weight of 8.4 kg for 6- to 11-month-old child

[a] Scenarios 1, 3: sum of 95th-percentile consumption of infant formula and mean consumption of jarred foods for each age group; scenarios 2, 4: sum of mean consumption of infant formula and 95th-percentile consumption of jarred foods for each age group. Note factor of 0.119 used to convert dry infant formula reported by Kersting et al. (1998) to formula made up.

[b] EFSA (2009).

[c] Body weights for boys assumed: 6.4 kg, 3-month-old infant; 8.1 kg, 6-month-old infant; 9.3 kg, 9-month-old infant; 10.3 kg, 12-month-old infant.

[d] EFSA (2004); Heppner & Schlatter (2007).

from 0.42 to 0.82 µg/kg bw per day for mean exposure and from 1.34 to 2.40 µg/kg bw per day at the 99th percentile. The lower estimate was based on actual food consumption amounts, and the upper estimate on recommended food consumption amounts for infants in Brazil (Arisseto, Vicente & Toledo, 2010).

6.3 Contributors to dietary furan exposure

For adults, coffee was the major contributor to dietary furan exposure, likely to be between 40% and 80% of total dietary exposure (Crews & Castle, 2007a; Heppner & Schlatter, 2007; Morehouse et al., 2008; EFSA, 2009; Fromberg, Fagt & Granby, 2009). In the EFSA (2009) estimates, other contributors to dietary furan exposure (>5%) were reported to be cereal and cereal products (3–16%), vegetables, nuts, pulses and others (2–14%), meat and meat products (4–13%) and milk and milk-based drinks (2–9%).

For Danish children aged 4–6 years, the major contributors to dietary furan exposure were breakfast cereals (40%), sauces (14%), cake (13%), stew (11%) and fruit juice (10%). If the higher furan levels recently reported by Fromberg, Fagt & Granby (2009) are used for toasted bread instead of the mean furan level for bread, this food group would make a higher contribution.

For infants from 6 months of age, baby food sold in jars or cans was the most important contributor to dietary furan exposure (Arisseto, Vicente & Toledo, 2010).

7 DOSE–RESPONSE ANALYSIS AND ESTIMATION OF CARCINOGENIC/TOXIC RISK

7.1 Identification of key data for risk assessment

7.1.1 Pivotal data from biochemical and toxicological studies

(a) Metabolism and activation

About 80% of radiolabelled furan administered by intragastric instillation to rats was rapidly eliminated in the first 24 h, with expired air, urine and faeces all being significant routes of excretion. The great majority of the administered dose remaining in tissues (19%) was found in the liver (13%), with much smaller amounts also found in kidney, blood and small and large intestines, each accounting for less than 1% of the initial dose. In plasma and liver, no unchanged furan was present, and 80% was not extractable with organic solvents, suggesting that radioactive label from furan was bound to macromolecules.

CYP2E1 was identified as the major route of oxidation of furan. Hepatocytes from mice oxidized furan at a greater rate than those from rats or humans. The history of the human donors suggested that the variability between individuals in the rate of oxidation of furan was due to ethanol induction of CYP2E1 in two of the three individuals. Such variability might affect the extent of first-pass uptake and bioactivation following oral exposure.

Production of carbon dioxide was postulated to occur through ring opening of furan by oxidation to BDA, with subsequent formation of maleic acid followed by metabolism to carbon dioxide. The rapid metabolism of furan to carbon dioxide suggested that some of the non-extractable radioactivity in tissues could be due to amino acids in proteins derived from citric acid intermediates. The formation of the oxidative metabolite, BDA, which is extremely reactive, was confirmed in rat liver microsomes both in vitro and in vivo, and BDA was found to be rapidly conjugated with GSH. Characterization of urinary metabolites of furan from the rat suggested that in addition to its reaction with GSH, BDA readily reacts with cysteine and lysine residues in protein. The characterization of the metabolites provided evidence for alkylation of proteins by BDA through internal protein cysteine–BDA–lysine crosslinking and GSH–BDA–protein crosslinking. Other evidence for crosslinking (protein–protein, protein–DNA or DNA–DNA) is available. This, of course, would be expected for BDA, an unsaturated dialdehyde. In addition, nucleoside adducts of BDA with dCyd and dAdo have been characterized from model systems and then identified in DNA digests treated with physiologically relevant concentrations of BDA, as well as in DNA isolated from *Salmonella typhimurium* strain TA104 that had been treated with mutagenic concentrations of BDA used in the Ames assay in a dose-related manner. The nucleoside adducts of dAdo were of particular concern because of its formation of substituted etheno adducts, in the process exposing a reactive aldehyde moiety. Such etheno adducts have been implicated in the induction of point mutations and the generation of DNA–DNA and DNA–protein crosslinks.

As an early, critical, cytotoxic effect in hepatocytes, furan was found to uncouple oxidative phosphorylation in mitochondria both in vitro and in vivo.

(b) Toxicity

Single-dose and short-term repeated-dose studies clearly identify the liver as the target organ of furan toxicity by the oral route in the mouse and rat. The rat was notably more sensitive than the mouse to the toxicity of furan in terms of time to onset of liver changes and severity and incidence of lesions at a particular dose. Typically, the lesions in the rat affected the caudate and left lateral lobes of the liver and were characterized by hyperplasia and cholangiofibrosis of the biliary tract, greenish-yellow to brown pigmentation of Kupffer cells and cytomegaly, degeneration and necrosis of hepatocytes. These lesions occurred at doses that were less than those associated with increased liver weights. Nodular hyperplasia was also observed in the rat. Although furan induced the same types of hepatocelluar lesions in both species, the most prominent lesions in the rat affected the biliary tract, whereas in mice, hepatocytes and the biliary tract were equally affected by furan administration. Also, at the higher doses in the rat, renal tubular lesions were noted, as well as atrophy of the thymus, testes and ovaries; none of these effects were noted in the mouse.

A 90-day (i.e. 13-week) study in F344 rats (Gill et al., 2009) extended the dose range from that used in the 13-week NTP (1993) assays (i.e. 2–8 mg/kg bw) to as low as 0.03 mg/kg bw per day, 5 days/week. Mild subcapsular hepatic lesions consisting of rare to occasional apoptosis of hepatocytes, Kupffer cells filled with

yellow pigment and microfoci of inflammatory cells were noted at the next highest dose of 0.12 mg/kg bw per day, 5 days/week. In addition, statistically significant dose-related changes in clinical chemistry parameters related to liver function, altered thymocyte maturation and dose-related increases in platelet count (both sexes) and dose-related increases of serum thyroxine, testosterone and LH levels and of intratesticular testosterone levels (males only) were noted. Although statistically increased intratesticular testosterone levels were found at all doses tested, no adverse effects on testosterone synthesis were noted. Thus, the lowest dose tested (0.03 mg/kg bw per day, 5 days/week) appears to be a no-observed-adverse-effect level (NOAEL). Preliminary results suggested that furan affects the mRNA expression levels of StAR protein, which regulates cholesterol transfer within the mitochondria, the rate-limiting step of steroid hormone production. This is relevant to the finding that uncoupling of oxidative phosphorylation in hepatocyte mitochondria is an early, critical event in the cytolethality of furan.

In long-term studies, male rats displayed reductions in red blood cell parameters at 9 months and bone marrow hyperplasia, whereas mice were less affected.

(c) Mutagenicity and clastogenicity

Furan has been extensively tested for genotoxicity, with mixed results. In in vitro tests, results were mainly negative for DNA alteration, bacterial mutagenicity and mammalian mutagenicity. The few positive results (e.g chromosomal alteration) were accompanied by negative results for the same end-point. In vivo, furan did not induce DNA alteration, mutagenicity in *Drosophila* or bone marrow chromosomal aberrations. Mixed results were reported for SCEs in mouse bone marrow but not rat bone marrow, and increased micronuclei were reported in mouse splenocytes, but not mouse bone marrow. BDA was positive in vitro for DNA alteration, bacterial gene mutation and mammalian cell gene mutation, but not mammalian cell micronucleus induction.

(d) Carcinogenicity

Furan was carcinogenic in mice and rats, inducing tumours of the liver in both species. In mice administered furan intragastrically for 2 years, hepatocellular adenomas and carcinomas were induced by 8 mg/kg bw per day (44/50) and 15 mg/kg bw per day (50/50) in males, compared with 26/50 in controls. In females, the incidences were 34/50 and 50/50, respectively, compared with 7/50 in controls. In rats dosed intragastrically for 2 years, furan induced primarily cholangiocarcinomas: in males, 43/50 at 2 mg/kg bw per day, 48/50 at 4 mg/kg bw per day and 49/50 at 8 mg/kg bw per day, compared with 0/50 in controls; and in females, 49/50 at 2 mg/kg bw per day, 50/50 at 4 mg/kg bw per day and 48/50 at 8 mg/kg bw per day, compared with 0/50 in controls. Hepatocellular adenomas and carcinomas were present in males at incidences of 1/50 controls, 5/50 low dose, 22/50 mid dose and 35/50 high dose. In females, the incidences were 0/50, 2/50, 4/50 and 8/50, respectively. In addition, mononuclear cell leukaemias were increased in both sexes.

The induction of cholangiocarcinomas in rats, which did not occur in mice, appears to be related to the earlier development of cholangiofibrosis only in rats.

(e) Special studies

Furan did not bind to DNA in the rat liver, although it bound to liver microsomal protein in vitro. In vitro, BDA reacted with amino acids, GSH and 2′-deoxynucleosides. In studies of DNA crosslinking, negative results were obtained for furan in vivo and mixed results for BDA in vitro.

7.2 BMD analysis of tumour dose–response data

Dosing experimental animals with furan in bioassays gave rise to increases in liver tumours and leukaemias. The neoplasms evaluated were as follows: cholangiocarcinomas in livers of male and female rats, hepatocellular neoplasms in male and female rats, mononuclear cell leukaemias in male and female rats and hepatocellular neoplasms in male and female mice.

The cholangiocarcinomas were seen only in rats and were associated with extreme hepatotoxicity and an early and marked biliary tract response. The relevance for humans of the cholangiocarcinomas is not clear, and the available data do not allow for an analysis of mode of action. Also, the high incidences of these neoplasms at all doses of furan precluded identification of a point of departure. The Committee was aware of ongoing studies in rats to extend the dose–response data and address mechanistic aspects.

The modelled data are shown in Table 18. In the dose–response analysis, the United States Environmental Protection Agency (USEPA) benchmark dose (BMD) software (BMDS version 2.0) was used. Benchmark doses for 10% extra risk (BMD_{10}) of tumours and their 95% lower confidence limits ($BMDL_{10}$) were calculated by fitting the nine different statistical models to the experimental data considered relevant. The slope parameter for the log-logistic and the log-probit models was restricted to be ≥1. Only BMD_{10} and $BMDL_{10}$ were considered further, which resulted from models with an acceptable fit to the data, based on statistical considerations (chi-squared test, $P > 0.1$). The BMD_{10} and $BMDL_{10}$ (in milligrams per kilogram of body weight per day) as well as the goodness of fit criteria resulting from fitting the models to the data are tabulated, and a graph resulting from the model giving the lowest BMDL is shown.

Table 19 shows the modelling output for hepatocellular adenoma and carcinoma for female mice treated with furan in the study of Moser et al. (2009). All models except the quantal-linear model gave an acceptable fit to the data, as indicated by the high *P*-value of the chi-squared test. The BMD_{10} and $BMDL_{10}$ giving the lowest and highest accepted values are indicated in bold, and a graphical representation of the model fit is shown for the multistage model, the model resulting in the lowest $BMDL_{10}$ (Figure 2). The BMD_{10} and $BMDL_{10}$ ranged from 1.87 to 2.86 mg/kg bw per day and from 1.34 to 1.89 mg/kg bw per day, respectively.

Table 18. Experimental data used for BMD modelling

Dose (mg/kg bw per day)[a]	Incidence	Sex	Species	Reference
Hepatocellular adenoma and carcinoma				
0	1/50	Male	Rat	NTP (1993)
2	5/50	Male	Rat	NTP (1993)
4	22/50	Male	Rat	NTP (1993)
8	35/50	Male	Rat	NTP (1993)
0	0/50	Female	Rat	NTP (1993)
2	2/50	Female	Rat	NTP (1993)
4	4/50	Female	Rat	NTP (1993)
8	8/50	Female	Rat	NTP (1993)
0	26/50	Male	Mouse	NTP (1993)
8	44/50	Male	Mouse	NTP (1993)
15	50/50	Male	Mouse	NTP (1993)
0	7/50	Female	Mouse	NTP (1993)
8	34/50	Female	Mouse	NTP (1993)
15	50/50	Female	Mouse	NTP (1993)
0	3/36	Female	Mouse	Moser et al. (2009)
0.5	8/72	Female	Mouse	Moser et al. (2009)
1	6/53	Female	Mouse	Moser et al. (2009)
2	5/41	Female	Mouse	Moser et al. (2009)
4	12/36	Female	Mouse	Moser et al. (2009)
8	29/39	Female	Mouse	Moser et al. (2009)
Leukaemias				
0	8/50	Male	Rat	NTP (1993)
2	11/50	Male	Rat	NTP (1993)
4	17/50	Male	Rat	NTP (1993)
8	25/50	Male	Rat	NTP (1993)
0	8/50	Female	Rat	NTP (1993)
2	9/50	Female	Rat	NTP (1993)
4	17/50	Female	Rat	NTP (1993)
8	21/50	Female	Rat	NTP (1993)

Table 18 (contd)

Dose (mg/kg bw per day)[a]	Incidence	Sex	Species	Reference
Cholangiocarcinoma				
0	0/50	Male	Rat	NTP (1993)
2	43/50	Male	Rat	NTP (1993)
4	48/50	Male	Rat	NTP (1993)
8	49/50	Male	Rat	NTP (1993)
0	0/50	Female	Rat	NTP (1993)
2	49/50	Female	Rat	NTP (1993)
4	50/50	Female	Rat	NTP (1993)
8	48/50	Female	Rat	NTP (1993)

[a] Dosing was 5 days/week.

Table 19. Modelling output for hepatocellular adenoma and carcinoma for female mice treated with furan in the Moser et al. (2009) study

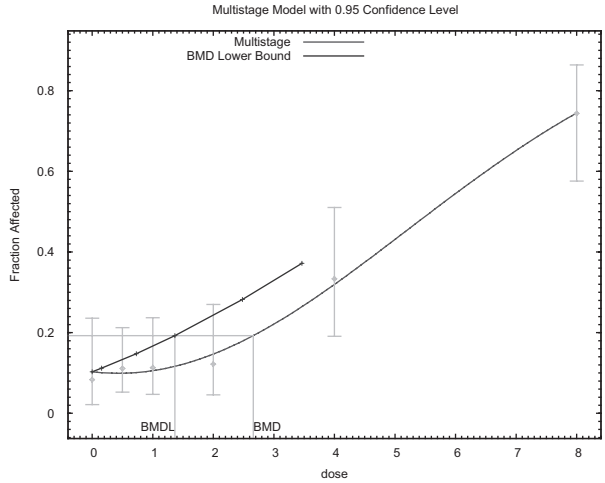
	Gamma	Logistic	Log-logistic	Log-probit	Multistage	Multistage cancer	Probit	Weibull	Quantal-linear
AIC	235.33	233.88	235.29	235.23	235.50	233.64	234.19	235.47	241.56
Chi-square	0.36	0.88	0.33	0.27	0.53	0.66	1.17	0.50	8.01
P-value	0.95	0.93	0.96	0.97	0.91	0.96	0.88	0.92	0.09
BMD_{10}[a]	2.76	2.03	2.78	**2.86**	2.66	2.34	**1.87**	2.62	0.96
$BMDL_{10}$[a]	1.65	1.71	1.77	**1.89**	**1.34**	1.34	1.59	1.53	0.74

AIC, Akaike's information criterion

[a] The BMD_{10} and $BMDL_{10}$ giving the lowest and highest accepted values are indicated in bold.

The modelling outputs for hepatocellular adenoma and carcinoma for male and female mice treated with furan in the NTP (1993) study are shown in Table 20 and Table 21, respectively. For male mice, six out of the nine models gave an acceptable fit, and the quantal-linear model resulted in the lowest $BMDL_{10}$ (Figure 3). The BMD_{10} and $BMDL_{10}$ ranged from 0.49 to 6.66 mg/kg bw per day and from 0.35 to 1.85 mg/kg bw per day, respectively. For female mice, five out of the nine models gave an acceptable fit, and the multistage model resulted in the lowest $BMDL_{10}$ (Figure 4). The BMD_{10} and $BMDL_{10}$ ranged from 1.63 to 6.88 mg/kg bw per day and from 1.07 to 4.20 mg/kg bw per day, respectively.

Figure 2. Multistage model for hepatocellular adenoma and carcinoma in female mice treated with furan in the Moser et al. (2009) study



Note: The lower line is the fit of the model to the experimental data. The vertical bars are the confidence intervals around the experimental data. The upper line is the upper bound for the response from which the lower confidence bound of the BMD (BMDL) can be defined.

Table 20. Modelling output for hepatocellular adenoma and carcinoma for male mice treated with furan in the NTP (1993) study

	Gamma	Logistic	Log-logistic	Log-probit	Multistage	Multistage cancer	Probit	Weibull	Quantal-linear
AIC	109.93	111.78	109.93	111.93	110.24	NC	111.02	111.93	112.50
Chi-square	0.00	1.17	0.00	0.00	0.18	NC	0.70	0.00	1.69
P-value	0.97	0.28	0.99	NA	0.67	NC	0.40	NA	0.19
BMD_{10}[a]	4.97	0.71	**6.66**	6.20	2.14	NC	0.78	4.13	**0.49**
$BMDL_{10}$[a]	0.45	0.53	**1.85**	1.43	0.42	NC	0.61	0.45	**0.35**

AIC, Akaike's information criterion; NA, not assessed; NC, not calculated, as the number of observations is lower than the number of parameters in the model

[a] The BMD_{10} and $BMDL_{10}$ giving the lowest and highest accepted values are indicated in bold.

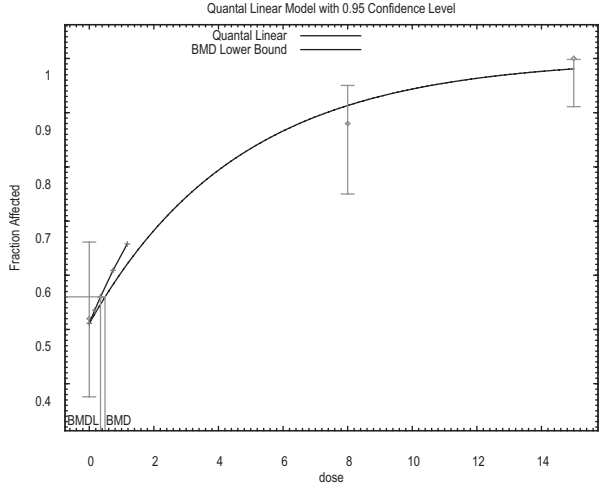
Table 21. Modelling output for hepatocellular adenoma and carcinoma for female mice treated with furan in the NTP (1993) study

	Gamma	Logistic	Log-logistic	Log-probit	Multistage	Multistage cancer	Probit	Weibull	Quantal-linear
AIC	107.22	110.65	107.18	109.18	109.23	NA	109.45	109.18	116.72
Chi-square	0.02	2.19	0.00	0.00	1.26	NA	1.49	0.00	6.56
P-value	0.90	0.14	0.99	NA	0.26	NA	0.22	NA	0.01
BMD_{10}[a]	5.37	1.77	**6.88**	6.60	2.44	NA	**1.63**	4.85	0.61
$BMDL_{10}$[a]	2.99	1.35	**4.20**	3.79	**1.07**	NA	1.29	2.17	0.48

AIC, Akaike's information criterion; NA, not assessed; NC, not calculated, as the number of observations is lower than number of parameters in the model

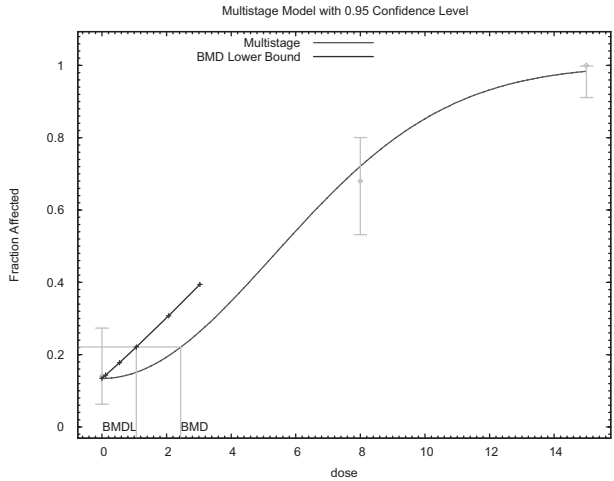
[a] The BMD_{10} and $BMDL_{10}$ giving the lowest and highest accepted values are indicated in bold.

Figure 3. Quantal-linear model for hepatocellular adenoma and carcinoma in male mice treated with furan in NTP (1993) study



Note: The lower line is the fit of the model to the experimental data. The vertical bars are the confidence intervals around the experimental data. The upper line is the upper bound for the response from which the lower confidence bound of the BMD (BMDL) can be defined.

Figure 4. Multistage model for hepatocellular adenoma and carcinoma in female mice treated with furan in the NTP (1993) study



Note: The lower line is the fit of the model to the experimental data. The vertical bars are the confidence intervals around the experimental data. The upper line is the upper bound for the response from which the lower confidence bound of the BMD (BMDL) can be defined.

The modelling outputs for hepatocellular adenoma and carcinoma for male and female rats treated with furan in the NTP (1993) study are shown in Table 22 and Table 23, respectively. For male rats, the model fits were generally poor, and only four out of the nine models just passed the goodness of fit test criterion of $P > 0.1$ and thus gave an acceptable fit. The Weibull model resulted in the lowest $BMDL_{10}$ (Figure 5), and the BMD_{10} and $BMDL_{10}$ ranged from 1.64 to 1.92 mg/kg bw per day and from 1.00 to 1.34 mg/kg bw per day, respectively. In female rats, all models gave an acceptable fit. The BMD_{10} for female rats tended to be higher than the BMD_{10} for the other sex–species combinations. The BMD_{10} and $BMDL_{10}$ ranged from 4.82 to 6.47 mg/kg bw per day and from 3.16 to 5.25 mg/kg bw per day, respectively. The log-logistic model resulted in the lowest $BMDL_{10}$ (Figure 6).

The modelling outputs for the mononuclear cell leukaemias for male and female rats treated with furan in the NTP (1993) study are given in Table 24 and Table 25, respectively. The mononuclear leukaemias in rats, which are notable in the strain used in the NTP (1993) bioassay, are of unknown pathogenesis, and the increases occurred against a background of unusually low incidences in the control groups. Moreover, studies of genotoxicity in rat bone marrow, where the progenitor cells of the leukaemias presumably arise, were negative, and there is no plausible mode of action. All models gave an acceptable fit to the data. For male rats, the BMD_{10} and $BMDL_{10}$ ranged from 1.66 to 2.47 mg/kg bw per day and from 0.97 to 1.98 mg/kg bw per day, respectively. For female rats, the respective figures are

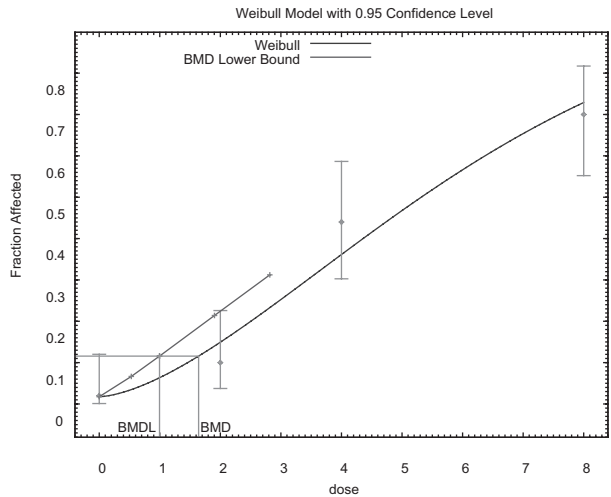
Table 22. Modelling output for hepatocellular adenoma and carcinoma for male rats treated with furan in the NTP (1993) study

	Gamma	Logistic	Log-logistic	Log-probit	Multistage	Multistage cancer	Probit	Weibull	Quantal-linear
AIC	180.07	183.40	179.41	179.11	181.16	181.16	182.14	180.58	183.15
Chi-square	2.06	7.25	1.40	1.11	3.11	3.11	6.18	2.53	6.16
P-value	0.15	0.03	0.24	0.29	0.08	0.08	0.05	0.11	0.05
BMD_{10}[a]	1.78	2.19	1.85	**1.92**	1.57	1.57	2.06	**1.64**	0.84
$BMDL_{10}$[a]	1.08	1.81	1.23	**1.34**	0.87	0.87	1.71	**1.00**	0.68

AIC, Akaike's information criterion

[a] The BMD_{10} and $BMDL_{10}$ giving the lowest and highest accepted values are indicated in bold.

Figure 5. Weibull model for hepatocellular adenoma and carcinoma in male rats treated with furan in NTP (1993) study



Note: The lower line is the fit of the model to the experimental data. The vertical bars are the confidence intervals around the experimental data. The upper line is the upper bound for the response from which the lower confidence bound of the BMD (BMDL) can be defined.

2.13–2.98 mg/kg bw per day and 1.18–2.29 mg/kg bw per day, respectively. For male and female rats, the Weibull and the log-logistic models resulted in the lowest $BMDL_{10}$, respectively (Figures 7 and 8).

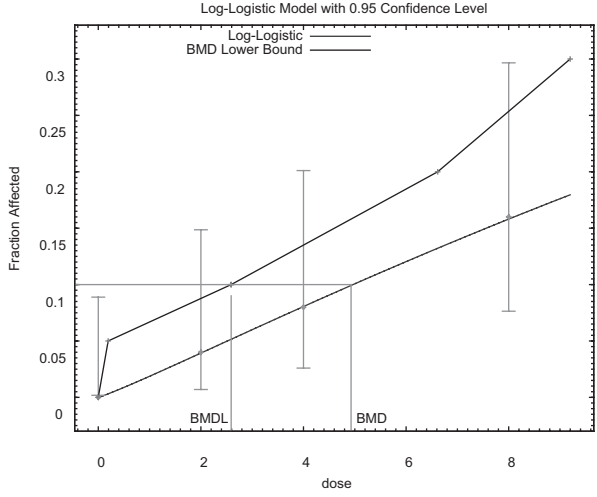
Table 23. Modelling output for hepatocellular adenoma and carcinoma for female rats treated with furan in the NTP (1993) study

	Gamma	Logistic	Log-logistic	Log-probit	Multistage	Multistage cancer	Probit	Weibull	Quantal-linear
AIC	92.64	94.98	92.64	92.65	94.64	94.64	94.65	92.64	90.65
Chi-square	0.00	1.53	0.00	0.01	0.00	0.00	1.33	0.00	0.01
P-value	1.00	0.47	1.00	1.00	1.00	1.00	0.52	1.00	1.00
BMD_{10}	4.96	**6.47**	4.93	**4.82**	5.00	5.00	6.23	4.96	4.94
$BMDL_{10}$	3.28	**5.25**	**3.16**	3.93	3.28	3.28	4.97	3.28	3.28

AIC, Akaike's information criterion

[a] The BMD_{10} and $BMDL_{10}$ giving the lowest and highest accepted values are indicated in bold.

Figure 6. Log-logistic model for hepatocellular adenoma and carcinoma in female rats treated with furan in NTP (1993) study



Note: The lower line is the fit of the model to the experimental data. The vertical bars are the confidence intervals around the experimental data. The upper line is the upper bound for the response from which the lower confidence bound of the BMD (BMDL) can be defined.

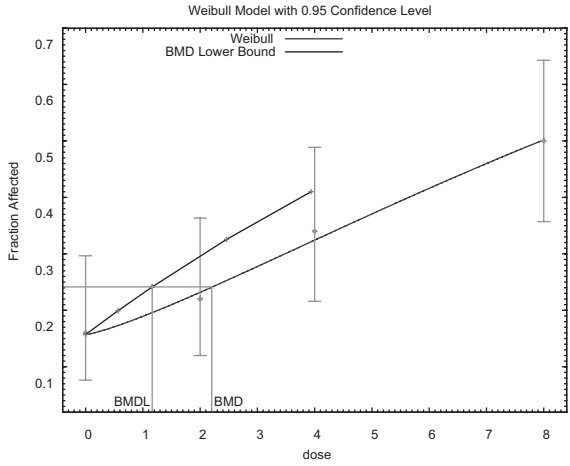
Table 24. Modelling output for leukaemias for male rats treated with furan in the NTP (1993) study

	Gamma	Logistic	Log-logistic	Log-probit	Multistage	Multistage cancer	Probit	Weibull	Quantal-linear
AIC	236.16	234.32	236.12	234.24	236.22	236.22	234.27	236.17	234.41
Chi-square	0.09	0.24	0.06	0.16	0.14	0.14	0.20	0.10	0.32
P-value	0.77	0.89	0.81	0.92	0.71	0.71	0.91	0.76	0.85
BMD_{10}[a]	2.24	**2.47**	2.26	2.32	2.11	2.11	2.35	2.20	**1.66**
$BMDL_{10}$[a]	1.16	1.98	**0.97**	**1.98**	1.16	1.16	1.88	1.16	1.15

AIC, Akaike's information criterion

[a] The BMD_{10} and $BMDL_{10}$ giving the lowest and highest accepted values are indicated in bold.

Figure 7. Weibull model for leukaemias in male rats treated with furan in the NTP (1993) study



Note: The lower line is the fit of the model to the experimental data. The vertical bars are the confidence intervals around the experimental data. The upper line is the upper bound for the response from which the lower confidence bound of the BMD (BMDL) can be defined.

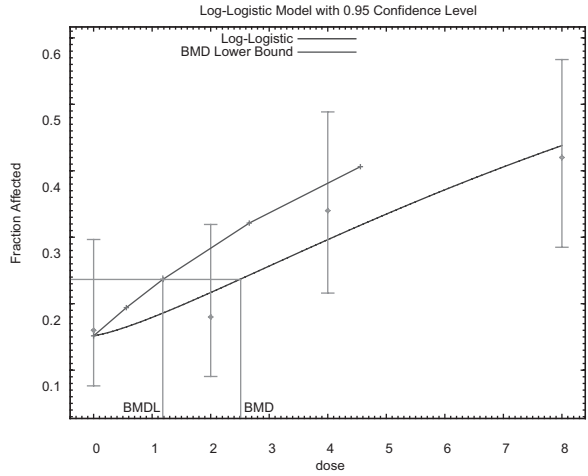
Table 25. Modelling output for leukaemias for female rats treated with furan in the NTP (1993) study

	Gamma	Logistic	Log-logistic	Log-probit	Multistage	Multistage cancer	Probit	Weibull	Quantal-linear
AIC	230.26	228.56	230.19	228.41	230.34	230.34	228.49	230.28	228.36
Chi-square	1.02	1.33	0.95	1.20	1.09	1.09	1.27	1.03	1.09
P-value	0.31	0.51	0.33	0.55	0.30	0.30	0.53	0.31	0.58
BMD_{10}[a]	2.54	**2.98**	2.51	2.58	2.27	2.27	2.86	2.49	**2.13**
$BMDL_{10}$[a]	1.40	**2.29**	**1.18**	2.32	1.39	1.39	2.18	1.40	1.39

AIC, Akaike's information criterion

[a] The BMD_{10} and $BMDL_{10}$ giving the lowest and highest accepted values are indicated in bold.

Figure 8. Log-logistic model for leukaemias in female rats treated with furan in the NTP (1993) study



Note: The lower line is the fit of the model to the experimental data. The vertical bars are the confidence intervals around the experimental data. The upper line is the upper bound for the response from which the lower confidence bound of the BMD (BMDL) can be defined.

Table 26 summarizes the modelling output for all end-points considered and lists the ranges of BMD_{10}s and $BMDL_{10}$s. The BMD_{10}s and $BMDL_{10}$s derived from the different data were broadly similar. Those for the hepatocellular adenomas and carcinomas in male mice were the lowest but varied over a broad range, and there was a high incidence of liver tumours in the control male mice. The study of Moser

Table 26. Ranges of BMD_{10} and $BMDL_{10}$ values for tumours associated with administration of furan by gavage

Tumour type	Study	Sex and species	BMD_{10}[a] (mg/kg bw per day)	$BMDL_{10}$[a] (mg/kg bw per day)
Hepatocellular adenomas and carcinoma	Moser et al. (2009)	Female mice	1.87–2.86	1.34–1.89
	NTP (1993)	Male mice	0.49–6.66	0.35–1.85
	NTP (1993)	Female mice	1.63–6.88	1.07–4.20
	NTP (1993)	Male rat	1.64–1.92	1.00–1.34
	NTP (1993)	Female rat	4.82–6.47	3.16–5.25
Leukaemia	NTP (1993)	Male rat	1.66–2.47	0.97–1.98
	NTP (1993)	Female rat	2.13–2.98	1.18–2.29

BMD_{10}, benchmark dose for 10% extra risk of tumours; $BMDL_{10}$, 95% lower confidence limit for the benchmark dose for 10% extra risk of tumours. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls.

[a] BMD_{10}s and $BMDL_{10}$s have not been adjusted for the dosing schedule of 5 days/week.

et al. (2009) had more and lower doses and a greater number of animals in the low-dose group compared with the NTP (1993) studies. A comparison of the BMD_{10}s and $BMDL_{10}$s derived from the Moser et al. (2009) and NTP (1993) studies with the dosages used in each study indicates that those derived from the Moser et al. (2009) study are much closer to the dosage levels than those in the NTP (1993) study. This indicates that the BMD_{10}s and $BMDL_{10}$s derived from the Moser et al. (2009) study had less uncertainty than those derived from the NTP (1993) studies. Therefore, the Committee decided to use the $BMDL_{10}$ of 1.3 mg/kg bw per day, derived from the hepatocellular adenoma/carcinoma data from the Moser et al. (2009) study, as the point of departure.

8. COMMENTS

8.1 Absorption, distribution, metabolism and excretion

Following oral administration to mice and rats, furan is rapidly absorbed, metabolized and eliminated in urine and faeces as metabolites and exhaled in air as unchanged furan and carbon dioxide formed as a result of ring opening. The initial ring-opened metabolite is BDA, which is formed in the liver in a reaction catalysed by CYP2E1. Furan-derived products are most abundant in the liver of dosed animals. A variety of identified urinary metabolites could arise from amino acid or protein crosslinking.

8.2 Toxicological data

The toxicity of orally administered furan has been extensively studied in mice and rats over a wide dose range. The primary site of toxicity of furan is the liver, although the kidneys and lungs are also affected at high doses (>30 mg/kg bw per day). In addition, changes in some haematological and hormonal parameters occur at doses as low as 0.12 mg/kg bw per day administered 5 days/week.

Regarding hepatotoxicity, uncoupling of hepatocyte mitochrondrial oxidative phosphorylation is an early critical event in cytolethality. Liver cell injury, including oxidative stress, progresses to cell death. This, in turn, gives rise to regenerative responses, including increased hepatocellular proliferation in mice and rats and, notably in the rat, an early proliferative reaction involving the biliary epithelium, referred to as cholangiofibrosis. These proliferative changes may be the basis for liver tumorigenicity, either alone or in combination with DNA alteration. Although furan is not genotoxic in a number of test systems and binding to rat liver DNA was not detectable, the metabolite BDA is highly reactive and binds to proteins and nucleic acids. BDA produced DNA strand breaks in cultured mammalian cells and was mutagenic in bacteria and cultured mammalian cells; being a dialdehyde, it also formed crosslinks with DNA of cultured cells. The in vitro genotoxicity of BDA allows the possibility that BDA formed in vivo from furan could react with DNA.

Several cancer bioassays of orally (gavage) administered furan in mice and rats have been performed. In mice, doses of 8 and 15 mg/kg bw per day, administered 5 days/week (NTP, 1993), and 0.5, 1.0, 2.0, 4.0 and 8.0 mg/kg bw per day, administered 5 days/week (Moser et al., 2009), were used. In rats, doses of 2, 4 and 8 mg/kg bw per day were administered 5 days/week (NTP, 1993). In livers of male and female rats, high incidences of cholangiocarcinomas were induced at all doses in the NTP (1993) study, accompanied by biliary tract hyperplasia, metaplasia and fibrosis. Hepatocellular neoplasms were increased at lower incidences. In both sexes of rat, furan also increased the incidences of mononuclear cell leukaemia, albeit against unusually low background incidences in control groups. In male and female mice in both studies, only hepatocellular neoplasms were increased.

8.3 Observations in humans

No epidemiological studies were available.

8.4 Analytical methods

GC-MS has been shown to be the most suitable technique for the reliable detection of low levels of furan in foods. GC-MS is usually preceded by HS extraction or HS-SPME. Both HS and HS-SPME approaches are simple and convenient and give satisfactory results for analyses of volatiles. Owing to the high volatility of furan, food samples and standards need to be chilled and handled quickly. Puréed, liquid samples or reconstituted powdered samples can be transferred directly to HS vials, whereas solid samples have to be homogenized. Most published methods include the use of deuterium-labelled furan as an internal standard, which is normally added to the homogenized sample before the extraction. LODs and LOQs from 0.1 to 5 ng/g and from 0.4 to 13 ng/g, respectively, have been reported for methods based

on HS extraction. Lower LODs and LOQs are reported for methods using HS-SPME. No certified reference material is currently available.

8.5 Formation, effects of processing and fate in foods

Furan can be formed in a variety of foods from different precursors by thermal and non-thermal processing (ionizing radiation). The proposed routes for furan formation are mainly based on 1) Maillard reactions, 2) thermal degradation of carbohydrates, 3) thermal degradation of certain amino acids, 4) thermal oxidation of ascorbic acid, polyunsaturated fatty acids and carotenoids and 5) free radical reactions during irradiation. Higher amounts of furan are normally formed under roasting conditions (dry heating, 200 °C, 10 min) compared with pressure-cooking conditions (sterilization, 121 °C, 25 min), and pH plays a complex role in the mechanism of furan formation. For coffee, the amount of furan formed in beans varies according to the level of roasting. Grinding may reduce furan levels by 10–60%, and further decreases occur in the production of instant coffee powder and in brewing.

Limited data are available on the formation of furan in home-cooked food as well as on the stability of furan during cooking, storing and reheating of meals. As furan appears to be well dissolved within the matrix, opening the jars (e.g. baby foods) exposes only a relatively small surface area. Therefore, despite furan's volatility, its evaporation is hindered by its slow diffusion inside the food matrix. However, if canned or jarred foods are heated in a saucepan under stirring, larger declines of furan content can be observed. Studies on the losses of furan during warming procedures for ready-to-eat foods have shown conflicting results, with some authors reporting losses of 29–85% and others finding that furan persists during normal heating practices. Losses of furan in heated foods left for cooling seem to be insignificant.

8.6 Levels and patterns of contamination in food commodities

Furan concentration data covering 21 countries were submitted by Australia, Brazil, Canada, the EU, Japan, the Republic of Korea, Switzerland and the USA. The total number of analytical results (single or composite samples) evaluated at the present meeting was 5662, with 59.8% from Europe, 16.7% from North America, 22.8% from Asia, 1.0% from Latin America and 0.7% from the Pacific region. The occurrence of furan has been investigated mainly in thermally processed foods, such as coffee, canned and jarred foods, including baby foods, soups and sauces. The ranges of national mean levels of furan for the foods with the highest contamination levels were as follows: roasted coffee (powder), 814–4590 µg/kg; instant coffee (powder), 90– 783 µg/kg; brewed roasted coffee, 34–113 µg/kg; jarred baby foods, 19–96 µg/kg; soya sauce, 16–52 µg/kg; canned fish, 6–76 µg/kg; and baked beans, 27–581 µg/kg. Lower levels have been found in other foods, including products from vegetables, meat, milk and cereals.

8.7 Food consumption and dietary exposure assessment

Although the presence of furan as a flavour component in food was first reported in 1979, dietary exposure assessments for furan were not undertaken until 2004, when data on furan concentrations in a variety of foods in the USA became available.

At the present meeting, the Committee considered dietary exposure estimates for furan submitted by the USA, the EU and Brazil, all of which were based on analysed data for foods and individual dietary records for the populations of interest. The dietary exposure estimates for the whole population, infants and young children in the USA and Denmark were considered by the Committee to underestimate dietary exposure, as furan levels were assigned to the specific foods analysed only and hence did not represent the whole food supply. In contrast, the dietary exposure estimates for adults submitted by the EU for 14 European countries were considered to be overestimates; as the mean furan levels from 2004–2009 results for individual foods were grouped and then assigned to the food consumption amount for the relevant wider food group, as described in the EFSA Concise European Food Consumption Database, some uncertainty was introduced in these dietary exposure assessments. For example, the furan level for coffee was assigned to the wider food group "coffee, tea and cocoa"; as levels of furan are much higher in coffee than in either tea or cocoa, this results in an overestimate of dietary exposure to furan from these beverages.

For infants and young children, concern has been expressed about potential dietary exposure to furan from the consumption of baby foods sold in jars or cans. Estimates of dietary exposure to furan for infants in Europe and Brazil assumed that all food consumed by the infants had been in jars or cans; these estimates were considered appropriate for infants fed solely on these products, but would be overestimates of dietary exposure for the whole infant population.

As furan occurs primarily in heat-processed foods, the Committee noted that international estimates using the GEMS/Food consumption cluster diets could not be generated, as appropriate food consumption data were not available for heat-processed foods.

In general, mean dietary exposure to furan from national assessments ranged from 0.25 to 1.17 µg/kg bw per day for adults, from 0.08 to 0.23 µg/kg bw per day for children 1–6 years of age and from 0.27–1.01 µg/kg bw per day for infants up to 12 months of age. For consumers at high percentiles of dietary exposure, estimates ranged from 0.60 to 2.22 µg/kg bw per day for adults and from 0.99 to 1.34 µg/kg bw per day for infants; no high-percentile dietary exposure data were available for children. Estimates of dietary exposure to furan are summarized in Table 27.

For adults, coffee was the major contributor to dietary furan exposures (40–80%), with cereals, vegetables, meats and dairy foods contributing more than 5% to total exposure. For children, breakfast cereals were the major contributor (40%). As reported furan levels were much higher for brewed coffee than for ready-to-drink instant coffee, the type of coffee consumed in a given population and the

Table 27. Estimates of dietary exposure to furan

Country	Dietary exposure estimate (µg/kg bw per day)	
	Mean	Upper percentile
Europe		
Europe[a]	0.29–1.17 adults 0.27–1.01 infants 3–12 months	0.60–2.22 adults (95th) 1.14–1.34 infants 6–9 months (95th)
Denmark[b]	0.95–1.02 adults 0.08 children 4–6 years	2.10–2.19 adults (95th)
North America		
USA[c]	0.25–0.26 adults 0.23 children 2–5 years 0.41 infants 0–12 months	0.61 adults (90th) 0.99 infants 0–12 months (90th)
South America		
Brazil[d]	0.46 infants 6–11 months	1.34 infants 6–11 months (99th)

[a] Individual dietary records for 14 European countries from the Concise European Food Consumption Database; analysed furan values from period 2004–2009.
[b] Individual dietary records from the Danish National Nutrition Survey; new furan data for some heat-processed foods; EFSA data for other foods.
[c] Individual dietary records from the USA 1994–1996, 1998 supplementary CSFII; analysed furan values from 2003 and 2007 surveys.
[d] Individual dietary records for infants; analysed data for baby food.

furan level for coffee influenced the dietary exposure estimates for adults. The lower values obtained in the USFDA estimates for adults compared with those from EFSA for European countries were largely explained by the lower furan level for brewed coffee in the USFDA estimates. Despite this, estimated dietary furan exposures available to the Committee were in the same order of magnitude.

For the purposes of risk characterization, a value of 1 µg/kg bw per day was taken to represent mean dietary exposure to furan, and a value of 2 µg/kg bw per day was taken to represent high dietary exposure. The Committee considered these values to be sufficient to cover potential dietary exposures of infants and children to furan.

8.8 Dose–response analysis

Dosing with furan in bioassays gave rise to increases in liver tumours and leukaemias. The neoplasms evaluated for dose–response analysis were as follows: cholangiocarcinomas in livers of male and female rats, hepatocellular neoplasms in male and female rats, mononuclear cell leukaemias in male and female rats and hepatocellular neoplasms in male and female mice.

The cholangiocarcinomas were seen only in rats and were associated with extreme hepatotoxicity and an early and marked biliary tract proliferative response.

The relevance for humans of the cholangiocarcinomas is not clear, and the available data do not allow for an analysis of the mode of action. Also, the high incidences of these neoplasms at all doses of furan precluded identification of a point of departure. The Committee was aware of ongoing studies in rats to extend the dose–response data and address mechanistic aspects for this end-point.

The mononuclear cell leukaemias in rats, which occur in high incidence in the strain used in the NTP (1993) bioassay, are of unknown pathogenesis, and the increases occurred against a background of unusually low incidences in the control groups. Moreover, studies of genotoxicity in rat bone marrow, where the progenitor cells of the leukaemias presumably arise, were negative, and the mode of action is unknown.

The hepatocellular neoplasms in rats in the NTP (1993) study and in mice in the NTP (1993) study and the study of Moser et al. (2009) and the leukaemias in rats in the NTP (1993) study were selected for modelling.

In the dose–response analysis using the USEPA BMD software (BMDS version 2.0), the nine different statistical models were fitted to the experimental data considered relevant for further consideration. Those resulting in acceptable fits based on statistical considerations (chi-squared test, $P > 0.1$) were selected to derive the BMD and BMDL for a 10% extra risk of tumours. This procedure resulted in a range of BMD_{10} and $BMDL_{10}$ values for each end-point considered (see Table 26 above).

The BMD_{10}s and $BMDL_{10}$s derived from the different data were broadly similar. Those for the hepatocellular adenomas and carcinomas in male mice were the lowest but varied over a broad range, and there was a high incidence of liver tumours in the control male mice. The study of Moser et al. (2009) had more and lower doses and a greater number of animals in the low-dose group compared with the NTP (1993) studies. For each study, a comparison of the BMD_{10}s and $BMDL_{10}$s derived with the dosages used indicates that those derived from the Moser et al. (2009) study are much closer to the dosage levels used in that study. This indicates that the BMD_{10}s and $BMDL_{10}$s derived from the Moser et al. (2009) study had less uncertainty than those derived from the NTP (1993) studies. Therefore, the Committee decided to use the $BMDL_{10}$ of 1.34 mg/kg bw per day, which corresponds to 0.96 mg/kg bw per day when adjusted from a 5 days/week dosing schedule to an average daily dose, in female mice derived from the hepatocellular adenoma and carcinoma data from the Moser et al. (2009) study as the point of departure.

9. EVALUATION

Margins of exposure (MOEs) were calculated at dietary exposures of 0.001 mg/kg bw per day, to represent the average dietary exposure to furan for the general population, and 0.002 mg/kg bw per day, to represent the dietary exposure to furan for consumers with high exposure. This estimate will also cover dietary exposure of children. Comparison of these dietary exposures with the $BMDL_{10}$ of 0.96 mg/kg bw per day for induction of hepatocellular adenomas and carcinomas

in female mice gives MOEs of 960 and 480 for average and high dietary exposures, respectively. The Committee considered that these MOEs indicate a human health concern for a carcinogenic compound that might act via a DNA-reactive genotoxic metabolite.

The furan levels can be reduced in some foods through volatilization (e.g. by heating and stirring canned/jarred foods in an open saucepan). However, there is currently a lack of quantitative data for all foods, and no information is available on other mitigation methods.

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APPENDIX 1. DATA ON FURAN CONCENTRATIONS IN FOODS FROM 2004 TO 2009

a. Australia

Coffee type	Total number of samples	Furan concentration (µg/l)	
		Mean	Minimum–maximum
Cappuccino	10	32.3	23–50
Latte	10	22.9	7–49
Flat white	8	33.2	25–53
Long black	4	42.0	16–77
Short black	2	112.5	15–210
Mocha	2	23.5	12–35
Instant black	1	2.0	—
Instant white	2	2.5	2–3
Ground coffee	2	23.0	22–24

Source of analytical data: Food Standards Australia New Zealand (FSANZ, 2008).

b. Brazil

Food groups	Total number of samples	Number of positive samples	Furan concentration (µg/kg)[a]			
			Mean (trace = 0)	Mean (trace = LOQ)	Minimum	Maximum
Baby food	31	20	17.8	18.6	2.5	95.5
Canned feijoada (black beans)	2	0	0	2.4	—	—
Canned vegetables	3	0	0	2.4	—	—
Canned peach	2	0	0	2.4	—	—
Canned tuna	3	2	5.6	6.4	7.7	9.2
Canned tomato purée	1	0	0	2.4	—	—

Brazil (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)[a]			
			Mean (trace = 0)	Mean (trace = LOQ)	Minimum	Maximum
Canned tomato sauce	3	0	0	2.4	—	—
Jam (strawberry)	1	0	0	2.4	—	—
Doce de leite (milk-based sweet)	2	1	1.9	3.1	—	—
Soya sauce	3	3	25.7	25.7	13.1	50.0
Roasted ground coffee (powder)	3	3	2998.1	2998.1	1946.4	5021.4
Bread	1	1	8.5	8.5	—	—

[a] All foods were analysed as bought. Two means are given in the table: the "lower bound" derived by assuming that not detected results and results between the LOD and the LOQ are zero (trace = 0), and the "upper bound" derived by assuming that not detected results and results between the LOD and the LOQ are equal to the LOQ of 2.4 μg/kg (trace = LOQ). If all analysed samples within the same food group showed quantifiable results, the two mean values are similar. Not detected results are less than the LOD of 0.7 μg/kg. Minimum and maximum values were derived assuming only quantifiable results.

Source of analytical data: Arisseto, Vicente & Toledo (2009)

c. Canada

Food groups	Total number of samples	Furan concentration (μg/kg)	
		Mean	Minimum–maximum
Juice and drinks (cocktail)	3	13.1	6.7–16.7
Apple sauce	5	11.1	6.3–19.2
Mixed fruits	4	27.2	4.8–51.2
Peaches	2	17.7	13.4–21.9
Pineapples	3	4.8	3.4–6.7
Asparagus	4	5.5	2.8–11.4
Beans	15	63.3	18.2–195
Beets	4	100.4	11.7–338

Canada (contd)

Food groups	Total number of samples	Furan concentration (μg/kg)	
		Mean	Minimum–maximum
Carrots	5	43.9	26.9–70.9
Corn	5	36.1	28.1–52.1
Mushrooms	5	17.2	11.3–26.0
Peas	5	40.3	26.8–73.5
Potatoes	4	65.2	20.2–114
Tomatoes & pasta sauces	22	51.9	6.3–200
Tomato/vegetable juices (clamato, cocktail)	8	8.5	4.3–14.1
Other vegetables (artichoke, palm)	4	9.5	1.1–27
Condiments & sauces (e.g. ketchup, chili)	12	60.7	7.7–286
Baked beans	5	580.6	368–824
Pasta	5	396.4	151–1230
Peanut butter	1	10.6	—
Tuna	5	21.8	15.4–27.9
Salmon	5	13.4	9.3–18.1
Sardines	4	33.5	17.5–65.5
Shellfish	1	171	—
Soup (condensed cream of mushroom)	1	93.5	—
Chili con carne	5	386.4	241–863
Meat spreads	5	100.7	51–172
Meat (luncheon meat, flakes)	5	22.3	13.9–39
Stew & meatball	4	309	34.6–1030
Ground roasted coffee (powder)	1	4590	—
Instant coffee (powder)	2	413	279–547
Baby food	17	96.3	8.5–331

Source of analytical data: Becalski et al. (2009)

d. European Union

Food groups	Total number of samples	Furan concentration (μg/kg)[a,b]		
		Mean (lower–upper)	Minimum	Maximum
Instant coffee	48	588–589	0–8	2200
Roasted bean coffee	9	2271–2272	0–5	4895
Roasted ground coffee	66	1112–1114	0–5	5749
Coffee (non-specified)	275	1691	0–2	6500
Baby food	985	24–25	0–0.03	215
Infant formula	35	18–19	0–2	56
Baked beans	42	25–27	0–4	80
Beer	86	4–6	0–1	28
Cereal product	99	10–14	0–0.2	168
Fish	9	8–10	0–4	24
Fruit juice	203	4–6	0–0.5	420
Fruits	84	2–7	0–0.6	27
Meat product	65	19–22	0–2	115
Milk product	20	13–15	0–1	80
Sauces	207	8–12	0–0.1	120
Soups	198	23–24	0–0.7	225
Soya sauce	51	23–25	0–10	78
Vegetable juice	45	2–7	0–1	20
Vegetables	95	7–12	0–1	74
Other products	286	22–23	0–1	164

[a] With the exception of the maximum value, a range is provided when there was a difference between the estimated lower- and upper-bound furan concentrations. The lower-bound value was derived by assuming that values below the LOD and values between the LOD and the LOQ were set to zero, and the upper-bound value was derived by assuming that values below the LOD and values between the LOD and the LOQ were set to the LOD or the LOQ, respectively.

[b] Only 16% of the results in the EFSA database were obtained after preparation of the sample as consumed before the analysis.

Source of analytical data: EFSA (2009)

e. Japan

Food groups	Total number of samples	Furan concentration (μg/kg)	
		Mean	Minimum–maximum
Baby foods	15	22.2	1.4–90
Infant formula[a]	11	3.9–4.6	nd–36

nd, not detected

[a] For infant formula, two means are given in the table: the "lower bound" derived by assuming that not detected results and results between the LOD and the LOQ are zero, and the "upper bound" derived by assuming that not detected results and results between the LOD and the LOQ are equal to the LOQ of 1 μg/kg.

Source of analytical data: Yoshida et al. (2007)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)[a]			
			Mean (trace = 0)	Mean (trace = LOQ)	Minimum	Maximum
Fruit juices	26	24	2.7	2.7	1.1	5.4
Grapefruit juice	1	1	1.8	1.8	—	—
Apple juice	10	8	2.1	2.1	0.6	5.6
Grape juice	4	3	0.8	0.8	0.7	1.3
Pineapple juice	2	2	1.5	1.5	1.1	1.9
Orange juice	3	1	0.5	0.7	—	—
Dried fish	23	16	4.9	4.9	1.6	22
Canned vegetables	15	15	16	16	1.5	79
Canned boiled soya beans	10	10	29	29	7.4	54
Canned fruits	15	14	2.7	2.7	1.6	6.8
Canned cooked foods	15	15	69	69	11	140
Canned sauces	5	5	73	73	18	130
Canned soups	10	10	19	19	7.7	44
Retort cooked foods	100	100	42	42	4.5	140

Japan (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)[a]			
			Mean (trace = 0)	Mean (trace = LOQ)	Minimum	Maximum
Retort ready-to-eat baby foods	40	40	30	30	8.7	86
Jarred infant cooked foods	35	35	21	21	3.6	55
Jarred infant cooked foods (fruits and vegetables)	5	5	43	43	5	140
Barley tea	3	3	17	17	4.4	30
Canned coffee	80	80	65	65	4.1	150
Canned brewed green tea	31	30	1.4	1.4	0.4	3.3
Soya sauces	30	30	40	40	16	100
Liquid seasonings made from soya sauce	20	20	45	45	19	100
Fermented soya bean pastes	30	30	34	34	2.0	290
Fermented soya bean pastes made with soya bean kouji	30	30	250	250	87	770
Fermented soya bean (i.e. natto)	30	0	0.0	0.4	—	—
Deep fried soya bean curds	30	1	0.0	1.0	—	—
Soya bean curds	10	5	1.1	1.1	1.4	2.3
Soya bean milks	10	2	0.3	0.5	0.6	2.4
Emulsified sauces	6	3	6.4	6.4	2.2	31
Other sauces	14	14	33	33	6.4	58
Canned fish	20	20	76	76	3.2	300
Salted and dried fishes	17	12	6.8	6.8	1.4	22

Japan (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)[a]			
			Mean (trace = 0)	Mean (trace = LOQ)	Minimum	Maximum
Steamed fish paste	20	1	0.065	0.48	—	—
Brewed barley tea	10	5	2.1	2.1	2.0	8.8
Brewed roasted tea	10	10	8.7	8.7	3.6	16
Brewed coffee	30	30	34	34	5.8	150
Japanese seasoning	10	10	8.8	8.8	4.3	13

[a] Two means are given in the table: the "lower bound" derived by assuming that not detected results and results between the LOD and the LOQ are zero (trace = 0), and the "upper bound" derived by assuming that not detected results and results between the LOD and the LOQ are equal to the LOQ (trace = LOQ). If not detected results and results between the LOD and the LOQ were not considered in calculations or all analysed samples within the same food group showed quantifiable results, the two mean values are similar. Canned cooked foods, canned soups and retort cooked foods were prepared according to the instructions on the package.

Source of analytical data: Japan Ministry of Agriculture, Forestry and Fisheries (MAFF, 2009a)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)	
			Median	Minimum–maximum
Fruit (can)	3	1	4.0	—
Vegetables (can)	3	3	8.0	4.0–47
Curry (can)	1	1	200	—
Stew (can)	1	1	58	—
Seasoned vegetables (can)	1	1	70	—
Meat sauce (can)	1	1	140	—
Soup (can)	2	2	36	25–47
Retort pouch curry (aluminium pouch)	10	10	46.6	30–110
Retort pouch stew (aluminium pouch)	1	1	16	—
Retort pouch hashed beef (aluminium pouch)	1	1	32	—

Japan (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)	
			Median	Minimum–maximum
Retort pouch soup (aluminium pouch)	2	2	22.5	20–25
Retort pouch pasta sauce (aluminium pouch)	4	4	47.5	10–100
Retort pouch rice bowl (aluminium pouch)	2	2	46.5	36–57
Infant and toddler foods (aluminium pouch)	8	8	16	9–57
Infant and toddler foods (glass bottle)	8	8	16	3–140
Infant and toddler drinks (glass bottle)	2	2	2.6	1.8–3.3
Infant and toddler drinks (PET bottle)	2	1	18	—
Coffee (can)	10	10	58.5	35–120
Coffee (PET bottle)	4	4	89.5	43–120
Coffee (carton)	2	2	61	39–83
Tea (can)	3	3	1.4	1.2–1.6
Tea (carton)	1	1	2.7	—
Tea (PET bottle)	2	1	1.3	—
Vegetable juice (can)	3	3	4.0	3.8–4.5
Vegetable juice (carton)	3	3	1.9	1.2–2.3
Soya sauce (glass bottle)	1	1	30	—
Soya sauce (PET bottle)	5	5	36	22–54
Noodle sauce (glass bottle)	2	2	67	40–94
Noodle sauce (PET bottle)	2	2	34.5	21–48
Soya bean paste (plastic container)	5	5	55	2–280
Soya bean paste (bag)	1	1	9.0	—
Emulsified sauce (plastic container)	1	0	—	—
Non-emulsified sauce (plastic container)	3	3	40	37–43

PET, polyethylene terephthalate
Source of analytical data: Japan Ministry of Agriculture, Forestry and Fisheries (MAFF, 2009b)

f. Republic of Korea

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)	
			Mean of positives	Minimum–maximum
Baby food (powdered milk)	47	29	3.8	1.0–20.7
Baby food (soup)	50	50	22.5	2.1–102.5
Baby food (beverages)	20	17	5.5	1.2–21
Sweet corn (canned, jarred)	9	9	8.6	2.1–23
Kidney bean (canned, jarred)	3	3	16.2	0.9–47.5
Bamboo shoot (canned, jarred)	2	2	2.1	0.9–3.6
Mushroom (canned, jarred)	3	3	11.4	2.3–30.9
Sesame leaf (canned, jarred)	3	3	44.1	23.1–58.3
Baby corn (canned, jarred)	1	1	5.7	5.6–5.8
Olive (canned, jarred)	6	6	3.3	1.3–6.3
Cucumber pickle (canned, jarred)	2	2	4.0	2.3–5.8
Caper (canned, jarred)	2	2	2.9	2.6–3.9
White peach (canned)	1	1	3.5	2.5–5.0
Mandarin (canned)	1	1	2.6	2.1–3.0
Mango (canned)	1	1	1.3	0.8–2.1
Cherry (canned)	1	1	4.0	2–8
Pork luncheon meat (canned)	12	12	9.2	2.8–17.8
Korean seasoned pork or beef (canned)	5	5	63.3	14–194
Chicken (canned)	2	2	17.7	15.3–22
Tuna (canned)	13	13	27.4	5.7–79.4
Mackerel (canned)	5	5	21.7	10.6–43.3
Saury (canned)	5	5	66.7	20.8–211.6
Whelk (canned)	4	4	38.6	28.4–60.6
Oyster (canned)	1	1	181.6	169.1–194.1
Crab (canned)	1	1	0.4	0.4–0.5

Republic of Korea (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)	
			Mean of positives	Minimum–maximum
Curry	10	7	10.7	7.7–15.1
Stir-fried bean paste	10	10	42.3	15.9–65.4
Instant soup	7	7	6.6	3.4–9.5
Thick beef soup	3	3	2.7	1.9–3.7
Soup (powder)	10	10	17.6	2.1–42.1
Soup (instant)	5	5	18.5	9.0–36.2
Soya sauce	5	5	16.3	1.5–28.4
Chili sauce	1	1	6.7	6.1–7.0
Pork rib sauce	1	1	12	10.–13.7
Pepper sauce	1	1	12.7	9.5–17.8
Oyster sauce	1	1	38.1	35.2–43.8
Spaghetti sauce	1	1	40.6	37.7–45.9
Jam (strawberry)	9	8	3.3	2.2–4.0
Jam (grape)	3	3	2.4	1.9–3.0
Jam (blueberry)	1	1	2.3	2.3–2.4
Jam (apple)	1	1	4.8	4.5–5.0
Jam (mixed fruits)	1	1	3.1	2.6–3.4
Bread	5	2	1.9	1.5–2.3
Biscuit	15	15	7.6	1.8–26.3
Snack	10	10	6.8	2.8–14.1
Orange juice (canned, jarred)	14	11	4.8	3.2–7.6
Grape juice (canned, jarred)	10	9	3.7	2.6–9.2
Pomegranate juice (canned, jarred)	2	2	3.6	2.7–4.6
Mango juice (canned, jarred)	2	2	3.3	2.8–3.8
Pear juice (canned, jarred)	1	1	3.0	2.9–3.0
Citrus juice (canned, jarred)	1	1	2.3	2.3–2.3

Republic of Korea (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)	
			Mean of positives	Minimum–maximum
Lemon juice (canned, jarred)	1	1	1.7	1.7–1.7
Apple juice (canned, jarred)	1	1	5.2	5.1–5.4
Aloe juice (canned, jarred)	1	1	5.5	5.4–5.7
Tomato juice (canned, jarred)	2	2	5.7	3.9–7.8
Japanese apricot juice (canned, jarred)	1	0	—	—
Mandarin juice (canned, jarred)	2	2	2.2	1.9–2.4
Mixture juice (canned, jarred)	2	2	3.0	1.9–4.3
Nutritional/diet drinks (vitamin)	4	4	7.4	4.9–10.1
Nutritional/diet drinks (red ginseng)	3	3	7.1	0.8–19.6
Nutritional/diet drinks (dietary fibre)	2	2	7.8	2.4–13.7
Nutritional/diet drinks (royal jelly)	1	1	6.0	3.5–7.4
Instant coffee (powder)	11	11	90.1	22.6–224.5
Instant coffee (liquid)	11	11	3.5	0.7–4.5
Coffee mix (powder)	10	10	54.2	26.4–99
Coffee mix (liquid)	10	10	3.6	1.1–5.5
Ground roasted coffee (powder)	10	10	814.1	267.1–2552.7
Ground roasted coffee (brewed)	4	4	48.5	30.7–67.1

Source of analytical data: Kim et al. (2009b)

g. Switzerland

Food groups	Total number of samples	Number of positive samples	Furan concentration (µg/kg)	
			Mean of positives	Minimum–maximum[a]
Baby food	101	101	21.7	1–153
Fruit and vegetable juices for babies	4	4	11.8	1–40
Canned or jarred vegetables	17	11	6.5	3–12
Canned fruits	2	1	6	—
Canned soup	2	2	31	19–43
Tins containing meat	2	2	9.0	4.0–14
“Sugo”, tomato and chili sauces	13	12	10.6	4–39
Soya sauce, hydrolysed vegetable protein	7	7	48.9	18–91
Vegetables, fresh	8	8	—	—
Vegetables, prepared	5	3	12.3	5.0–19
Bread, toast	39	30	46.4	5.0–193
Savory snacks	27	24	59	9–143
Sweet, pastry and biscuit	7	3	17.3	7.0–24
Sugar, caramel, pudding	15	11	312	4–1956
Dried fruits and nuts	16	1	7.0	—
Meat and meat products	5	1	10	—
Roasted coffee (brewed)	16	15	82.9	13–199
Roasted coffee (powder)	18	18	1979	22–5938
Instant coffee (brewed)	17	17	17	1–51.3
Instant coffee (powder)	11	11	783.3	44–2150
Green coffee	6	0	—	—
Coffee substitute (brewed)	4	4	6.8	1–15
Coffee substitute (powder)	4	4	586.3	102–1770
Dark beer	1	1	3	—
Cola soda pop	1	0	—	—

Switzerland (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (µg/kg)	
			Mean of positives	Minimum–maximum[a]
Hot chocolate and malt beverage	2	0	—	—
Whole milk ultra-high temperature	1	0	—	—
Plum beverage	1	0	—	—
Beetroot juice with fruit juice	1	0	—	—
Potato flakes, not prepared	1	0	—	—
Instant gravy	1	0	—	—
Hash browns, not prepared	1	0	—	—
Milk chocolate	1	1	21	—
Spice spread	1	1	24	—
Dried French beans	1	1	5	—
“Eierflädli” (cut omelette for soups)	1	0	—	—
Liquorice	4	3	17.7	13–25
Burnt almonds	1	1	15	—
Flour and starch	5	0	—	—
Flour and starch (roasted)	5	5	1932.8	1050–3708
“Basler Mehlsuppe”, not prepared (powder)	1	1	5	—
Oxtail soup, not prepared (powder)	1	1	8	—
Compact black garnish paste	1	1	9	—

[a] Minimum and maximum values were derived assuming only quantifiable results.
Source of analytical data: Reinhard et al. (2004); SFOPH (2004)

Switzerland (contd)

Food groups	Total number of samples	Furan concentration (µg/kg)	
		Median	Minimum–maximum
Baby foods (jarred)	20	29	12–69
Baby foods (powder)	4	13	1.0–38
Honey	5	4	3.0–10
Coffee (extract)	4	98	73–125
Coffee (beans)	4	4400	2650–5050
Sweet corn (canned)	1	4	—
Peanuts (roasted, in honey)	1	4	—
Jam (apricot)	1	1	—

Source of analytical data: Kantonales Laboratorium Basel (2004)

h. United States of America

Food groups	Total number of samples	Number of positive samples	Furan concentration (µg/kg)	
			Mean of positives	Minimum–maximum[a]
Baby food (juices)	11	11	3.4	1.4–8.2
Baby food (canned/ jarred)	132	130	38.1	1.3–112
Baby food (cereal)	5	2	5.2	3.9–6.4
Infant formula	42	28	11.9	2.5–26.9
Roasted coffee (brewed)	8	8	46.7	33.6–84.2
Instant coffee (brewed)	6	2	6.0	4.8–7.2
Soup	36	36	36.6	6.7–125
Canned fish	9	6	5.6	1.5–8.1
Canned vegetables	49	46	21.9	0.8–85.6
Vegetables, fresh	4	1	2.1	—
Vegetable juices	7	7	5.3	3.2–7.6
Bread	12	0	—	—
Baked products	43	11	6.7	1.2–30.1

United States of America (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)	
			Mean of positives	Minimum–maximum[a]
Crackers and crispbread	4	4	12.2	4.2–18.6
Breakfast cereal	25	16	18.5	2.3–47.5
Meat products	33	20	6.2	0.3–39.2
Topping	5	5	3.5	1.5–6.6
Mayonnaise	3	0	—	—
Ketchup	1	1	12.3	—
Honey	2	2	9.6	8.3–10.4
Marshmallows	4	0	—	—
Syrup	7	6	22.9	0.5–88.3
Sugar	4	4	9.2	1.0–17.7
Candy bars	9	8	2.5	1.0–5.5
Nuts and nut butter	7	7	5.2	2.1–7.5
Desserts	12	11	4.7	1.5–13
Beverages (e.g. tea, soda)	14	9	0.7	0.4–1.4
Evaporated milk	3	3	12.5	10.9–15.3
Condensed milk	2	0	—	—
Whole milk	1	0	—	—
Cheese	6	5	1.3	0.4–2.9
Sweet-based milk	1	1	3.8	—
Jam, jelly and preserves	46	44	7.5	0.9–37.4
Gravies	8	8	47.8	13.3–173.6
Snacks	32	32	17.8	1.6–64.7
Liquid eggs	1	0	—	—
Fat and oils	2	1	5.4	—
Sauces	33	30	11.9	3.3–46
Ready-to-eat meals (e.g. pasta, chili)	12	12	45.4	9.5–94.4

United States of America (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (µg/kg)	
			Mean of positives	Minimum–maximum[a]
Fruits (canned)	19	18	4.9	1.1–10.7
Fruit juices	35	21	4.8	0.5–30.5
Apple sauce	9	9	1.6	0.5–4.3
Dried fruits	8	7	1.3	0.6–2.2
Nutrition drinks	21	19	33.6	2.3–174
Beer	8	4	1.9	0.8–4.4
Chocolate drinks	10	4	4.1	0.5–10.3
Baked beans	15	15	60.4	23.3–122
Soya sauce	5	5	52.1	17.2–75.6

[a] Minimum and maximum values were derived assuming only quantifiable results.
Source of analytical data: USFDA (2009)

MERCURY (addendum)

First draft prepared by

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1. EXPLANATION

Mercury occurs naturally in the earth's crust, usually in the form of the mineral cinnabar (mercury(II) sulfide). It can be released into the global environment through a number of processes, both natural and anthropogenic. Global natural emissions of mercury have been estimated at up to 2400 tonnes per year (Bergan & Rohde, 2001), whereas mercury emissions from anthropogenic sources in the year 2000

were approximately 2200 tonnes (Pacyna et al., 2006). While relatively chemically inert, mercury occurs in three valence states: elemental mercury (also known as metallic mercury), monovalent mercurous ion and divalent mercuric ion (Horvat, 2005), elemental mercury and the divalent ion being the most important in nature. Inorganic mercury salts are usually found in the forms of mercury(II) sulfide (HgS), mercury(II) oxide (HgO) and mercury(II) chloride ($HgCl_2$) (Table 1). There are several organic mercury compounds; by far the most common in the environment and in the aquatic food-chain is methylmercury.

Table 1. Elemental and inorganic mercury compounds

Molecular formula	Relative molecular mass	Synonyms	CAS No.
Hg	200.59	Elemental	7439-97-6
		Quicksilver	
		Colloidal mercury	
$HgCl_2$	271.52	Mercuric chloride	7487-94-7
		Mercury(II) chloride	
		Corrosive sublimate	
		Mercuric bichloride	
		Mercury perchloride	
$Hg_2(NO_3)_2$	525.19	Mercurous nitrate	10415-75-5
		Mercury(I) nitrate	
		Mercury protonitrate	
$Hg(NO_3)_2$	324.66	Mercuric nitrate	10045-94-0
		Mercury(II) nitrate	
		Mercury pernitrate	
Hg_2O	417.18	Mercurous oxide	15829-53-5
		Mercury(I) oxide	
		Mercury oxide	
HgO	216.59	Mercuric oxide	21908-53-2
		Mercury(II) oxide	
		Santar	
$HgSO_4$	296.68	Mercuric sulfate	7783-35-9
		Mercury(II) sulfate	
		Mercury bisulfate	
HgS	232.66	Mercuric sulfide	1344-48-5
		Mercury(II) sulfide	

Table 1 (contd)

Molecular formula	Relative molecular mass	Synonyms	CAS No.
Hg_2Cl_2	472.08	Vermillion Cinnabar Mercurous chloride Mercury(I) chloride Calomel Calogreen	10112-91-1

CAS, Chemical Abstracts Service

Mercury was previously evaluated by the Committee at its tenth, fourteenth, sixteenth and twenty-second meetings (Annex 1, references *13*, *22*, *30* and *47*). At its sixteenth meeting, the Committee allocated a provisional tolerable weekly intake (PTWI) of 0.3 mg of total mercury (5 µg/kg body weight [bw]), of which no more than 0.2 mg (3.3 µg/kg bw) should be in the form of methylmercury, based primarily on the relationship between the intake of mercury from fish and mercury levels in blood and hair associated with the onset of clinical disease. The sixteenth meeting of the Committee noted that almost all dietary exposure to methylmercury is from fish and seafood and that methylmercury is probably by far the most toxic form of mercury in food; therefore, other forms of mercury could be given less weight when establishing a tolerable intake for mercury. The original PTWI for methylmercury (3.3 µg/kg bw) was revised at the sixty-first meeting (Annex 1, reference *166*) to 1.6 µg/kg bw, based on an assessment of results from various epidemiological studies involving fish-eating populations and developmental neurotoxicity. At the sixty-seventh meeting (Annex 1, reference *184*), the Committee provided further clarifications as to the relevance of the new methylmercury PTWI for different subgroups of the population.

At the sixty-first meeting, the Committee recommended that the total mercury PTWI be reviewed.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

During its original assessment of mercury (Annex 1, reference *31*), the Committee considered that the chemical form of mercury defined the nature of its biological and toxic effects, based on known differences in absorption, biotransformation, retention and excretion. Absorption and bioavailability of inorganic mercury salts in food were reported to be less than 15% in experimental animals, whereas human volunteers who ingested an oral tracer dose (approximately 6 µg

of mercury) of mercury(II) nitrate given either in an aqueous solution or protein bound (calf liver protein) absorbed an average of 5–10% of the dose (Rahola et al., 1973). After 58 days of monitoring, no significant radioactivity was found in the head region. In comparison, human volunteers ingesting an oral dose of 1.0 g of pulverized dental amalgam material (50% elemental mercury) absorbed only about 0.04% of the mercury dose (Sandborgh-Englund et al., 2004). Whereas distribution and rate of uptake of inorganic mercury compounds can vary, in general, the kidney, specifically the proximal renal tubules, has been shown to be the main organ for deposition and bioaccumulation (Yokel et al., 2006; Berlin, Zalups & Fowler, 2007).

(a) Mice

Various strains of mice (inbred, H-2-congenic A.SW, B10.S and F_1/F_2 hybrids) were exposed to mercury(II) chloride (mixed with ^{203}Hg) in drinking-water (2.7 mg/l) for 6 weeks and then sacrificed. Whole-body retention of mercury was on average 1.4% of the total mercury dose (252 µg), whereas kidney and liver were found to be the organs with the highest accumulated mercury levels in all strains (kidney on average had 13-fold higher mercury levels than liver) (Ekstrand et al., 2010).

Varying the oral dose of mercury(II) chloride given to mice has been shown to have only a minimal effect on estimated whole-body retention. Bom:NMRI strain male mice, 7–8 weeks of age, given a single oral dose of mercury(II) chloride ranging from 0.27 to 27 mg/kg bw retained from 1.2% to 2.8% of the initial dose as measured 14 days after dosing. Per cent retention was inversely related to magnitude of dose, which the authors suggested may have been related to renal damage and increased urinary excretion. The time required to eliminate 70% of the initial dose ranged from 19 to 37 h (Nielsen & Andersen, 1989).

Mice (male Albino) exposed to a single oral dose (gavage) of mercury(II) chloride (analytical reagent grade) (4 mg/kg bw) exhibited significant increases in both hepatic and renal metallothionein levels within 24 h (Tandon et al., 2001). Inhibition of γ-glutamylcysteine synthetase activity prior to exposure to inorganic mercury results in decreased renal mercury accumulation and increased urinary excretion (Berndt et al., 1985; Tanaka, Naganuma & Imura, 1990).

(b) Rats

Wistar strain female rats (n = 4) administered a single oral dose (gavage) of ^{203}Hg-labelled mercury(II) chloride at 0.2–20.0 mg/kg bw were reported to retain between 3.0% and 8.7% of the dose when assessed up to 120 h post-dosing (Piotrowski et al., 1992). Earlier studies have shown that age can be an important factor in metal absorption from the gastrointestinal tract. Suckling rats (1 week old, Albino strain) given an oral dose of ^{203}Hg (4 µg of mercury) in cows' milk retained on average 38% of the dose, compared with older animals (18 weeks of age), which retained only 6.7% of the dose (Kostial et al., 1978). In a similar experiment by the same authors, it was suggested that the higher whole-body retention in animals dosed with mercury in a milk vehicle may be related to higher gut retention, longer residency times and decreased mercury elimination (Kostial et al., 1981).

When female mice were dosed on lactation day 10 with 0.5 mg of ^{203}Hg-labelled mercury(II) chloride intravenously and the mercury transfer was followed in their offspring until the end of lactation (day 21), it was estimated that approximately 8% of the total dose was excreted in milk, and 15% of this was absorbed by the offspring (Sundberg, Oskarsson & Bergman, 1991).

Age dependency of mercury absorption by the duodenum has also been seen in rats. Following a dose of mercury(II) chloride of 16 µg/kg bw, absorption specifically by the duodenum measured 1 h later was highest in 6-day-old SD male rats (18.1%), compared with only 7.3% in 23-day-old weanlings or 3.6% in 7-week-old animals (Walsh, 1982).

Absorption of monovalent mercurous compounds has been reported to be less than the absorption of mercuric or divalent forms, likely due to solubility (Friberg & Nordberg, 1973). Mercuric salts (halides, sulfates, nitrates) are relatively water soluble, and Hg^{2+} ions in biological systems can form stable complexes with various moieties containing sulfhydryl groups (glutathione [GSH], cysteine, albumin, metallothionein, etc.) (Berlin, Zalups & Fowler, 2007). Mercury in the form of mercury(I) sulfide, a relatively water-insoluble inorganic mercury compound, has a much lower bioavailability compared with water-soluble mercury(II) chloride (Paustenbach, Bruce & Chrostowski, 1997; ATSDR, 1999). In contrast, organic mercurials are almost completely absorbed from the gastrointestinal tract. Organic mercury, specifically methylmercury, is readily bioavailable, with up to 94% of an oral dose, in the form of either methylmercury(II) chloride or methylmercury in fish tissue, absorbed by human volunteers (Magos & Clarkson, 2006).

Up to 50% of either a non-toxic dose (135 µg/kg bw) or a moderately nephrotoxic dose (500 µg/kg bw) of mercury(II) chloride administered intravenously to male SD rats (n = 4) was found in the kidneys within 3 h (Zalups, 1993).

In pregnant SD rats (n = 12) provided drinking-water containing mercury(II) chloride at 0.2 µg/ml from gestation day 0 to postnatal day 20, the majority of the accumulated mercury was found in the kidneys (52.7%); the organ with the next highest content was the liver (38.7%) (Feng et al., 2004). Although organ mercury concentrations were considerably lower in the offspring, the highest mercury levels were also found in the kidneys and liver, as well as in the spleen. In a similar experiment, following exposure of female SD rats (n = 3–4) to a single oral dose of ^{203}Hg-labelled mercury(II) acetate on lactation day 11 (0.1–5.8 mg/kg bw), increasing concentrations of mercury could be detected in milk; the concentrations were positively related to both dose and whole blood levels (Sundberg, Oskarsson & Bergman, 1991). At the highest dose, milk mercury levels were approximately 15.6% of whole blood mercury concentrations 24 h after dosing. By 72 h after dosing, mercury levels in milk had decreased to 8.6% of whole blood levels.

Excretion of inorganic mercury (mercuric) compounds in rats has been described as biphasic. In the first phase, approximately 35% of a non-toxic dose will be excreted within a few days, whereas the second phase involves a slower excretion rate, with a total half-life of 30 days (Nielsen, 1992).

SD rats (five of each sex per dose group), 45–50 days old, were dosed by gavage with mercury(II) chloride at 0, 2, 4, 6, 8 or 10 mg/kg bw per day for 14 consecutive days. Body weights were recorded on dosing days 0, 4, 7, 10 and 14 (sacrifice). At sacrifice, various organs were collected for mercury analysis. Significant mortality occurred in the three highest dose groups, with only one female rat surviving per group until termination. For males, two rats died in the 4 and 6 mg/kg bw per day dose groups, three in the 8 mg/kg bw per day dose group and all rats in the highest dose group. Kidney had the highest mercury content of all organs (three highest dose groups not analysed), with similar values obtained for both the 2 and 4 mg/kg bw per day doses (Khan et al., 2001).

Male Wistar rats, approximately 4 months old, were continually exposed to mercury(II) chloride at 15 mg/l in drinking-water for 6 months ($n = 5$) or 10 months ($n = 6$) (estimated dose 2.3 mg/kg bw per day). In a separate experiment, a similar group of rats was treated with the same dose of mercury(II) chloride for 1 year, and then kidneys were removed and analysed for dry weights and water content. At the end of the 4- and 6-month dosing periods, the rats were sacrificed and kidney tissues prepared for light and electron microscopic analysis. Relative kidney weights were increased by 27–30% in the groups exposed to mercury(II) chloride for 6 and 10 months. The 1-year study determined that the increase in relative kidney weight was not related to water retention. After 6 months of mercury exposure, there was an increase in the relative amount of interstitial tissue; after 10 months, the absolute volume of proximal tubule lumina and glomeruli in the mercury-treated rats had increased by approximately 50%, and the absolute volume of the proximal tubule cells had increased by 30% (Madsen & Maunsbach, 1981).

Groups of male SD rats (six per dose group) were exposed to mercury(II) chloride (^{203}Hg-labelled) in drinking-water at concentrations of 0, 5, 50 or 500 µmol/l for 8 weeks. Mercury intakes were reported as approximately 0, 21, 212 and 1526 µg/day or 0, 0.1, 1.0 and 7.3 mg/kg bw per day, respectively. Urine and faeces were collected on a daily basis, whereas body weights were measured weekly. Maximum blood mercury levels were achieved relatively quickly in all dose groups within the first 2 weeks. It was further estimated that steady-state mercury levels in the kidney were reached by 15 days in the 5 µmol/l dose group and by 30 days in the 50 µmol/l dose group (Morcillo & Santamaria, 1981).

Accumulation of mercury in the kidney has been related to both induction of, and binding to, metallothionein species and mercuric conjugates of GSH (Zalups, 2000; Zalups & Koropatnick, 2000; Berlin, Zalups & Fowler, 2007; Holmes, James & Levy, 2009). Exposure of male SD rats to a single subtoxic dose of mercury(II) chloride (135 µg/kg bw intraperitoneally) caused significant increases in γ-glutamylcysteine synthetase activity and related GSH-dependent enzymes in renal proximal tubule cells (Lash & Zalups, 1996).

Genetic polymorphisms in humans associated with reduced GSH production and mercury–GSH conjugation activities have been reported to influence the retention and excretion of mercury (Custodio et al., 2005; Gundacker et al., 2007; Ekstrand et al., 2010). Additional details on renal and hepatic accumulation and transport of inorganic mercury can be found in Berlin et al. (2007).

The major portion of absorbed inorganic mercury is excreted by the kidney (urine) and, to a lesser extent, through bile and faeces. The latter route involves formation of low molecular weight conjugates of mercury and GSH prior to secretion into bile (Ballatori & Clarkson, 1984). Lower rates of mercury secretion into bile by weanling rats, compared with adult animals, are thought to be related to their decreased ability to secrete sulfhydryl groups into bile. Available information suggests that excretory routes for both metallic mercury and inorganic mercury compounds are similar in humans and experimental animals (ATSDR, 1999).

Following parturition, SD rat pups (five of each sex) were injected subcutaneously with ^{203}Hg-labelled mercury(II) chloride at 5 mg/kg bw on postnatal day 22 or 29, and mercury elimination rates were followed for 5 days post-treatment by whole-body gamma counting. Mercury elimination curves were similar between the two groups, within almost 50% of the original dose excreted by 120 h post-injection (Daston et al., 1986). Mortality was observed in the treated rats, with those rats excreting 20–22% of the initial dose surviving until the end of the observation period.

Overall estimates of inorganic mercury half-lives in both experimental animals and humans range from 1 to 2 months (IPCS, 2003; Holmes, James & Levy, 2009).

2.1.2 Biotransformation

Following absorption from the gastrointestinal tract, inorganic mercury compounds in blood are bound, to a large extent, to sulfhydryl groups of haemoglobin and plasma proteins. Based on limited lipophilicity, neither mercury(I) nor mercury(II) is able to effectively cross the blood–brain barrier, in contrast to methylmercury. It has been hypothesized that thiol-conjugated mercury uptake and distribution may involve amino acid transporters, which may ultimately play a role in organ toxicity (Wei et al., 1999; Foulkes, 2000; Bridges et al., 2004; Lash et al., 2005; Bridges, Battle & Zalups, 2007; Rooney, 2007).

Actual metabolism of inorganic mercury compounds is limited, other than the previously described thiol and sulfhydryl conjugation reactions. Mercuric ions tend to be non-diffusible, and therefore binding facilitates transport. There is some limited evidence suggesting that mercury(II) (Hg^{2+}) can be reduced to metallic mercury and eliminated as metallic mercury vapour. Although not regarded as a major route of mercury elimination, reduction of mercury(II) to elemental mercury vapour was detected following exposure of adult CBA/J strain male mice to ^{203}Hg-labelled mercury(II) chloride at a single intraperitoneal dose of 0.5 mg/kg bw, as mercury (Dunn, Clarkson & Magos, 1981). Within 30 min after dosing, the mice had exhaled less than 5 ng of mercury. In comparison, elemental mercury can be readily oxidized by catalase and hydrogen peroxide to inorganic mercury (Hg^{2+}) (Clarkson, 1989; Rooney, 2007), whereas methylmercury can be demethylated through the action of reduced nicotinamide adenine dinucleotide phosphate (NADPH)–cytochrome P450 reductase to inorganic mercury (Clarkson & Magos, 2006). Generally, inorganic mercury present in the brain is the result of either in situ oxidation of elemental mercury or demethylation of organic mercury, both of which are lipid soluble and can cross the blood–brain barrier.

2.2 Toxicological studies

2.2.1 Acute toxicity

The oral median lethal dose (LD_{50}) of various inorganic mercury compounds ranges from 25 to 205 mg/kg bw (ATSDR, 1999; IPCS, 2003) (Table 2). The features of acute toxicity usually consist of shock, cardiovascular collapse, acute renal failure and severe gastrointestinal damage (IPCS, 1991). In female Sprague-Dawley rats, mercury(II) chloride at a single mercury dose of 7.4 or 9.2 mg/kg bw by gavage in water caused significant decreases in haemoglobin, erythrocytes and haematocrit at autopsy. A significant decrease in lactate dehydrogenase activity and mild to moderate renal effects, consisting of protein casts, cellular casts and interstitial sclerosis, were also observed. In female Bom:NMR mice, no effects were seen with mercury(II) chloride at a single mercury dose of 5 mg/kg bw by gavage (IPCS, 2003).

Table 2. Oral LD_{50} values for mercury compounds

Mercury compound	Species	LD_{50} (mg/kg)
Mercury(I) chloride (Hg_2Cl_2)	Rat	166
	Mouse	180
Mercury(II) chloride ($HgCl_2$)	Rat	37
	Human (LD_{lo})	10–42
Mercury(II) cyanide ($Hg(CN)_2$)	Rat	25
	Mouse	33
Mercury(I) sulfate (Hg_2SO_4)	Rat	205
	Mouse	152
Mercury(II) sulfate ($HgSO_4$)	Rat	57
	Mouse	25

LD_{lo}, lowest reported lethal dose
Source: Adapted from Von Burg (1995)

2.2.2 Short-term studies of toxicity

Four short-term studies in rodents (Druet et al., 1978; Bernaudin et al., 1981; Andres, 1984; NTP, 1993) were identified by several agencies from available toxicology databases and used to derive toxicological reference values: lowest-observed-adverse-effect level (LOAEL), reference dose (RfD), minimal risk level (MRL) and tolerable daily intake (TDI). Of these four studies, the first was used to estimate an oral LOAEL of 15.8 mg of mercury per person per day, based on the LOAEL of 0.05 mg/kg bw per day, as mercury(II) chloride, administered subcutaneously in rats (IPCS, 1991); the remaining three studies were used to derive the United States Environmental Protection Agency (USEPA) oral RfD for mercury of 0.3 µg/kg bw per day, based on LOAELs in rats ranging from 0.226 to 0.633 mg/kg bw per day, as mercury, with the application of an uncertainty factor of 1000 (IRIS, 1995). The last study was used to derive an intermediate MRL

for mercury of 2.0 µg/kg bw per day (ATSDR, 1999) and a TDI for mercury of 2.0 µg/kg bw per day (IPCS, 2003), based on the no-observed-adverse-effect level (NOAEL) of 0.23 mg/kg bw per day, as mercury(II) chloride, in rats following 5 days/week administration by gavage.

(a) Mice

In the NTP (1993) study, B6C3F1 mice (10 animals of each sex per group) were administered mercury(II) chloride (>99.5% purity) by gavage at 0, 1.25, 2.5, 5, 10 or 20 mg/kg bw per day, 5 days/week, for 6 months (0, 0.92, 1.9, 3.7, 7.4 or 14.8 mg/kg bw per day, as mercury). A decrease in body weight gain was reported only in the males of the highest dose group. Significant increases in absolute and relative kidney weights of male mice occurred at 3.7 mg/kg bw per day or greater and at 7.4 and 14.8 mg/kg bw per day, respectively. The kidney weight changes corresponded to an increased incidence of cytoplasmic vacuolation of renal tubule epithelium in males exposed to at least 3.7 mg/kg bw per day. The exposed female mice did not exhibit any histopathological changes in the kidneys.

(b) Rats

In the Druet et al. (1978) study, both male and female Brown Norway rats were divided into groups of 6–20 animals each and received subcutaneous injections of mercury(II) chloride 3 times weekly for 8 weeks, at 0, 100, 250, 500, 1000 or 2000 µg/kg bw. An additional group was injected with a 50 µg/kg bw dose (15.8 µg/kg bw per day, as mercury) for 12 weeks. Antibody formation was measured by the use of kidney cryostat sections stained with a fluoresceinated sheep anti-rat immunoglobulin G (IgG) antiserum. In the first phase, rats developed antiglomerular basement membrane antibodies. During the second phase, the patterns of fixation of antisera changed from linear to granular. The immune response was accompanied by proteinuria and, in some cases, by a nephrotic syndrome. Tubular lesions were observed at the higher dose levels. Proteinuria was reported at doses of 100 µg/kg bw and above, but not at 50 µg/kg bw. Proteinuria was considered a highly deleterious effect, given that affected animals developed hypoalbuminaemia and many died. IgG antiserum was detected in all groups (including the 50 µg/kg bw dose level), except controls.

The Bernaudin et al. (1981) study involved the ingestion (gavage) of mercury(II) chloride at either 0 or 3000 µg/kg bw per week by male and female Brown Norway rats for up to 60 days. No organ abnormalities were reported using standard histological techniques in either experimental or control rats. Immunofluorescence histology revealed that 80% (4/5) of the mercury(II) chloride–exposed rats were observed to have a linear IgG deposition in the glomeruli after 15 days of exposure. After 60 days of mercury(II) chloride exposure, 100% (5/5) of the rats were seen with a mixed linear and granular pattern of IgG deposition in the glomeruli and granular IgG deposition in the renal arteries. Weak proteinuria was observed in 60% (3/5) of the rats fed mercury(II) chloride for 60 days. The control rats were observed to have no deposition of IgG in the glomeruli or arteries, as well as normal urinary protein concentrations.

In the Andres (1984) study, mercury(II) chloride (3 mg/kg bw in 1 ml of water) was given by gavage to five Brown Norway rats and two Lewis rats (sex not specified) twice a week for 60 days. A sixth Brown Norway rat was given only 1 ml of water by gavage twice a week for 60 days. After 2–3 weeks of exposure, the mercury(II) chloride–treated rats started to lose weight, and two rats died 30–40 days after dosing. No rats were observed to develop detectable proteinuria during the 60-day period. The kidneys appeared normal in all animals when evaluated using standard histological techniques, but examination by immunofluorescence showed deposits of IgG present in the renal glomeruli of only the mercury-treated Brown Norway rats. The treated Brown Norway rats were also observed with mercury-induced morphological lesions of the ileum and colon with abnormal deposits of immunoglobulin A (IgA) in the basement membranes of the intestinal glands and of IgG in the basement membranes of the lamina propria. All observations in the Lewis rats and the control Brown Norway rat appeared normal.

Adult male Wistar rats (n = 6) were given mercury(II) chloride at a dose of 0.25 mg/kg bw per day for periods of 15, 30, 45 or 60 days by gavage. At termination, blood and liver samples were collected for analysis of various biochemical parameters. Plasma glucose (>30 days), cholesterol, triglycerides and total protein (>15 days) were all reduced (by up to 50%), whereas urea concentrations were increased (>15 days). Hepatic GSH levels were slightly reduced by approximately 10% (>30 days) (Merzoug et al., 2009).

Exposure of weanling Wistar rats (five per dose group) to mercury(II) chloride in the diet (0, 75, 150 or 300 µg/g) for 4 weeks induced significant increases in relative kidney weights in both males and females at all doses but had no effect on relative liver weights (Jonker et al., 1993).

In the NTP (1993) study, Fischer rats (10 animals of each sex per group) were administered mercury(II) chloride in deionized water by gavage at 0, 0.312, 0.625, 1.25, 2.5 or 5 mg/kg bw per day, 5 days/week, for 6 months (0, 0.23, 0.46, 0.92, 1.9 or 3.7 mg/kg bw per day, as mercury). Survival was not affected, although body weight gains were decreased in males at the highest dose and in females at or above 0.46 mg/kg bw per day. Absolute and relative kidney weights were significantly increased in both sexes with exposure to at least 0.46 mg/kg bw per day. In males, the incidence of nephropathy (characterized by foci of tubular regeneration, thickened tubular basement membrane and diluted tubules with hyaline casts) was 80% in the controls and 100% for all treated groups; however, severity was assessed to be minimal in the controls and two lowest dose groups and minimal to mild in the 0.92 mg/kg bw per day group and higher. In females, there was a significant increased incidence of nephropathy only in the high dose group (4/10 with minimal severity). No treatment-related effects were observed in the other organs; however, histopathology on the other organs was performed only on control and high-dose rats. A NOAEL from this study was identified as 0.23 mg/kg bw per day, as mercury(II) chloride (0.20 mg/kg bw per day, as mercury) (NTP, 1993). A concurrent study was conducted in which groups of Fischer rats (30 per group) were dosed with the low, middle and high dose groups from the main 6-month study and sacrificed at 2-, 4- and 6-month intervals (10 per time point) for tissue residue analysis. As expected, kidneys were the main organ bioaccumulating

mercury, with maximum kidney mercury concentrations reached in all dose groups by 4 months (Dieter et al., 1992).

Groups of male Wistar rats (44 animals per group) were exposed to mercury(II) chloride at 0, 50 or 100 mg/l for 90 days through oral administration in the drinking-water (calculated intakes were approximately 0, 4 and 8 mg/kg bw per day, respectively). Mercury exposure for 90 days resulted in an increase in the absolute and relative wet weights of the testis and a decrease in the absolute and relative wet weights of the accessory sex glands in both treated groups, compared with the matched control. Marked perturbation in testosterone levels in serum was also detected in treated groups during the study. Cauda epididymal sperm count or motility decreased significantly in both treated groups, and qualitative examination of testicular sections revealed fewer mature luminal spermatozoa in comparison with the control. When the mercury-treated males were mated with normal cyclic females, mercury exposure resulted in a decline of the reproductive performance of male rats. These effects were associated with a significant increase in mercury content of testes and blood in a time-dependent and dose-dependent fashion, respectively. Evidence of induction of oxidative stress was reflected in terms of perturbations in antioxidant defence as measured by the activities of antioxidant enzymes (superoxide dismutase and catalase) and a significant dose-dependent increase in the testicular lipid peroxidation as a consequence of pro-oxidant exposure. These results suggest that an increase in free radical formation relative to loss of the antioxidant defence system after mercury exposure may render the testis more susceptible to oxidative damage, leading to their functional inactivation (Boujbiha et al., 2009). In comparison, adult rats (n = 20) and guinea-pigs (n = 12) (strain not identified) given mercury(II) chloride at a dose of 1 mg/kg bw per day intraperitoneally for 30 consecutive days did not exhibit changes in testicular weight (Chowdhury & Arora, 1982).

Adult Sprague-Dawley rats were used to investigate the effect of mercury(II) chloride on testicular and epididymal morphological alterations and interferon-gamma (IFN-γ) and interleukin-4 (IL-4) levels in serum. Groups of males (five per group) were given drinking-water containing mercury(II) chloride at 0 (control), 0.01, 0.05 or 0.1 µg/ml in deionized water (approximately equivalent to 0, 1.5, 7.5 and 15.0 µg/kg bw per day, as mercury) for 1, 2 or 3 months. Some rats also received mercury(II) chloride for 7 months. No effects on body weight, testis weight or epididymis weight were observed. Morphological alterations, however, were found in the epididymis and testis. After 1 month of mercury administration, degenerative changes, such as peritubular cell dissociation, were noted at 0.1 µg/ml. Seminiferous tubules in testis tissue sections from rats receiving mercury for 1 month showed epithelium disorganization, loss of cohesion and germ cell shedding independent of the dose. After 2 months of exposure to either 0.05 or 0.1 µg/ml, progressive degeneration with spermatogenic arrest at the spermatocyte stage and reduction in sperm density and hypospermatogenesis were observed in seminiferous epithelium by light and electron microscopy. Leydig cells showed cytoplasmic vacuolation and nuclear signs of cell death. Loss of peritubular cell aggregation was observed in the epididymis. Mercury accumulation was detected in both organs by mass spectroscopy. Rats showed increased IFN-γ levels in serum

compared with controls, but they reached significance only after 7 months of mercury administration (no information on whether there was a dose-dependent response within the test dose range). The results demonstrate that sublethal concentrations of mercury(II) chloride can induce morphological and ultrastructural modifications in male reproductive organs of rats. These contribute to functional alterations of spermatogenesis, with arrest at the spermatocyte stage, hypospermatogenesis and possibly impaired steroidogenesis, which together could affect male fertility (Penna et al., 2009). Note that this study suggested the 0.01 µg/ml dose, or approximately 1.5 µg/kg bw per day, as mercury, to be an effect level; however, based on the results presented, effects at this dose appear to be equivocal, due to inconsistent dose-dependent responses.

Adult male Wistar rats (five per dose group) were dosed by gavage with mercury(II) chloride at 0, 1 or 2 mg/kg bw per day for 30 consecutive days. At sacrifice, the right testis was removed and weighed, and the testicular plasma membrane was isolated. Whereas the highest dose caused an approximate 10% decrease in relative testis weight, no weight change was noted in the low dose group.

The activities of various membrane-bound enzymes were either increased (alkaline phosphatase, γ-glutamyl transferase) or decreased (5′ nucleotidase, Ca^{2+}-adenosine triphosphatase [ATPase], Mg^{2+}-ATPase, Na^{+}-K^{+}-ATPase) by the mercury treatments (Ramalingam & Vimaladevi, 2002).

2.2.3 Long-term studies of toxicity and carcinogenicity

An evaluation by IPCS (2003) based on various long-term studies in rodents indicated that the 2-year NTP (1993) bioassay in rats and mice appeared to be the most relevant and appropriate for assessing the carcinogenicity of mercury(II) chloride.

(a) Mice

In the 2-year NTP (1993) study, B6C3F1 mice (60 animals of each sex per group) were administered mercury(II) chloride (>99.5% purity) by gavage at 0, 5 or 10 mg/kg bw per day, 5 days/week, for 104 weeks (0, 3.7 and 7.4 mg/kg bw per day, as mercury). An interim sacrifice (10 animals of each sex per dose) was conducted after 15 months of exposure. The survival rates in the controls, low dose group and high dose group were, respectively, 72%, 72% and 62% for males and 82%, 70% and 62% for females. Female mice exhibited a significant increase in the incidence of nephropathy (21/49, 43/50 and 42/50 in control, low-dose and high-dose females, respectively). Nephropathy was observed in 80–90% of the males in all groups, with the severity increasing as the dosage increased. The incidences of renal tubular hyperplasia were 1/50, 0/50 and 2/49 in control, low-dose and high-dose males, respectively. The combined incidences of renal tubular adenomas and adenocarcinomas were 0/50, 0/50 and 3/49 in control, low-dose and high-dose males, respectively. Although no tumours were seen in the low-dose males, a statistically significant positive trend for increased incidence of renal tubular malignancies with increased dose was observed. These observations were

considered important, because renal tubular hyperplasia and tumours in mice are rare (IRIS, 1995). It was considered that there was equivocal evidence of carcinogenic activity in male mice (renal tubular adenomas and adenocarcinomas) and no evidence in female mice (NTP, 1993).

(b) Rats

Fischer 344 rats (60 animals of each sex per group) were administered mercury(II) chloride (>99.5% purity) by gavage in water at doses of 0, 2.5 or 5 mg/kg bw per day, 5 days/week, for 104 weeks (0, 1.9 and 3.7 mg/kg bw per day, as mercury) (NTP, 1993). An interim sacrifice (10 animals of each sex per group) was conducted after 15 months of exposure. Survival after 24 months was 43%, 17% and 8% in control, low-dose and high-dose males, respectively, and 58%, 47% and 50% in control, low-dose and high-dose females, respectively. During the second year of the study, body weight gains of low-dose and high-dose males were 91% and 85% of those of controls, respectively, and body weight gains of low-dose and high-dose females were 90% and 86% of those of controls, respectively. At study termination, nephropathy was evident in both treated and control rats, with incidences of 6/50, 29/50 and 29/50 in control, low-dose and high-dose males, respectively. The incidences of forestomach squamous cell papillomas in the control, low-dose and high-dose groups were 0/50, 3/50 and 12/50 in males and 0/50, 0/49 and 2/50 in females, respectively. The incidence of papillary hyperplasia of the stratified squamous epithelium lining of the forestomach was elevated at a statistically significant rate in all dosed males (3/49, 16/50 and 35/50 in control, low-dose and high-dose males, respectively) and in high-dose females (5/50, 5/49 and 20/50 in control, low-dose and high-dose females, respectively). The incidence of thyroid follicular cell carcinomas, adjusted for survival, showed a significant positive trend in males; the incidence was 1/50, 2/50 and 6/50 in control, low-dose and high-dose groups, respectively. The combined incidence of thyroid follicular cell neoplasms (adenoma and/or carcinoma) was not significantly increased (2/50, 6/50 and 6/50 in control, low-dose and high-dose males, respectively). In female rats, a significant decrease in the incidence of mammary gland fibroadenomas was observed (15/50, 5/48 and 2/50 in control, low-dose and high-dose females, respectively). The high mortality in both groups of treated males indicates that the maximum tolerated dose (MTD) was exceeded in these groups and limits the value of the study for assessment of carcinogenic risk. NTP (1993) considered the forestomach tumours to be of limited relevance to humans because of structural considerations and the observation that the tumours did not appear to progress to malignancy. The relevance of the thyroid carcinomas was also questioned, because these neoplasms are usually seen in conjunction with increased incidences of hyperplasia and adenomas, which were not observed in this study (IRIS, 1995).

2.2.4 Genotoxicity

Several in vitro studies have shown that mercury(II) chloride induces single-strand breaks in the deoxyribonucleic acid (DNA) of Chinese hamster ovary cells (Cantoni, Evans & Costa, 1982; Cantoni & Costa, 1983; Cantoni et al., 1984a,b; Christie et al., 1984, 1986), spindle disturbance in human lymphocytes, cell

transformation in Syrian hamster cells (Casto, Myers & DiPaolo, 1979) and sister chromatid exchanges (Howard et al., 1991) and chromosomal aberrations in both Chinese hamster ovary cells and human lymphocytes (Morimoto, Iijima & Koizumi, 1982; Verschaeve, Kirsch-Volders & Susanne, 1984). In vitro studies with human lymphocyte cultures detected various genotoxic effects by inorganic mercury (mercury(II) chloride or mercury(II) nitrate), including micronuclei, sister chromatid exchange, increased levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), decreased proliferation rate index and decreased mitotic index (Ogura, Takeuchi & Morimoto, 1996; Lee et al., 1997; Rao et al., 2001; Silva-Pereira et al., 2005).

(a) In vitro

Lymphocyte cultures prepared from blood samples collected from 10 healthy adults were incubated with mercury(II) chloride at concentrations ranging from 0.1 to 1000 µg/l for 9 h. Following cytogenetic analysis, the relative frequency of cells with gaps, breaks and gaps plus breaks was determined. There was an approximate 2-fold increase in cells with chromosomal alterations (mainly gaps), but only in the lowest and highest dose groups. In comparison, at the same doses, methylmercury(II) chloride produced a stronger response in all dose groups (up to an 18-fold increase). Also, no cells with polyploidy aberrations were seen in the mercury(II) chloride lymphocyte cultures (Silva-Pereira et al., 2005).

Cytokine-activated peripheral blood mononuclear cell cultures from three human volunteers were incubated in the presence of mercury(II) chloride at 0.1–50 µmol/l (0.27–13.6 mg/l) for 24 h before collection and analysis for 8-OHdG, micronuclei and chromosomal aberrations. Significant cytotoxicity was observed at the highest dose. Aberrations (excluding gaps) were increased at the two highest doses of mercury(II) chloride that were scored (10 and 20 µmol/l) and were significantly related to loss in cell viability. At lower doses, the main chromosomal aberration was described as single chromatid breaks. Micronuclei were also increased, but only at doses of mercury(II) chloride at which significant toxicity was observed (20 µmol/l). A significant increase in 8-OHdG (approximately 2- to 3-fold) was also observed at higher doses of mercury(II) chloride (10 and 20 µmol/l). The authors suggested that the observed increase in chromosomal aberrations was likely due to mercury(II) chloride acting as an inhibitor of the mitotic spindle, whereas an increase in 8-OHdG arose due to oxidative DNA damage (Ogura, Takeuchi & Morimoto, 1996).

Although culturing human peripheral blood lymphocyte cultures with mercury(II) chloride at concentrations ranging from approximately 1 to 10.5 µmol/l (0.27–2.8 mg/l) for 72 h produced a slight increase in sister chromatid exchanges (less than 2-fold compared with controls), simultaneous exposure to ascorbic acid completely blocked the effect (Rao et al., 2001).

Mercury(II) chloride and mercury(I) chloride were also positive in the *Bacillus subtilis* rec-assay (Kanematsu, Hara & Kada, 1980). In general, Ames assay results with *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and mercury(II) chloride have been negative, both with and without S9 metabolic activation (NTP, 1993).

Chinese hamster ovary cells were cultured in the presence of mercury(II) chloride (up to 100 µmol/l or 27 mg/ml) for 60 min and then assessed for cellular GSH content and DNA single-strand breaks by alkaline elution. Concentrations of mercury(II) chloride greater than 25–50 µmol/l were very cytotoxic, whereas lower concentrations produced single-strand DNA breaks similar to X-ray treatment (1.5–6.0 Gy) (Cantoni, Evans & Costa, 1982).

(b) In vivo

In an in vivo study with male Swiss albino mice (five animals per group) administered a single oral dose of mercury(II) chloride (analytical grade) (0, 3, 6 or 12 mg/kg bw), a dose-related increase in bone marrow chromosomal aberrations was observed 24 h following dosing (Ghosh et al., 1991). Chromatid breaks and gaps were the most commonly observed aberrations. The mitotic index was also significantly reduced by all mercury doses. As simultaneous exposure to chlorophyllin significantly reduced the incidence of chromosomal aberrations, it was suggested that a possible mechanism of mercury(II) chloride DNA damage may involve generation of free radicals.

In other studies, no increase in chromosomal aberrations was observed in bone marrow or spermatogonia of mice (Poma, Kirsch-Volders & Susanne, 1981).

Male Swiss albino mice, 12 weeks of age, were treated intraperitoneally with a single dose of mercury(II) chloride at 0, 2, 4 or 6 mg/kg bw, with bone marrow cells and spermatogonia sampled 12, 24, 36 and 48 h after injection for cytogenetic analysis. The frequencies of gaps, breaks and exchanges in either bone marrow cells or spermatogonia were not significantly increased compared with the controls (Poma, Kirsch-Volders & Susanne, 1981).

In a similar experiment using the same model, 30 min following a single intraperitoneal dose of mercury(II) chloride at 6 mg/kg bw, the mice were also treated with an intraperitoneal dose of ethyl methanesulfonate (EMS) at 200 mg/kg bw. Whereas EMS caused a significant increase in bone marrow chromosomal aberrations, no effect was observed when mercury(II) chloride was administered either alone or in combination with EMS (Poma, Kirsch-Volders & Susanne, 1984).

In rats, a weak, but dose-related, increase in dominant lethal mutations was reported after repeated oral administration of mercury(II) chloride (Zasukhina et al., 1983).

Groups of male Wistar rats (five per group) were treated by gavage with a single dose of mercury(II) chloride (0.0054, 0.0108, 0.0216, 0.0432 or 0.0864 mg/kg bw). At various times after mercury treatment, beginning at 24 h and extending to 2 weeks, blood samples were collected, and leukocyte DNA damage was determined by the comet assay (Grover et al., 2001). All mercury doses were shown to produce an increase in DNA comet tail lengths by 24 h, which gradually decreased to near control levels by 72 h, without significantly affecting cell viability.

Adult female Wistar rats (eight per dose group) were administered mercury(II) chloride orally at a dose of 0.068, 0.136 or 0.272 mg/kg bw for 5 consecutive days. Three days after the last dose, the animals were killed, and

mercury levels in the liver and the kidney were measured. A significant increase in mercury concentration in the kidney was observed in the two highest dose groups, whereas liver mercury burden showed a significant increase only in the highest dose group, compared with controls. Blood samples were analysed using the comet assay and the acridine orange supravital staining micronucleus test. Mean tail length and tail moment in lymphocytes and micronucleated reticulocytes were significantly higher or more frequent in the treated rats than in control rats, regardless of the dose of mercury(II) chloride, whereas the difference between the treated groups for both comet assay and micronucleus parameters was not statistically significant (Rozgaj, Kasuba & Blanusa, 2005).

(c) Summary

In vitro data show that mercury(II) chloride has genotoxic potential. Despite mixed results, chromosomal aberrations have been observed in an in vivo study using the oral exposure route (Ghosh et al., 1991). Although direct interaction with DNA has not been conclusively seen, indirect DNA damage induced by inorganic mercury may involve free radicals and oxidative stress, disruption of microtubules and inhibition of DNA repair mechanisms (Crespo-López et al., 2009).

2.2.5 Reproductive and developmental toxicity

A number of studies on the reproductive effects of mercury(II) chloride or mercury(II) acetate in rodents were evaluated in IPCS (2003). Most of these were conducted with non-oral exposure, and the lowest effect levels were observed following a single intraperitoneal injection of mercury(II) chloride at 1 mg/kg bw, as mercury, in male mice, with decreased conceptions in females, and at 0.74 mg/kg bw, as mercury, in rats, with seminiferous tubular degeneration (Lee & Dixon, 1975). Only one study was carried out by oral exposure, in which pregnant hamsters receiving mercury(II) acetate at a single dose of 22 mg/kg bw, as mercury, on gestation day 8 showed an increase in the incidence of resorptions and small and oedematous embryos in the presence of histological damage in the liver and kidney in the dams (Gale, 1974).

(a) Mice

Groups of male and female C57BL/6 mice (25 of each sex per group) were exposed by gavage to mercury(II) chloride (>99.5% purity) at doses of 0.00, 0.25, 0.50 or 1.00 mg/kg bw per day, and males were paired with females receiving the same dose. Dosing continued for males throughout mating, whereas dosing in females continued throughout mating, gestation and lactation. The males were necropsied at the end of mating, following 81 days of exposure, and the females were necropsied at the conclusion of lactation (total exposure duration 79 days). Fertility indices, parturition, gestation, live birth litter size, survival indices and implantation efficiency were recorded and statistically analysed. Fertility indices were significantly ($P < 0.05$) reduced in all treated groups. The fertility indices, however, for all dose groups were 16% compared with 44% in the control group and showed no dose-dependent response. Exposure of parental mice to mercury(II) chloride did not affect the litter size, and only the high dose showed a decrease in

the live birth index. No evidence of mercury-induced target organ toxicity was seen in either the clinical pathology parameters or the histomorphological evaluations. It was indicated that oral exposure to mercury(II) chloride at 0.25–1.00 mg/kg bw per day produced adverse effects on the reproductive performance of mice, in the absence of overt mercury toxicity, with a LOAEL of 0.25 mg/kg bw per day, as mercury(II) chloride, or 0.185 mg/kg bw per day, as mercury (Khan et al., 2004).

CD-1 male mice, 3 months of age, were exposed to mercury(II) chloride in drinking-water (4 mg/l) for 12 weeks. The estimated daily intake was 0.65 mg/kg bw per day. At sacrifice, significant decreases in both body weight gain (28%) and relative testes weight (46%) were observed. Histopathological testes examination revealed that testicular necrosis, focal hyperplasia and interstitial oedema were evident in the mercury-treated animals. Average epididymal sperm numbers were also significantly reduced (55%) (Orisakwe et al., 2001).

Adult male albino Swiss strain mice dosed with mercury(II) chloride by gavage at 1.25 mg/kg bw per day for 45 days showed no change in body weight gain, but relative testis and cauda epididymal weights were significantly reduced (29% and 44%, respectively) compared with controls (Rao & Sharma, 2001). After a 45-day recovery period, testes weights had recovered to 90% of control values, whereas cauda epididymal weights were no longer reduced. Caudate epididymis sperm count, sperm motility and sperm viability were significantly reduced by the mercury treatment (63%, 49% and 39%, respectively), with some recovery also observed following termination of treatment (21%, 26% and 0% reduced compared with controls). Serum testosterone levels were reduced by mercury treatment (61%), with the inhibition persisting after the recovery period (48% reduced compared with controls). When the mercury-treated male mice were mated with control estrous females, mating success (per cent sperm-positive females per mated male) was reduced to 0%. However, after the recovery period, mating success had increased to 50%, compared with 92.5% for control males.

(b) Rats

Several short-term studies in rats in which reproductive effects were observed are discussed in section 2.2.2.

White female rats (strain not identified) were mated with the same variety of male rats that had been chronically exposed to mercury(II) chloride (0, 0.025, 0.25 or 2.5 mg/kg bw per day) by gavage for 12 months. At day 20 of gestation, the female rats were sacrificed, and embryonic material was analysed. No effects were observed in the total number of implantation sites or number of live embryos, but the two highest mercury(II) chloride doses produced an increase in the number of resorptions compared with controls (Zasukhina et al., 1983).

Sprague-Dawley rats (20 per group), approximately 7 weeks of age, were treated daily with mercury(II) chloride by gavage in a two-generation reproduction study (Atkinson et al., 2001). Males (F_0) were treated with mercury(II) chloride at 0.00, 0.50, 1.00 or 2.00 mg/kg bw per day and mated with females dosed with mercury(II) chloride at 0.00, 0.75, 1.50 or 3.00 mg/kg bw per day. F_0 males were dosed throughout the pre-cohabitation (60 days) and cohabitation period (21 days).

As clinical signs of toxicity were observed on day 43 (males) and day 27 (females), the high doses were reduced from 2.00 to 1.5 mg/kg bw per day in males and from 3.00 to 2.5 mg/kg bw per day in females. F_0 females were dosed throughout the pre-cohabitation (16 days), cohabitation (21 days), gestation and lactation periods. Following lactation, selected F_1 males and females were exposed to the same doses received by their parents (F_0).

Body weight gain was significantly reduced beginning in treatment week 7 in the high-dose males, whereas body weights were reduced in both the mid- and high-dose females from weeks 5 to 10. Following a 21-day cohabitation period, pregnant females were allowed to deliver their litters, with sufficient F_1 offspring selected for the F_1 breeding. Dosing of F_1 animals was the same as for the F_0 generation, except only the low and middle dose groups were used (insufficient offspring from high dose group). Significant dose-dependent reductions were seen in the F_0 generation fertility index (95%, 63.2%, 36.8% and 11.8% for controls, low dose group, middle dose group and high dose group, respectively), but not in the F_1 generation animals. Implant efficiency was also reduced in all dose groups for the F_0 generation (23.2%, 11.3%, 7.2% and 3.6% for controls, low dose group, middle dose group and high dose group, respectively), but only in the middle dose group for F_1 animals. Body weight gain for F_1 pups was decreased during lactation for all dose groups, an effect that persisted in adulthood. However, body weight gain of the F_2 pups was not significantly changed compared with controls. With the exception of a slightly reduced relative seminal vesicle weight in the mid- and high-dose F_0 males, no additional male sex organ (testis, prostate, epididymis) changes were noted in low-dose F_0 or low- and mid-dose F_1 animals.

Further details noted from the Atkinson et al. (2001) study included the following: the male and female dosages were not administered consistently throughout the study; no subsequent decreases in fertility index were observed in the F_1 generation, even with continued exposure to mercury(II) chloride at 1.0–1.5 mg/kg bw per day; there were variable sex ratios in the F_1 offspring (0.75–2.00) that were not related to dose; and the total number of reported live F_1 males equalled the total number of live offspring surviving until the end of the experiment (which contradicts the reported sex ratio).

Groups of pregnant female Wistar rats (two per group) were treated, by gavage, with mercury(II) chloride (99.5% purity), dissolved in distilled water, at 0.4, 0.8 or 1.6 mg/kg bw, as mercury, 1) from day 5 to day 15 during pregnancy (P protocol); 2) from day 5 to day 15 of pregnancy plus for 4 weeks of lactation (P+L protocol); and 3) from day 5 to day 15 of pregnancy plus for 4 weeks of lactation, with the offspring being further treated for 8 weeks post-weaning (P+L+P protocol). The authors reported that there were minor body weight decreases in the pups from dams treated in the two highest dose groups (data not provided), but the treatment, in general, failed to cause major signs of general intoxication in the rats (Papp, Nagymajtenyi & Vezer, 2005).

Weanling female SD rats (20 per dose group) were treated with mercury(II) chloride (0, 1 or 2 mg/kg bw per day) by gavage beginning on postnatal day 21 for 60 days prior to mating with unexposed males. Mating (10 per dose group)

proceeded with proven fertile males, and the females were euthanized on gestation day 13. By 30 days of mercury(II) chloride exposure, body weight gain in exposed females was slightly reduced in both dose groups (<10%); however, no indication as to whether the fertility index was affected was provided. Total implantations were slightly decreased in only the high dose group compared with controls, whereas non-viable implantations (i.e. sites on the uterus that showed signs of resorption in addition to sites that had not developed into a live fetus) were increased in the same dose group only. A slight decrease was also noted in plasma progesterone levels, whereas luteinizing hormone, expressed on a microgram per pituitary gland basis, was increased, both only in the high dose group (Heath et al., 2009).

2.2.6 Special studies

(a) Immunological effects

(i) Mice

Administration of mercury(II) chloride in drinking-water at a dose of 0.7 mg/kg bw per day, as mercury, for 2 weeks resulted in an increased lymphoproliferative response after stimulation with T-cell mitogens in a strain of mice particularly sensitive to the autoimmune effects of mercury (SJL/N). In the same experiment, a strain of mice that is not predisposed to the autoimmune effects of mercury (DBA/2) showed no increase in lymphocyte proliferation (Hultman & Johansson, 1991).

Male BALB/c mice (four per group) were exposed to mercury(II) chloride continuously at 0, 0.3, 1.5, 7.5 or 37.5 mg/l, as mercury, in drinking-water for 14 days. Body weight was reduced in the highest dose group, whereas the relative kidney and spleen weights were significantly increased. The dose range of mercury used did not cause hepatotoxicity, as indicated by circulating alanine aminotransferase and aspartate aminotransferase levels. Circulating blood leukocytes were elevated in the highest dose group. Exposures from 1.5 to 37.5 mg/l dose-dependently decreased CD3+ T lymphocytes and both CD4+ and CD8+ single-positive lymphocyte populations in the spleen (Table 3). Exposure to 7.5 and 37.5 mg/l, as mercury, decreased the CD8+ T lymphocyte population in the thymus, whereas double-positive CD4+/CD8+ and CD4+ thymocytes were not altered. Mercury altered the expression of inflammatory cytokines (tumour necrosis factor-alpha, IFN-γ, IL-12, c-myc and major histocompatibility complex II) in various organs, especially at 1.5 mg/l (300 µg/kg bw per day, as mercury) and higher. Results indicated that a decrease in T lymphocyte populations in immune organs and altered cytokine gene expression may contribute to the immunotoxic effects of inorganic mercury. A no-observed-effect level (NOEL) of 0.3 mg/l in water (60 µg/kg bw per day, as mercury) was indicated (Kim, Johnson & Sharma, 2003).

In three independent experiments, pregnant BALB/c strain mice ($n = 6$, 3 and 3 for experiments 1, 2 and 3, respectively) were exposed to mercury(II) chloride in drinking-water (10 mg/l) throughout the entire gestation period, with mercury dosing stopped at parturition. Offspring ($n = 6$) from the mercury-exposed females were weaned and at 10 weeks of age examined for immunophenotype and function of

Table 3. Effects of mercury on lymphocyte populations in the spleen of male BALB/c mice

Mercury concentration in water (mg/l)	Splenocytes ($\times 10^{5}$) per spleen expressing receptor		
	CD3+	CD4+	CD8+
0	47.69 ± 0.93	31.29 ± 0.64	14.08 ± 0.27
0.3	43.59 ± 0.64	28.75 ± 0.55	12.93 ± 0.53
1.5	41.01 ± 1.65*	26.92 ± 1.20*	12.02 ± 0.53*
7.5	37.91 ± 1.81*	25.04 ± 1.30*	10.89 ± 0.58*
37.5	35.56 ± 0.94*	23.14 ± 0.61*	10.69 ± 0.35*

* $P < 0.05$, significantly different from the control

spleen and thymus cells (Pilones, Tatum & Gavalchin, 2009). Mercury(II) chloride intake by the dams, although not provided, could be estimated as approximately 2 mg/kg bw per day (reported 5 ml water consumption per day). No body weight changes were seen in maternal animals or offspring as a result of mercury exposure, and thymus cellularity and spleen cellularity were not affected. Proliferative responses of splenocytes to both T cell (concanavalin A) and B cell (lipopolysaccharide) mitogens were slightly increased in the mercury-exposed mice compared with controls, along with increased production of some cytokines (IFN-γ and IL-4). In the female mice, an increase was also seen in the number of splenic CD4+ IdLNF1-specific T cells, which have been associated with the development of nephritis in susceptible mice strains.

(b) Neurological effects

(i) Rats

A study with rats exposed to mercury(II) chloride at a mercury dose of 0.74 mg/kg bw per day for up to 11 weeks resulted in neurological disturbances consisting of severe ataxia and sensory loss (Chang & Hartmann, 1972), but there was no indication as to whether the route of exposure was subcutaneous or oral (IPCS, 2003).

Electrophysiological parameters (electrocorticogram, cortical evoked potentials, conduction velocity and refractory periods of peripheral nerve) of the male offspring at the age of 12 weeks (eight per group per treatment protocol) from dams in the groups treated according to the protocols described in the Papp, Nagymajtenyi & Vezer (2005) study (see section 2.2.5) were investigated. The rats' spontaneous and evoked electrophysiological activity underwent dose- and treatment-dependent changes (increased frequency of spontaneous activity, lengthened latencies and duration of evoked potentials, lower conduction velocity of the peripheral nerve, etc.). However, compared with the controls, the effects were not significant ($P < 0.05$) at mercury(II) chloride doses up to 1.6 mg/kg bw per day

(high dose), with the exception that the auditory evoked potential was decreased and the refractory periods were longer in the 0.4 mg/kg bw dose group treatment protocol (P+L+P).

(ii) In vitro

In a study to characterize the effects that chelators have on the cytotoxic effects of metals, mixed cortical cell cultures containing both neuronal and glial cells were prepared from fetal mice. The cells were cultured with inorganic mercury (mercury(II) chloride), methylmercury, thimerosal, lead chloride or iron citrate in media and plated. After 24 h, the level of cell death was determined by the lactate dehydrogenase release assay. The cell deaths caused by mercury(II) chloride at 0.1, 1 and 5 µmol/l were 5%, 40% and 100%, respectively, compared with 0.4% in the controls (Rush, Hjelmhaug & Lobner, 2009). In a study to elucidate the role of intracellular GSH in protecting against mercury toxicity, mercury(II) chloride was shown to reduce the viability of Neuro-2A neuroblastoma cells (derived from the brain of an albino strain A mouse) grown in Dulbecco's Modified Eagle's Medium and reduced cellular GSH and oxidized GSH (recycled by GSH reductase back to GSH) concentrations at 1 µmol/l and higher (Becker & Soliman, 2009).

2.3 Observations in humans

2.3.1 Sources and pathways of exposure

In addition to the consumption of food containing inorganic mercury, exposure occurs through use of mercury-containing medicinal and ethnic or folk products. These include skin-lightening creams, topical creams for acne and skin cleansing, impetigo, syphilis and psoriasis, laxatives, ear drops, worming powders, teething powders, diaper powders, diuretics and cathartics, antimicrobial, antifungal and antihelminthic preparations, and traditional, ethnic or herbal medicines and folk remedies, such as Ayurvedic medicines from India and traditional medicines from China (Kang-Yum & Oransky, 1992; Hardy et al., 1995; CDC, 1996; Cooper et al., 2007; Risher & De Rosa, 2007; Liu et al., 2008; Martena et al., 2010).

2.3.2 Biomarkers of exposure

The absorption of ingested inorganic mercury from the gastrointestinal tract is estimated to vary from 2% to 38% in adults, depending on the mercury species, with most estimates being in the range of 10–15% (Holmes, James & Levy, 2009). Different mercury salts vary in solubility, with mercury(II) salts being more soluble than mercury(I) salts, which likely influences their relative absorption and toxicity. Inorganic mercury does not easily cross cell membranes such as the blood–brain barrier and placenta, although it may do so more easily by forming complexes with selenium. Moreover, metallic and organic species of mercury can undergo metabolic interconversion to inorganic forms in situ by means of oxidation and demethylation, respectively, resulting in an accumulation in tissues such as the brain and the fetus.

Excretion of inorganic mercury occurs primarily via the urine and, to a lesser extent, the bile and faeces. The half-life of inorganic mercury in the blood is only a few days, making it useful as a biomarker of recent exposure only. Moreover, total blood mercury reflects both organic and inorganic forms, limiting the information it provides solely about exposure to inorganic forms. Organic forms account for 80% of the total mercury concentration in hair, making it of limited use as a biomarker of inorganic mercury (George et al., 2010). The greatest tissue accumulation of inorganic mercury occurs in the kidneys, where it is bound to metallothionein and filtered from the blood. Accordingly, the preferred biomarker for estimating chronic exposure to inorganic mercury is considered to be the concentration in urine. A recent study in which a variety of mercury biomarkers, including total mercury, inorganic mercury and methylmercury in whole blood, red cells, plasma, hair and urine, were measured in adults confirmed total urinary mercury as the best biomarker for inorganic mercury exposure (Berglund et al., 2005). The concentration of mercury in the first morning void correlates well with the concentration in a 24 h urine sample (R^2 = 0.85), and correction for creatinine did not improve the correlation (Cianciola et al., 1997). The concentration in a spot sample, adjusted for creatinine, is more weakly correlated with the concentration in a 24 h sample (R^2 = 0.37) (Martin et al., 1996).

There are limited data on genetic influences on the metabolism of inorganic mercury. In one study of polymorphisms in glutamyl-cysteine ligase and glutathione *S*-transferase genes, conducted in gold miners (Custodio et al., 2005), individuals with genotypes associated with decreased GSH had greater retention, as reflected in higher total mercury concentrations in whole blood, plasma and urine. In studies of dental professionals exposed to elemental mercury, polymorphisms in brain-derived neurotrophic factor and coproporphyrinogen oxidase have been reported to modify the expressions of mercury neurotoxicity (Echeverria et al., 2005, 2006; Heyer et al., 2006). However, polymorphisms in the serotonin transporter gene (*5-HTTLPR*) or the catechol-*O*-methyltransferase gene do not modify the expressions of mercury neurotoxicity (Heyer et al., 2008, 2009).

There does not appear to be a consensus regarding the normal reference range for urinary mercury concentration, with levels ranging from 5 to 20 µg/l (Abbaslou & Zaman, 2006; Mahajan, 2007; Risher & De Rosa, 2007).

2.3.3 Clinical observations

Case reports of inorganic mercury intoxication suggest that acute ingestion of large amounts of mercury(II) oxide, mercury(II) chloride or mercury(II) bromide can be fatal (Ly, Williams & Clark, 2002; Triunfante et al., 2009). Although the lethal dose is uncertain, the cases of intentional ingestion suggest a mercury range of 10–>50 mg/kg bw. In two cases, the postmortem blood mercury levels were 11.7 µg/ml and 2.95 µg/ml (Triunfante et al., 2009). In other reports, individuals survived the ingestion of approximately 40 g of mercury(II) oxide (Ly, Williams & Clark, 2002), 0.9 g of mercury(II) chloride (Yoshida et al., 1997) and 100 g of mercury(II) chloride (Boscolo et al., 2009).

Acute exposure via ingestion can result in corrosive gastroenteritis, oropharyngeal burns, nausea, haematemesis, severe abdominal pain, anaemia, liver enzyme elevations, gingivitis, haematochezia, acute tubular necrosis, immunological glomerulonephritis, acute renal failure, pulmonary oedema and cardiovascular collapse (Mahajan, 2007).

The effects of chronic exposure differ somewhat depending on the form of inorganic mercury (e.g. mercury(I) versus mercury(II) chloride). In general, inorganic mercury exposure is associated with a classic triad of signs, including tremor, neuropsychiatric disturbances and gingivostomatitis. A fine intention tremor, which usually begins with the hands, can progress to choreoathetosis and spasmodic ballismus. Sensorimotor neuropathy (e.g. paraesthesias, particularly of glove and stocking sensory loss) and visual disturbances might also be present. The central nervous system signs and symptoms include emotional lability (particularly irritability and excessive shyness), delirium, headache, memory loss, insomnia, anorexia and fatigue. Renal dysfunction is also prominent and can be manifested in forms ranging from asymptomatic proteinuria (e.g. elevation of β_2-microglobulin or *N*-acetyl-D-glucosaminidase) to oliguria or anuria and to nephrotic syndrome. The dose–effect relationships are uncertain. Holmes, James & Levy (2009) stated that detectable changes in renal function begin to occur at urinary mercury concentrations of >5–10 µg/g creatinine, with "clear toxicity" at 35 µg/g creatinine.

Some data suggest that occupational exposure to mercury (mining, refining) is associated with increased risk of overall mortality, death from hypertension, non-ischaemic heart disease, pneumoconiosis, and nephritis and nephrosis (Boffetta et al., 2001). However, limited exposure information was available.

Acrodynia is a primarily cutaneous disease that can result from inorganic or elemental mercury poisoning, either from dermal exposure or from gastrointestinal absorption. It is most often observed in infants but has also been reported in children and adolescents. It is characterized by a painful, pink oedematous swelling of the feet and/or hands, with skin desquamation, morbilliform rashes, severe muscle cramping in the legs, arterial hypertension and tachycardia. Other signs can include photophobia, ulceration of mucosa, alopecia, nail loss, excessive perspiration and salivation, irritability, sleep disturbance and muscle weakness. The dose–response relationship for acrodynia is not well established. In case reports of children presenting with acrodynia, the urinary mercury levels, prior to chelation, range widely, often as high as several hundred micrograms per litre, but rarely below 30 µg/l (Torres, Rai & Hardiek, 2000; Horowitz et al., 2002; Weinstein & Bernstein, 2003; Beck et al., 2004; Abbaslou & Zaman, 2006; Michaeli-Yossef, Berkovitch & Goldman, 2007; Koh, Kwong & Wong, 2009).

In children, a variety of neurological effects have also been reported following inorganic mercury intoxication, including developmental delay and regression, poor sleep, affective disturbance and self-directed aggression (Chrysochoou et al., 2003). In two randomized trials of dental amalgam involving elemental mercury exposure, children in the amalgam groups, in which the peak mean urinary mercury concentrations were <1 and 3.2 µg/g creatinine, respectively, followed over the

5- to 7-year follow-up periods, had neuropsychological and behavioural outcomes that were not significantly different from those of children in the control group (Bellinger et al., 2006, 2007a,b, 2008; DeRouen et al., 2006; Lauterbach et al., 2008). A meta-analysis of 12 studies involving adults occupationally exposed to mercury concluded that adverse neuropsychological effects on the adult central nervous system, expressed as deficits in memory, attention and psychomotor functions, are consistently found at urinary mercury concentrations greater than 35 µg/g creatinine (Meyer-Baron, Schaeper & Seeber, 2002). However, in a study involving dentists (mean urinary mercury concentration 3.3 µg/l, standard deviation [SD] = 4.9 µg/l) and dental assistants (mean urinary mercury concentration 2.0 µg/l, SD = 2.3 µg/l), significant associations were found between urinary mercury level and scores on a variety of tests of attention, concentration, visual memory, executive functions, fine motor function and sensory function (Echeverria et al., 2006). Mercury exposure in the dental profession is mainly from elemental mercury used in amalgam materials and/or mercury.

Although some studies of occupational exposure have suggested associations between inorganic mercury exposure and some cancers, the data are limited, and IARC (1993) has classified inorganic mercury compounds in Group 3 (not classifiable as to their carcinogenicity to humans).

Evidence that exposure to inorganic mercury causes immunotoxicity in humans is very limited. In a study of chloralkali workers, despite higher urinary mercury levels than in the referent group, no differences were found in IgG levels, antinuclear autoantibodies or circulating immune complexes (Barregard et al., 1997). In another group of workers with a median urinary mercury concentration of 25.4 µg/g creatinine, no differences were seen, compared with the control group, in terms of several markers of immune function (IgA, IgG, immunoglobulin M [IgM], autoantibodies [antiglomerular basement membrane and antilaminin autobodies]) (Langworth et al., 1992). Some work has suggested a role for mercury as a cofactor in autoimmune disease, interacting with genetic predisposition or some other triggering event (e.g. an acquired event, such as malaria infection) (Silbergeld, Silva & Nyland, 2005; Vas & Monestier, 2008). Increased expression of antinuclear and antinucleolar autoantibodies was reported in people living in a gold mining area in Amazonian Brazil (Silva et al., 2004), as well as a positive interaction between mercury exposure and malaria (Silbergeld, Silva & Nyland, 2005). Other reports, usually in occupational cohorts, have linked increased mercury exposure to increased T cell proliferation (Moszczynski et al., 1995), antilaminin antibodies (Lauwerys et al., 1983) and antifibrillarin antibodies (scleroderma patients) (Arnett et al., 2000). In a recent in vitro study using human peripheral mononuclear cells, mercury(II) chloride disrupted cytokine signalling pathways, stimulating the production of pro-inflammatory cytokines and reducing the release of anti-inflammatory cytokines (Gardner et al., 2009).

Case series and case reports have demonstrated an association between the topical use of mercury(II) ammonium chloride–containing creams and nephrotic syndrome. Among 60 nephrotic syndrome patients, 15–56 years of age, in Kenya, 53% admitted using a skin-lightening cream for a mean duration of 13 months before the onset of symptoms (range 1–36 months) (Barr et al., 1972). The urinary mercury

level ranged from 0 to 250 µg/l, with the highest levels among patients who were using the cream at the time (mean concentration of 150 µg/l, compared with mean concentrations of 29 µg/l among patients who had discontinued use and 6 µg/l among patients who had never used the cream). Percutaneous renal biopsy material was available for 34 patients, 50% of whom showed a "minimal change glomerular lesion", 38% a proliferative glomerulonephritis and 12% a membranous glomerulonephritis. Follow-up of 26 patients after 6 months to 2 years showed complete remission in 50% of the patients, with most (77%) occurring spontaneously. Remission occurred 3–11 months (mean of 6 months) after a patient stopped using the cream.

3. ANALYTICAL METHODS

3.1 Chemistry

The chemistry of mercury relevant to its presence in foods has been recently well described (EFSA, 2008).

3.2 Description of analytical methods

Sample handling is generally critical only for water samples. The best materials for water sample storage and processing are polytetrafluoroethylene and fluorinated ethylene-propylene. Fresh samples are usually stored deep-frozen, lyophilized in darkness or sometimes sterilized. It has been reported that methylmercury may be decomposed in some food matrices with repeated freezing and unfreezing (particularly in bivalves). However, relatively little is known about the effect of storage on the stability of methylmercury in food samples (Leermakers et al., 2005).

Whatever the method used, it should be noted that it must be used in accordance with Good Laboratory Practice, including analytical quality assurance, the use of fully validated methods (acceptable performance criteria for limit of detection [LOD], limit of quantification [LOQ], specificity, fidelity, accuracy and precision), the use of suitable reference materials (if possible, certified reference materials) and external quality assurance (Jorhem, 2008). Participation in proficiency testing programmes and intercomparison exercises of appropriate sample matrices is highly recommended for laboratories producing results for mercury in food as an integral part of their quality control schemes.

3.2.1 Methods of analysis for determining total mercury content of foods

Most data regarding mercury in food relate to total mercury. Following acidic digestion of samples, cold vapour atomic absorption spectrometry (CV-AAS) or cold vapour atomic fluorescence spectrometry (CV-AFS) has been widely used for the determination of total mercury in several food matrices (Sánchez Uria & Sanz-Medel, 1998). An LOQ of about 30 µg/kg dry mass in foods may be obtained by CV-AAS. Further sensitivity enhancement may be obtained by CV-AFS. The main advantages of the cold vapour technique are the separation of the analyte from the

potentially interfering sample matrix and its comparatively low cost. The most frequently occurring interference in CV-AAS or CV-AFS is that of nitrites and nitric oxides reducing the signal of mercury, requiring either stripping the sample digest with inert gas or treating it with reducing agents (Nunes et al., 2005). With an LOQ of about 10 µg/kg dry mass and greater selectivity, inductively coupled plasma mass spectrometry (ICP-MS) is increasingly being used with an addition of gold chloride to mercury standard solutions to avoid the mercury memory effects (Noël et al., 2005; Julshamn et al., 2007). Although the instrumentation is expensive to purchase and to operate, the ability of ICP-MS to provide low LOQs, to provide a wide dynamic linear range and to measure many elements simultaneously can offset these cost factors.

In conclusion, CV-AAS or CV-AFS and increasingly ICP-MS have been used for a wide variety of food samples with good results, although some modifications or care may be required for certain types of matrix.

3.2.2 Methods of analysis for determining organic mercury levels in food

The implementation of a technical analysis of mercury compounds requires procedures that typically involve the following steps: extraction and/or enrichment of the matrix, derivation of non-volatile ionic species during gas chromatographic (GC) separation, cleaning (if necessary), chromatographic separation and selective detection. Each step is critical to the viability and comparability of final results. Immediately after the sampling stage, it is recommended that the samples be placed in a freezer to reduce the risk of degradation during storage, until the analysis is made (Yu & Yan, 2003; Leermakers et al., 2005; Parker & Bloom, 2005).

Basically, all the speciation methodology is generally targeted on the separation and determination of methylmercury, and there has been no conclusive identification of other species. Numerous separation and detection techniques that have been coupled to perform the speciation analysis of mercury have been recently reviewed (Carro & Mejuto, 2000; Szpunar et al., 2000; Cornelis et al., 2003, 2005; Siepak & Boszke, 2004; Leermakers et al., 2005; Stoichev et al., 2006; Björn et al., 2007; Krystek & Ritsema, 2007; EFSA, 2008). However, no standardized method of mercury speciation exists, and consequently there is a real need for the development of a fully validated method according to standardized international criteria for the determination of reliable organic mercury levels in foods.

GC with both packed and capillary columns has been the most widely used technique for the separation of mercury species, whereas high-performance liquid chromatography (HPLC) is increasingly being applied (Sánchez Uria & Sanz-Medel, 1998; Carro & Mejuto, 2000; Harrington, 2000). The detection of mercury species by GC has mainly been carried out by electron capture detector, which is, however, not specific to mercury. CV-AAS and CV-AFS are therefore more appropriate for detection, together with microwave-induced plasma atomic emission spectrometry (MIP-AES), inductively coupled plasma atomic emission spectrometry (ICP-AES), mass spectrometry (MS) and, increasingly, ICP-MS (Sánchez Uria & Sanz-Medel, 1998; Carro & Mejuto, 2000; Willoud et al., 2004; Monperrus et al., 2008). All these techniques provide sufficiently good sensitivity to be used for the analysis of certain

food samples: LODs were about 40 µg/kg for MS, 10 µg/kg for CV-AAS, 1 µg/kg for CV-AFS, 5 µg/kg for MIP-AES or ICP-AES and less than 3 µg/kg for ICP-MS. Besides its high sensitivity and selectivity, ICP-MS yields more accurate and precise results by speciated isotope dilution mass spectrometry (SID-MS) (Leermakers et al., 2005; Monperrus et al., 2008). In recent years, the use of ICP-MS in speciation analysis has increased tremendously; this is evident from the large number of publications devoted to the use of ICP-MS in the speciation of mercury (Leermakers et al., 2005). However, such advanced instrumentation is not always available in the laboratories of some countries with important fish catches, and the most commonly used method reported was CV-AAS, which is a well-established and proven method for determining the mercury content of foods. Extraction of the mercury species from its matrix is one of the most critical steps, because two conflicting issues need to be addressed: obtaining high extraction efficiency and preventing losses.

Extraction procedures vary, but most are based on the initial work of Westöö (1966, 1967, 1968), where the sample is treated with hydrochloric acid to release methylmercury from sulfhydryl groups and sodium chloride to enable its recovery into the organic phase (benzene or toluene). Inorganic mercury remains in the aqueous phase. The organic phase is further back-extracted to aqueous cysteine solutions to purify the extract. Modifications have included other organic phases, thiosulfate instead of cysteine, application of copper(II) to release methylmercury from proteins, use of bromide or chelating agents to improve extraction, further purification by back-extraction into the organic phase and defatting the samples prior to digestion to prevent emulsifications (Sánchez Uria & Sanz-Medel, 1998; Carro & Mejuto, 2000). Some workers have analysed the extracted mercury species as for total mercury, denoting it as organic mercury, and the aqueous phase of the sample for Hg^{2+}. Other workers differentiate between inorganic and organic mercury compounds by selective reduction, where the samples are treated with tin(II) chloride, reducing Hg^{2+} to Hg^0 and leaving mercury–carbon bonds intact. After complete purging of Hg^0, it is analysed by CV-AAS or CV-AFS, while the remaining sample, assumed to contain only organic mercury, is analysed as for total mercury. Instead of extraction, biological samples treated with sulfuric and iodoacetic acids have been subjected to steam distillation, where volatile methylmercury iodide is distilled off. The distillate is usually derivatized with sodium tetraethylborate (forming methylethylmercury) to improve sensitivity and performance of the GC analysis. However, the steam distillation may produce methylmercury from Hg^{2+} as an artefact (Bloom, Colman & Barber, 1997). Alkaline digestions, usually in the presence of cysteine to avoid losses of methylmercury hydroxides and to stabilize the mercury–carbon bond, with subsequent acidification and extraction of methylmercury as above, have also been used. The hydroxide releases methylmercury quantitatively from proteins. This procedure is often followed by derivatization with sodium tetraethylborate prior to GC analysis. However, in the presence of high levels of inorganic mercury, Hg^{2+} may be converted to methylmercury during derivatization (Delgado et al., 2007).

By using HPLC instead of GC for separation, the derivatization procedure may be omitted, and the cleanup becomes less critical, with LODs similar to those

for GC methods (Sánchez Uria & Sanz-Medel, 1998; Carro & Mejuto, 2000; Harrington, 2000; Leermakers et al., 2005; Hight & Cheng, 2006). Digestion may be carried out in aqueous cysteine hydrochloride directly at 60 °C and the solution analysed for methylmercury and Hg^{2+} with reversed-phase HPLC after simple filtration (Chiou, Jiang & Daadurai, 2001; Hight & Cheng, 2006; Percy et al., 2007). Precision and accuracy in single-laboratory validations have been shown to be satisfactory, but validation by way of intercomparison and/or interlaboratory studies is required. Although these methods appear promising, they have only recently been introduced and are therefore currently not in widespread use.

3.2.3 General conclusions

The techniques most frequently used to release mercury species from solid samples are acid leaching or alkaline digestion, with the option of applying ultrasonic or microwave energy to assist in the procedure. In alkaline media, methylmercury appears to be more stable than in acid media, and proteins are easily hydrolysed.

GC has been the most widely used technique for the separation of mercury species, whereas HPLC is increasingly being applied (Leermakers et al., 2005; Hight & Cheng, 2006). The methods of detection (LOD in parentheses) of CV-AAS (10 µg/kg), CV-AFS (1 µg/kg), MIP-AES or ICP-AES (5 µg/kg), MS (40 µg/kg) and ICP-MS (<3 µg/kg) all have sufficient sensitivity for food samples. The advantage of MS and ICP-MS is their multielement and multi-isotope capabilities that can yield more accurate and precise results by SID-MS, which can also check for species transformations and extraction recoveries (Krata & Bulska, 2005; Leermakers et al., 2005; Monperrus et al., 2008). Once in solution, methylmercury may decompose when exposed to light, low pH and high storage temperatures (Devai et al., 2001; Yu & Yan, 2003; Hight & Cheng, 2006; Delgado et al., 2007). Other factors, such as the type of storage container, may also affect the stability. Dimethylmercury is, for several reasons, not reliably determined by most of the methods above (Leermakers et al., 2005).

Available certified reference materials and proficiency testing schemes or intercomparison exercises exist for both total mercury and methylmercury to demonstrate and maintain analytical quality assurance. However, there is a current need for fully validated, standardized methods for determination of methylmercury and inorganic mercury.

4. SAMPLING PROTOCOLS

Some authorities have regulations with regard to specific sampling protocols for mercury and other contaminants. For example, the European Commission has regulated the number and size of incremental samples, size of the aggregate sample and precautions to be taken for control purposes (EC, 2007).

5. LEVELS AND PATTERNS OF CONTAMINATION IN FOOD COMMODITIES

At its present meeting, the Committee reviewed data from eight countries on the occurrence of mercury in different food commodities analysed between 1997 and 2009. The total number of analytical results for total mercury was more than 106 740, with 93% coming from Europe (Finland, France, Spain), 5% from Asia (China, Japan), 1% from the Americas (Brazil, Canada) and 1% from Oceania (Australia), for water (85%), fish (6%), shellfish (2%) and other food groups (6%). The 2128 samples analysed for methylmercury came from France, China and Japan for fish (94%), shellfish (2%) and other products (4%). Each of the dossiers contained analyses on individual samples, except for the dossier from China. The Committee obtained additional analytical data from the published literature. However, the Committee did not receive any occurrence data on inorganic mercury levels in foods or water. Finally, owing to the lack of required information, such as LOD, LOQ, analytical quality assurance, analytical methods, results below LOD and no results given, the data submitted to the Committee from the Netherlands and Singapore could not be used for this report.

5.1 Australia

The dossier from Australia (20th Australian Total Diet Study [TDS]) contained aggregated analytical results on total mercury from 1076 samples comprising 51 finfish collected in 2002: 21 fish fillets, raw, unfrozen (mean 0.018 mg/kg, median 0.016 mg/kg and range 0.005–0.050 mg/kg) and 21 fish portions and 9 canned tuna (mean 0.742 mg/kg, median 0.250 mg/kg and range 0.042–3.50 mg/kg) (FSANZ, 2003). No more indication of the fish species analysed was given. No indication was given of the number of species found to contain total mercury at concentrations greater than 0.5 or 1 mg/kg.

The maximum concentration found in foods other than fish was 0.048 mg/kg for prawns (mean 0.021 mg/kg and median 0.016 mg/kg).

Very low levels of organic mercury were detected in fish portions (mean 0.808 µg/kg and range not detected [ND] to 2.7 µg/kg) and in tuna, canned (mean 0.918 µg/kg and range ND–2.2 µg/kg). Surprisingly, using the maximum concentration found in fish portions or canned tuna, the percentages of methylmercury were estimated to be only 0.077% and 0.71%, respectively.

Updated data from the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) database indicated a mean total mercury concentration of 0.37 mg/kg (range 0.1–0.71 mg/kg) in 24 samples of shark sampled in 2007. No indication was given of the number of species found to contain total mercury at concentrations greater than 0.5 mg/kg.

5.2 Brazil

The dossier from Brazil (Morgano et al., 2005, 2007) contained analytical results on total mercury from 550 samples of 45 non-predatory fish species (covering 6 species of freshwater fish from pisciculture establishments) collected in

2001–2006. Fifty-one per cent of the data were below the LOQ of 0.0007 mg/kg, and the maximum concentration found was 0.878 mg/kg (mean 0.095 mg/kg, median 0.06 mg/kg). Only two species—meca (*Xiphias gladius*) and bagre (*Genidens barbus*)—contained total mercury at concentrations greater than 0.5 mg/kg.

5.3 Canada

Data from Canada (Dabeka, McKenzie & Bradley, 2003) indicated that total mercury was measured in 259 total diet food composites from two Canadian cities: Whitehorse and Ottawa. Levels were generally low, with 46% of the composites having concentrations below the LOD, which ranged from 0.026 to 0.506 µg/kg. The fish category ($n = 8$) contained the highest mercury concentrations, which averaged 67 µg/g and ranged from 24 to 148 µg/kg, with the highest level being in the canned fish from Whitehorse. All composites were below the Canadian guideline for total mercury in fish of 0.5 mg/kg.

The methylmercury levels in predatory fish species marketed in Canada were also estimated (Forsyth et al., 2004). Mercury was detected in all analysed samples of swordfish, marlin, shark and tuna purchased from major supermarket outlets and fish retailers in three cities across Canada. Total mercury and methylmercury levels ranged up to 3.85 mg/kg and 2.35 mg/kg, respectively. Swordfish contained the highest levels, followed by shark, fresh/frozen tuna and marlin. Levels in canned tuna were considerably less than those in the other examined samples (Table 4).

Table 4. Summary of total mercury and methylmercury concentrations in predatory fish from Canada

Species	*n*	Mean concentration (µg/kg)		Concentration range (µg/kg)	
		MeHg	HgT	MeHg	HgT
Marlin	3	489	842	212–881	336–1743
Shark	12	849	1360	285–1538	390–2729
Swordfish	10	1080	1822	300–2346	399–3845
Tuna, all canned	37	98	157	9–411	20–587
Tuna, canned, unidentified	5	25	47	10–43	25–69
Tuna, canned, albacore	16	166	260	105–229	193–384
Tuna, canned, skipjack	5	47	93	23–98	36–174
Tuna, canned, yellowfin	11	57	85	9–411	20–587
Tuna, fresh, frozen	13	662	929	61–1319	77–2121

HgT, total mercury; MeHg, methylmercury

It was estimated that methylmercury represents between 30% (in tuna skipjack, canned) and 94% (in fresh shark and tuna) of total mercury. Canned tuna products contained significantly less mercury and methylmercury than did retail fresh/frozen tuna, swordfish, marlin and shark samples. Canned albacore tuna, however, did contain significantly more mercury and methylmercury than did other canned tuna products sold in Canada. The percentage of mercury present in tuna as methylmercury was positively correlated with the total mercury levels, so that older, larger tuna tend to have most of the mercury present as methylmercury. This trend was not found in retail samples of shark or swordfish.

5.4 China

The dossier from China (2007 Chinese TDS) contained analytical results on total mercury and methylmercury in aquatic food and 11 other food groups (cereals, legumes, potatoes, meat, eggs, milk, vegetables, fruits, sugar, beverages and waters, alcoholic beverages) of 12 provinces of four regions (Table 5). Methylmercury was found only in aquatic food groups from all 12 provinces. The ranges for total mercury and methylmercury contents of aquatic foods were 4.77–46.0 µg/kg and 3.29–31.6 µg/kg, respectively, with means of 18.5 µg/g and 12.6 µg/kg, respectively, all well below the national maximum levels for aquatic food (500 µg/kg for non-predatory fish, 1000 µg/kg for predatory fish). The highest total mercury content (46.0 µg/kg) and methylmercury content (31.6 µg/kg) were found for Ningxia, an interior province in North 2 region. The second highest total mercury content (41.4 µg/kg) and methylmercury content (29.8 µg/kg) were found in Guangxi province, a coastal province in South 2 region. The mean total mercury and methylmercury contents for coastal provinces (Liaoning, Hebei, Shanghai, Fujian, Guangxi) were 21.0 µg/g and 14.0 µg/kg, respectively, and those for the interior provinces were 18.2 µg/g and 11.5 µg/kg, respectively. The orders of mean total mercury and methylmercury contents of four regions are both South 2 > North 2 > South 1 > North 1. The percentages of methylmercury in total mercury in aquatic foods ranged from 50% to 87%, with a mean of 68%. The total mercury mean results for the other food groups were less than 5 µg/kg (compared with 18.5 µg/kg for aquatic foods), and the per cent contributions of food composites to the corresponding national maximum levels were generally low, except in Shanghai province (more than 25% for cereals, legumes, potatoes, meat, eggs, vegetables and fruits).

In another study, in Zhoushan, China, the average total mercury and methylmercury concentrations in all species of fish (n = 148) were 0.26 mg/kg and 0.18 mg/kg, respectively (Cheng et al., 2009). Total mercury and methylmercury levels measured in all fish samples ranged from lows of 0.01 mg/kg and 0.0004 mg/kg wet mass, respectively, found in a specimen of *Monopterus albus* (mean concentrations of 0.13 mg/kg and 0.09 mg/kg, respectively). Approximately 15% and 19% of the samples, respectively, showed total mercury and methylmercury levels that exceeded the limits established by the Chinese National Standards Agency (CNSA) (0.3 mg/kg and 0.2 mg/kg, respectively; CNSA, 1994). The proportion of methylmercury relative to total mercury in the fish ranged from 59% to 84%, with a mean of 74%, which indicated, according to the authors, that fish accumulate more organic mercury (methylmercury) than inorganic mercury.

Table 5. Total mercury and methylmercury in foods from 12 provinces of China in 2007 Chinese TDS

Food groups	Total mercury concentrations (μg/kg)[a]													1990 Chinese TDS[b]	2000 Chinese TDS[c]
	Heilong-jiang	Liaoning	Hebei	Shanxi	Ningxia	Henan	Shanghai	Hubei	Fujian	Sichuan	Guangxi	Jiangxi	Mean	Mean	Mean
Cereals	0.13 (0.7)	0.35 (1.8)	0.10 (0.5)	0.26 (1.3)	0.51 (2.6)	ND	12.03 (60.2)	0.36 (1.8)	0.78 (3.9)	0.58 (2.9)	0.10 (0.5)	0.10 (0.5)	1.39	13	9.0
Legumes	0.31 (3.1)	0.43 (4.3)	ND	0.13 (1.3)	0.12 (1.2)	ND	13.05 (130.5)	0.20 (2.0)	0.11 (1.1)	ND	0.92 (9.2)	ND	1.91	10	4.0
Potatoes	0.10 (1.0)	ND	ND	0.13 (1.3)	0.12 (1.2)	ND	10.45 (104.5)	ND	0.13 (1.3)	0.14 (1.4)	0.14 (1.4)	ND	1.60	7	4.9
Meat	1.46 (2.9)	0.43 (0.9)	0.41 (0.8)	0.38 (0.8)	0.45 (0.9)	0.19 (0.4)	13.51 (27.0)	1.01 (2.0)	0.39 (0.8)	0.42 (0.8)	1.45 (2.9)	1.30 (2.6)	1.78	13	5.6
Eggs	0.20 (0.4)	0.32 (0.6)	0.24 (0.5)	6.08 (12.2)	0.32 (0.6)	0.15 (0.3)	38.10 (76.2)	8.30 (16.6)	1.11 (2.2)	1.15 (2.3)	0.41 (0.8)	0.45 (0.9)	4.74	29	5.0
Aquatic foods	4.77 (1.0)	13.35 (2.7)	10.71 (2.1)	8.47 (1.7)	46.04 (9.2)	10.45 (2.1)	28.06 (5.6)	10.30 (2.1)	11.54 (2.3)	14.49 (2.9)	41.41 (8.3)	22.22 (4.4)	18.48	40	24.4
MeHg	**3.29**	**11.7**	**8.03**	**5.31**	**31.6**	**7.46**	**14.0**	**7.34**	**6.39**	**9.76**	**29.8**	**15.9**	**12.55**		
Milk	ND	ND	ND	0.15 (1.5)	0.17 (1.7)	ND	1.77 (17.7)	2.23 (22.3)	ND	ND	ND	0.46 (4.6)	0.96	0	1.8
Vegetables	0.10 (1.0)	0.30 (3.0)	0.18 (1.8)	0.24 (2.4)	ND	ND	13.49 (134.9)	0.25 (2.5)	0.46 (4.6)	0.45 (4.5)	1.82 (18.2)	0.48 (4.8)	1.78	3	3.2

Table 5 (contd)

Food groups	Total mercury concentrations (μg/kg)[a]													1990 Chinese TDS[b]	2000 Chinese TDS[c]
	Heilong-jiang	Liaoning	Hebei	Shanxi	Ningxia	Henan	Shanghai	Hubei	Fujian	Sichuan	Guangxi	Jiangxi	Mean	Mean	Mean
Fruits	ND	0.26 (2.6)	0.20 (2.0)	0.13 (1.3)	0.18 (1.8)	0.17 (1.7)	3.84 (38.4)	ND	0.11 (1.1)	0.14 (1.4)	0.24 (2.4)	ND	0.59	4	2.7
Sugar	ND	0.16	0.22	0.15	0.19	0.45	0.95	ND	0.22	0.10	0.25	ND	0.30	0	5.0
Beverages and water	ND	ND	ND	ND	ND	ND	0.73	ND	ND	ND	ND	ND	0.06	0	0.3
Alcoholic beverages	ND	ND	ND	0.51	ND	ND	4.12	ND	0.11	ND	0.21	0.10	0.42	2	1.3

MeHg, methylmercury; ND, not detected

[a] Figures in parentheses are the per cent contributions (%) of food composites to the corresponding national maximum levels. There were no national maximum levels for sugar, beverages and water, or alcoholic beverages.

[b] Data from Chen & Gao (1993).

[c] Data from Li, Gao & Chen (2006).

Table 6. Summary of total mercury and methylmercury concentrations in fish from Hong Kong SAR

Species	*n*	Mean concentration (µg/kg)		Concentration range (µg/kg)	
		MeHg	HgT	MeHg	HgT
Local whole fish	224	55	69	3–349	3–374
Imported whole fish	42	140	179	21–1010	29–1370
Tuna, canned	14	144	181	27–430	37–469
All samples	280	72	91	3–1010	3–1370

HgT, total mercury; MeHg, methylmercury

Total mercury and methylmercury levels in crops, poultry, milk, drinking-water, food oil and salt samples (n = 88) were all below the corresponding CNSA limits (total mercury: 0.02 mg/kg for grain, 0.01 mg/kg for vegetables, 0.05 mg/kg for both egg and meat, 0.01 mg/kg for milk and 1000 ng/l for drinking-water; CNSA, 1994).

Another study from Hong Kong Special Administrative Region (SAR) estimated the total mercury and methylmercury concentrations in 280 samples of fish purchased from different commercial outlets (covering 89 species of whole fish and three types of canned tuna) (Tang et al., 2009). For total mercury, the median concentration was 63 µg/kg (range 3–1370 µg/kg), and for methylmercury, the median concentration was 48 µg/kg (range 3–1010 µg/kg) (Table 6). Total mercury and methylmercury were detectable in all 280 samples, of which 277 fish samples (99%) contained total mercury (range 3–469 µg/kg) and methylmercury (range 3–430 µg/kg) at levels below the regulatory limit of 500 µg/kg. Only three samples of imported alfonsino (*Beryx splendens*) had mercury levels higher than 500 µg/kg (total mercury: mean 1053 µg/kg, range 609–1370 µg/kg; methylmercury: mean 827 µg/kg, range 509–1010 µg/kg).

5.5 Finland

The dossier from Finland (Finland, 2010) contained analytical results on total mercury from 74 samples, comprising 31 milk and milk products (31% of samples above the LOQ) and 43 finfish (all above the LOQ), collected in 2006–2009. The only fish species found to contain total mercury at concentrations greater than 1.0 mg/kg was pike (1.53 mg/kg). One additional species, tuna, was found to contain total mercury at concentrations greater than 0.5 mg/kg (Table 7).

5.6 France

The dossier submitted by France (France, 2010) contained individual analytical results on total mercury from 999 samples of foods other than fish products, 90 545 aggregated samples of water, 3499 finfish and 1892 shellfish, collected in 1997–2007.

Table 7. Data on mercury concentrations in finfish from Finland

Mean concentrations of total mercury:				
Samples	No. of samples	Mean (mg/kg)		
All samples	43	0.108		
<1 mg/kg	42	0.074		
<0.5 mg/kg	41	0.060		
Mean concentrations of total mercury by species:				
Species	No. of samples	Mean (mg/kg)		% reduction
		All samples	Violative samples removed	
>1 mg/kg				
Pike	1/2	0.94	0.35	63

Of the 999 samples of foods other than fish products, 86% were less than the LOQs (range 0.003–0.011 mg/kg), and the maximum concentration found was 0.050 mg/kg in fungi (mean 0.022 mg/kg, median 0.018 mg/kg). Ninety-eight per cent of water samples were below the LOQ of 0.02 µg/l, with a maximum of 4.3 µg/l (mean lower bound 0.005 µg/l; mean upper bound 0.339 µg/l).

The only shellfish species found to contain total mercury at concentrations greater than 0.5 mg/kg was common scallop, with a concentration of 0.86 mg/kg, and 20% of the data were below the LOQ (maximum 0.1 mg/kg). Only 1.4% of the data were below the LOQ (maximum 0.1 mg/kg) for the fish species. The fish species found to contain total mercury at concentrations greater than 1.0 mg/kg (with the percentage within a species that exceeded either of the two limits in parentheses) were lamprey (100%), Portuguese dogfish (92%), swordfish (89%), shark (83%), marlin (50%), picked dogfish (35%), tuna (24%), catshark (22%), scabbardfish (14%), ling (14%), pike (2.2%) and ray (2.9%). The maximum content of 4.3 mg/kg was found in tuna. Additional species were found to contain mercury at concentrations greater than 0.5 mg/kg: emperor (39%), Atlantic bonito (25%), smooth hounds (20%), megrim (18%), barbell (9.1%), sheatfish (6.3%), mullet (6.3%), pike perch (8.1%), grenadier (5.4%), gurnard (2.7%), seabass (2.7%), eel (2.4%), anglerfish (2.3%), pout (2.2%), European perch (1.9%), whiting (1.1%), cod (1.0%) and trout (0.1%) (Table 8).

The dossier also contained analytical results on methylmercury from 153 samples of fish products (105 finfish, 44 shellfish and 4 seafood-based dishes) collected in 2005 (Sirot et al., 2008). No shellfish species contained methylmercury at concentrations greater than 0.5 mg/kg (range 0.002–0.451 mg/kg), with the maximum concentration found in edible crab. Of the 105 individual finfish samples analysed, all were quantified, and only 7 (6.7%) were found to contain methylmercury at concentrations greater than 0.5 mg/kg, with one (1%)

Table 8. Data on mercury concentrations in finfish from France

Mean concentrations of total mercury:				
Samples	No. of samples	Mean (mg/kg)		
All samples	4480	0.185		
<1 mg/kg	4398	0.144		
<0.5 mg/kg	4228	0.115		
Mean concentrations of total mercury by species:				
Species	No. of samples	Mean (mg/kg)		% reduction
		All samples	Violative samples removed	
>1 mg/kg				
Lamprey	7/11	1.13	0.75	34
Portuguese dogfish	23/26	1.99	0.27	86
Swordfish	21/36	1.14	0.66	42
Shark	4/6	1.76	0.57	68
Marlin	2/4	1.95	0.19	90
Picked dogfish	1/21	0.39	0.30	23
Tuna	17/411	0.37	0.32	14
Catshark	2/59	0.43	0.39	9
Scabbardfish	1/21	0.36	0.33	8
Ling	1/50	0.31	0.29	6
Pike	1/46	0.14	0.11	21
Ray	1/69	0.12	0.10	17
Mean concentrations of methylmercury:				
Samples	No. of samples	Mean (mg/kg)		
All samples	105	0.167		
<1 mg/kg	104	0.154		
<0.5 mg/kg	98	0.121		
Mean concentrations of methylmercury by species:				
Species	No. of samples	Mean (mg/kg)		% reduction
		All samples	Violative samples removed	
>1 mg/kg				
Swordfish	1/4	0.94	0.79	16

concentration greater than 1 mg/kg. The only species found to contain methylmercury at concentrations greater than 1.0 mg/kg (the percentage within a species that exceeded either of the two limits shown in parentheses) was swordfish (100%), with a concentration of 1.42 mg/kg (Table 8). Two additional species were found to contain mercury at concentrations greater than 0.5 mg/kg: emperor fish (67%) and tuna (20%).

It was estimated that methylmercury is between 90% and 105% of total mercury.

5.7 Japan

The dossier submitted by Japan (Japan, 2010) contained individual analytical results on total mercury from 3877 samples of foods other than fish products (cereals, fruits and vegetables) collected in 2002–2009 and on total mercury and methylmercury from 1275 finfish collected in 2007–2009.

The maximum concentration found in foods other than fish products (20% of samples above the LOQ; mean 0.003 mg/kg, median 0.002 mg/kg) was 0.013 mg/kg in rice.

The fish species (100% above the LOQ) found to contain total mercury at concentrations greater than 1.0 mg/kg (with the percentage within a species that exceeded the limit in parentheses) were swordfish (66%), splendid alfonsino (31%), blue shark (14%), marlin (11%) and tuna (2.6%). The maximum content of 11.4 mg/kg was found in striped marlin. No additional species were found to contain total mercury at concentrations greater than 0.5 mg/kg (Table 9).

Of the 1275 individual finfish samples analysed (100% above the LOQ), 408 (32%) were found to contain methylmercury at concentrations greater than 0.5 mg/kg, with 111 (8.7%) containing methylmercury at concentrations greater than 1 mg/kg. The species found to contain methylmercury at concentrations greater than 1.0 mg/kg (with the percentage within a species that exceeded either of the two limits in parentheses) were swordfish (54%), splendid alfonsino (17%), blue shark (13%), tuna (2.0%) and marlin (1.0%) (Table 9). The maximum methylmercury content of 2.8 mg/kg was found in swordfish. No additional species were found to contain methylmercury at concentrations greater than 0.5 mg/kg.

Of the 1275 individual finfish samples analysed, methylmercury ranged between 38% and 100% of total mercury, except for 6 samples of the same species, blue marlin (n = 50), where methylmercury was only 8–11% of total mercury. It should be noted that these six samples contained the highest levels of total mercury (range 2.0–11.4 mg/kg). The other blue marlin species had methylmercury contents ranging from 38% to 75% of total mercury.

5.8 Spain

The mercury contents of 25 samples of fish and shellfish products most frequently consumed in Spain were determined (Sahuquillo et al., 2007). There was wide variability, not only among the mercury levels of different fish species, but also for different samples of the same species—with the methylmercury content ranging

Table 9. Data on mercury concentrations in finfish from Japan

Mean concentrations of total mercury:				
Samples	No. of samples	Mean (mg/kg)		
All samples	1275	0.546		
<1 mg/kg	1117	0.390		
<0.5 mg/kg	736	0.249		
Mean concentrations of total mercury by species:				
Species	No. of samples	Mean (mg/kg)		% reduction
		All samples	Violative samples removed	
>1 mg/kg				
Swordfish	79/120	1.30	0.65	50
Splendid alfonsino	37/120	0.77	0.54	30
Blue shark	13/90	0.66	0.54	18
Marlin	12/110	0.70	0.37	47
Tuna	17/655	0.45	0.42	7
Mean concentrations of methylmercury:				
Samples	No. of samples	Mean (mg/kg)		
All samples	1275	0.444		
<1 mg/kg	1164	0.353		
<0.5 mg/kg	867	0.248		
Mean concentrations of methylmercury by species:				
Species	No. of samples	Mean (mg/kg)		% reduction
		All samples	Violative samples removed	
>1 mg/kg				
Swordfish	65/120	1.10	0.64	42
Splendid alfonsino	20/120	0.65	0.53	18
Blue shark	12/90	0.59	0.49	17
Marlin	1/110	0.30	0.29	3
Tuna	13/655	0.39	0.37	5

from below 54 to 596 µg/kg wet mass (Table 10). Total mercury contents in fish analysed in this study did not exceed the maximum levels established by the European Union. The highest mercury levels corresponded to predatory fish species located at the highest level of the food-chain (tuna, swordfish). According to the authors, despite their small size compared with tuna, salmon and swordfish, the relatively high mercury contents in mollera (poor cod, *Trisopterus minutes*) and pagre (common sea bream, *Pagrus pagrus*) could be explained by the fact that they came from Valencian coastal waters at the mouth of the Albufera lake. The comparison of methylmercury contents in fresh and canned tuna shows a mean difference of 17%. In samples of the same brand of canned tuna, differences between batches, perhaps ascribable to differences in the origin of the tuna, were found. Methylmercury contents were higher in canned natural tuna than in tuna in vegetable oil. The differences cannot be ascribed to the brine; considering the high variability in methylmercury content in the same fish species, the possibility that the difference could be due to the fish origin cannot be ruled out. However, the fact that the lower content corresponded to tuna in oil (with values even lower than those corresponding to the analysed fresh tuna) could also be explained by partial dissolution of organic mercury in the oil.

Table 10. Summary of methylmercury concentrations in fresh or canned fish and shellfish from Spain

Fish species	Mean methylmercury concentration (mg/kg)
Fresh species	
Tuna, fresh	0.596
Swordfish	0.479
Mollera	0.199
Pagre	0.153
Hake	0.143
Serrano	0.131
Perch	0.070
Mackerel	0.064
Chucla	0.059
Anchovy	0.058
Salmon	<0.054
Salmon, smoked	<0.054
Sardine	<0.054
Sole	<0.054
Prawn, cooked	<0.054

Table 10 (contd)

Fish species	Mean methylmercury concentration (mg/kg)
Prawn, fresh	<0.054
Llisa	<0.054
Mussel	<0.054
Canned species	
Tuna, natural	0.609
Tuna, in vegetable oil A	0.455
Tuna, in vegetable oil B	0.207
Tuna, in vegetable oil C	0.423
Mackerel	0.094
Mussel	<0.054
Octopus	<0.054

Finally, according to the network Rapid Alert System for Food and Feed, the number of foods that exceeded regulatory limits on mercury in imported foods by the European community is 449 over the period 2002–2008.

5.9 General conclusions

Total mercury levels in foods other than fish products were generally low (range 0.0001–0.050 mg/kg), with about 80% of the 6183 samples containing levels below the LOQs. The highest levels were found in fungi. Mean methylmercury levels reported by China in non-fish samples ranged from 0.001 to 0.023 mg/kg, with a maximum concentration found in poultry. No other information on methylmercury in non-fish samples was received from other countries. In water, total mercury levels in 98% of 90 545 samples analysed in France were below the LOQ of 0.02 µg/l, with a maximum of 4.3 µg/l.

Total mercury levels in 1892 shellfish samples (80% above LOQ) ranged from 0.002 to 0.86 mg/kg. No shellfish species contained methylmercury at concentrations greater than 0.5 mg/kg (range 0.002–0.451 mg/kg), with the maximum concentration found in edible crab.

Total mercury levels in 6114 fish samples ranged from 0.001 to 11.4 mg/kg, with the maximum concentration found in marlin. About 5% exceeded 1 mg/kg, particularly for lamprey, Portuguese dogfish, swordfish, shark, marlin, splendid alfonsino, picked dogfish, tuna, catshark, scabbardfish, ling, pike and ray.

The proportion of total mercury contributed by methylmercury generally ranged between 30% and 100%, depending on species of fish, size, age and diet. Furthermore, in about 80% of these data, methylmercury accounted for more than 80% of total mercury. However, a few submitted data showed proportions of methylmercury of about 10% or less.

6. FOOD CONSUMPTION AND DIETARY EXPOSURE ESTIMATES

6.1 General considerations on exposure to mercury from food

From a food perspective, the predominant human exposure to mercury occurs through the consumption of fish and shellfish. Analysis of a wide variety of foodstuffs collected from 12 different countries has shown that mercury concentrations in fish and shellfish are approximately 10–100 times greater than those in other foods, including cereals, potatoes, vegetables, fruits, meat, poultry, eggs, milk and milk products (Toro et al., 1994) (see also section 5).

The contribution of methylmercury to total mercury in fish varies with respect to age, size and trophic level of the fish species. In general, the percentage of total mercury contributed by methylmercury is usually 80–100% in fish muscle, but it can be significantly lower in organs such as liver and kidney (Storelli & Marcotrigiano, 2000; Storelli, Stuffler & Marcotrigiano, 2002). Additional studies have confirmed that for the majority of fish species studied, organic mercury represents the major fraction of total mercury in muscle tissue (Bloom, 1992; Holsbeek, Das & Joiris, 1997; Krystek & Ritsema, 2005; Storelli, Busco & Marcotrigiano, 2005; Storelli et al., 2005; Houserova et al., 2007; Afonso et al., 2008). For herbivorous fish, methylmercury can account for up to 70% of total mercury, whereas for piscivorous species, a maximum 100% contribution is possible (Lasorsa & Allen-Gil, 1995; Mason, Reinfelder & More, 1995). In a 5-year survey of canned tuna, up to 89% of the total mercury was considered to be methylmercury (Burger & Gochfeld, 2004), whereas a wide range of percentages (30–79%; average 62.4%) was reported in 37 samples of canned tuna in another survey (Forsyth et al., 2004). Analysis of 89 different species of fish and 3 varieties of canned tuna collected from commercial markets in Hong Kong SAR (n = 280 samples) gave a median methylmercury to total mercury percentage of 76% (Tang et al., 2009).

For marine mammals, a significant fraction of total mercury detected in organs (liver and kidney) is in the form of inorganic mercury (Wagemann et al., 1998). In general, the concentration of total mercury for marine mammals is greatest in liver, followed by kidney and then muscle tissue. As with fish, total mercury in muscle tissue is dominated by methylmercury; for liver, while variable, depending on species and age of the animal, the average contribution of organic mercury to total mercury is only approximately 15% (range 9–40%) (Wagemann et al.,1998).

Analysis of various bivalve species has shown variability in the ratio of methylmercury to total mercury, ranging from approximately 20% to 89% (Liang et al., 2003).

While limited details were available at the time for mercury speciation in foods other than fish and shellfish, earlier reports (IPCS, 1976) indicated that total mercury levels usually did not exceed 60 µg/kg, with methylmercury predominating, except in some samples of organ meats (liver and kidney). In the IPCS (1991) report on inorganic mercury, it was reported that inorganic mercury levels in most foods were usually below the LOD (20 ng/g). Based on the results of various TDSs, it was estimated that the average dietary inorganic mercury exposure was approximately 4.2 µg/day (IPCS, 1990). In all food items not related to fish, total mercury was

presumed to be only inorganic mercury, whereas for fish, 20% of total mercury exposure (3.0 µg/day) was considered to be exposure to inorganic mercury.

Inorganic forms of mercury appear to have limited potential for uptake by terrestrial plants (Bloom, 1992).

An additional source of dietary inorganic mercury can be from human milk. In a study that investigated the total and inorganic mercury content of human milk and blood in relation to fish consumption and amalgam fillings, an average of 51% of total mercury detected in milk (0.6 ng/g) was in the inorganic form, compared with only 26% in blood (Oskarsson et al., 1996). Mercury levels in milk were correlated to the number of amalgam fillings, but not methylmercury intake. Other studies have reported that there is a significant correlation between the concentration of mercury in breast milk and the number of amalgam surfaces in mothers reporting low fish consumption (one fish meal per month) (Da Costa, Malm & Dórea, 2005).

6.2 National estimates

6.2.1 Total mercury

Most of the available dietary exposure assessments for mercury were from national TDSs. These include the following TDSs: Australia (2000–2001), Canada (1998–2000), China (2007), Czech Republic (2000), France (2001–2002), Japan (2008), New Zealand (2003–2004), the Republic of Korea (2005), the United Kingdom (2006) and the USA (1991–2005). Published data from other studies focusing on special subpopulations were also available. These include TDSs conducted in Chile (Santiago) and Spain (Catalonia), as well as studies of fishermen and their household members in Zhoushan Island (China), residents of Changchun city in north-east China, secondary-school students in Hong Kong SAR, frequent seafood consumers in France (the CALIPSO study, or the Fish and Seafood Consumption Study and Biomarkers of Exposure to Trace Elements, Pollutants and Omega-3), exposures from fish and shellfish in Spain and modelled exposure estimates for fish consumers in the USA.

In general, most studies available allowed for the estimation of dietary exposure to total mercury from fish and shellfish, as well as from other foods.

(a) Australia

Total diet total mercury exposures reported in the 2000–2001 TDS (FSANZ, 2003) ranged from 0.01–0.08 µg/kg bw per day for adult females and 12-year-old girls to 0.01–0.25 µg/kg bw per day for infants (9 months of age). Total mercury exposures from foods other than fish and shellfish ranged from 0–0.07 µg/kg bw per day for adult females and 12-year-old girls to 0–0.24 µg/kg bw per day for infants (Table 11). The lower limits of the exposure ranges correspond to estimates derived assuming that samples with non-detectable concentrations have total mercury concentrations of 0 µg/kg, whereas upper limits assume that samples with non-detectable concentrations have total mercury concentrations equal to the LOD. It should be noted that, except for bacon, the only foods with detectable total mercury concentrations were fish and shellfish foods. Further, in the case of bacon, the

Table 11. Total diet total mercury exposures from the 2000–2001 Australian TDS

Subpopulation	Exposure from total diet (µg/kg bw per day)	Exposure from fish and shellfish[a] (µg/kg bw per day)	Exposure from foods other than fish or shellfish[a] (µg/kg bw per day)
Adult males (25–34 years)	0.01–0.09	0.01	0–0.08
Adult females (25–34 years)	0.01–0.08	0.01	0–0.07
Boys (12 years)	0.01–0.10	0.01	0–0.09
Girls (12 years)	0.01–0.08	0.01	0–0.07
Toddlers (2 years)	0.01–0.20	0.01	0–0.19
Infants (9 months)	0.01–0.25	0.02	0–0.24

[a] Derived by combining food consumption data and median total mercury levels reported in the Food Standards Australia New Zealand 20th Australian TDS report (FSANZ, 2003).

majority of the samples (17 out of 21) had non-detectable concentrations. Hence, all lower limits for exposure values are zero, and all upper limits overestimate exposures; therefore, it is not possible to estimate the contribution of individual foods to the total exposure to total mercury.

(b) Canada

Total diet total mercury exposure estimates for the Canadian population varied from 0.010–0.012 µg/kg bw per day (females 65+ years) to 0.055–0.062 µg/kg bw per day (infants 0–1 month). Total mercury exposures from foods other than fish and shellfish ranged from 0.004–0.006 µg/kg bw per day (females 65+ years) to 0.019–0.026 µg/kg bw per day (infants 2–3 months) (Table 12). The lower limits of the ranges correspond to estimates derived assuming that samples with non-detectable concentrations have a total mercury concentration of 0 µg/kg, whereas upper limits assume that samples with non-detectable concentrations have concentrations equal to the LOD. Foods other than fish and shellfish contributing most to total mercury exposures are dairy products (in the case of the children and infant subpopulations) and meats (for the older subpopulations). However, these contributions were based on the upper limit estimates that assumed that samples with non-detectable concentrations have concentrations equal to the LOD; hence, the contributions of highly consumed foods may be artificially inflated.

(c) Chile

Total diet total mercury exposure estimates for the population of Santiago, Chile, available from a TDS conducted between 2001 and 2002, ranged from 0.059

Table 12. Total diet total mercury exposures from the 1998–2000 Canadian TDS

Subpopulation	Total diet exposure[a] (µg/kg bw per day)	Exposure from fish and shellfish (µg/kg bw per day)	Exposure from foods other than fish or shellfish[a] (µg/kg bw per day)	Food other than fish and shellfish contributing most to total exposure[b]
M & F 0–1 month	0.055–0.062	0.038	0.017–0.024	Dairy products (44%)
M & F 2–3 months	0.019–0.026	0	0.019–0.026	Baby foods (52%)
M & F 4–6 months	0.018–0.027	0	0.018–0.027	Dairy products (58%)
M & F 7–9 months	0.021–0.028	0.003	0.018–0.025	Dairy products (44%)
M & F 10–12 months	0.015–0.023	0	0.015–0.023	Dairy products (49%)
M & F 1–4 years	0.033–0.042	0.017	0.016–0.025	Dairy products (36%)
M & F 5–11 years	0.032–0.038	0.020	0.011–0.018	Dairy products (28%)
M 12–19 years	0.022–0.026	0.014	0.008–0.012	Meat and meat products (24%)
M 20–39 years	0.027–0.030	0.019	0.008–0.011	Meat and meat products (30%)
M 40–64 years	0.018–0.021	0.012	0.006–0.009	Meat and meat products (26%)
M 65+ years	0.017–0.019	0.012	0.005–0.007	Meat and meat products (23%)
F 12–19 years	0.023–0.026	0.017	0.006–0.009	Dairy products (22%)
F 20–39 years	0.017–0.019	0.011	0.007–0.008	Meat and meat products (24%)
F 40–64 years	0.026–0.028	0.021	0.005–0.007	Meat and meat products (21%)
F 65+ years	0.010–0.012	0.006	0.004–0.006	Meat and meat products (19%)

F, female; M, male

[a] Lower limits of the ranges assume a zero concentration when the mercury concentration for individual composites fell below the LOD, whereas the upper limits assume a concentration equal to the LOD for these composites.

[b] Contributions based on exposure estimates derived assuming a concentration equal to the LOD when the mercury concentration for individual composites fell below the LOD.

Source: Dabeka, McKenzie & Bradley (2003)

Table 13. Total diet total mercury exposures for the population of Santiago, Chile, using a TDS

	Total mercury exposure[a] (µg/kg bw per day)
Exposure from fish and shellfish	0.024
Exposure from foods other than fish and shellfish	0.035–0.055
Total exposure	0.059–0.079

[a] Estimates were derived by combining consumption estimates and mean total mercury concentrations reported in Muñoz et al. (2005) and assume a body weight of 65 kg. Lower limits of the ranges assume a zero concentration for foods where the mean mercury concentration was below the LOD, whereas the upper limits assume a concentration equal to the LOD for these foods.

Source: Muñoz et al. (2005)

to 0.079 µg/kg bw per day. Total mercury exposures from foods other than fish and shellfish ranged from 0.035 to 0.055 µg/kg bw per day (Table 13). Lower limits of the ranges assume a zero concentration for foods where the mean mercury concentration was below the LOD, whereas the upper limits assume a concentration equal to the LOD for these foods. Bread is the highest contributor to the total mercury exposure from food other than shellfish. Its contribution ranged from 27% when the LOD is used for foods with mean concentration below the LOD to 43% when a zero concentration is assumed for these foods.

(d) China

Total diet total mercury exposure for an average adult Chinese male was estimated to be 0.08 µg/kg bw per day in the 2007 TDS. Other estimates from Changchun city for adults 18–77 years old are comparable (0.10 µg/kg bw per day); however, estimates for a subpopulation of fishermen and their families were much higher (0.92 and 0.47 µg/kg bw per day for adult males and females, respectively, and 0.67 µg/kg bw per day for children). Total mercury exposures from fish and shellfish for the general population are generally low: 0.01 µg/kg bw per day (2007 TDS) and 0.08 µg/kg bw per day among secondary-school children in Hong Kong SAR. However, exposures from fish and shellfish were well above these levels for the subpopulation of fishermen and their families (Table 14).

(e) Czech Republic

Total diet total mercury exposure estimates for the general population of the Czech Republic were 0.008 µg/kg bw per day in 2000 and 0.009 µg/kg bw per day in 2001. No estimates were submitted for the contribution of the various foods to total exposure to total mercury (Table 15).

Table 14. Total diet total mercury exposures from the 2007 China TDS and published studies

Study	Population	Total dietary exposure (µg/kg bw per day)	Exposure from fish and shellfish (µg/kg bw per day)	Exposure from foods other than fish and shellfish (µg/kg bw per day)
2007 TDS[a]	Adult male (average)	0.08	0.01	0.07
	Adult male (97.5th percentile)	0.51	NA	NA
Changchun city[b]	Adults 18–77 years	0.10	0.01	0.09
Fishermen and families in Zhoushan Island[c]	Adult males	0.92	0.87	0.05
	Adult females	0.47	0.41	0.06
	Children	0.67	0.57	0.10
Hong Kong SAR[d] (2000)	Secondary-school children (consumers with average exposure)	NA	0.08	NA
	Secondary-school children (consumers with high exposure)	NA	0.25	NA

NA, not available
[a] China (2010).
[b] Li, Wang & Luo (2006).
[c] Cheng et al. (2009).
[d] Tang et al. (2009).

(f) France

Total diet total mercury mean exposure estimates for the average French 3- to 4-year-old child and adult as estimated by the TDS were 0.26 µg/kg bw per day and 0.16 µg/kg bw per day, respectively. The corresponding estimates of total mercury exposures from foods other than fish and shellfish were 0.24 µg/kg bw per day and 0.15 µg/kg bw per day (Table 16). These estimates were derived assuming ½ LOD for foods with non-detectable concentrations and ½ LOQ for foods with concentrations below the LOQ. Hence, exposure estimates for foods with non-detectable concentrations are artificially overinflated and cannot be used to estimate contributions of individual foods. Total mercury exposures from fish for frequent fish consumers were much higher (Table 17), ranging from 0.87 µg/kg bw per week (0.12 µg/kg bw per day) to 1.75 µg/kg bw per week (0.25 µg/kg bw per day).

Table 15. Total diet total mercury exposures from the 2000 and 2001 Czech TDS

Country	Total diet study	Population	Mean total mercury exposure (µg/kg bw per day)[a]
Czech Republic	Czech TDS 2000	General population (0–88 years)	0.008
Czech Republic	Czech TDS 2001	General population (0–88 years)	0.009

[a] From TDS studies submitted to the GEMS/Food database.

Table 16. Total diet total mercury exposures from the first French TDS (2000–2001)

	Exposure (µg/kg bw per day)			
	Child 3–4 years		Adult 15+ years	
	Mean	95th percentile	Mean	95th percentile
Fish and shellfish	0.02	NA	0.02	NA
Foods other than fish and shellfish	0.24	NA	0.15	NA
Total diet	0.26	0.41	0.16	0.25

NA, not applicable
Source: Leblanc et al. (2005)

(g) Japan

Total mercury exposure estimates for the Japanese population for the period ranging from 1977 to 2008 were submitted to the Committee. The data from the period 2000–2008 are presented in Table 18. Total dietary total mercury exposures were estimated to be 0.14 µg/kg bw per day in 2000 and increased to 0.17 µg/kg bw per day in 2008. Exposures from foods other than fish and shellfish are low (0.02 µg/kg bw per day in 2000 and 0.01 µg/kg bw per day in 2008). The meats and eggs group is the highest contributor to total mercury exposures from foods other than fish and shellfish.

(h) New Zealand

Total diet total mercury exposures for New Zealand range from 0.066 µg/kg bw per day for female adolescents (11–14 years) to 0.16 µg/kg bw per day for infants (6–12 months). The 2003–2004 New Zealand TDS report (NZFSA, 2005) indicates that fish products contributed 74% of the dietary mercury exposure for young males and 65% for toddlers (Table 19).

Table 17. Total mercury and methylmercury exposures from fish foods for frequent seafood consumers in France (the CALIPSO study)

Location	Age/sex group	Total mercury exposure (µg/kg bw per week)			Methylmercury exposure (µg/kg bw per week)		
		Mean	SD	95th	Mean	SD	95th
LeHavre[a]	M 18–64 years	0.87	0.55	1.94	0.88	0.57	1.93
	F 18–64 years	1.17	1.15	2.69	1.17	1.17	2.69
	M & F 65+ years	1.25	1.22	3.45	1.26	1.31	3.45
	F 18–44 years	1.04	0.96	2.28	1.07	1.02	2.27
	All	1.12	1.08		1.13	1.11	
Lorient[a]	M 18–64 years	1.4	0.21	3.11	1.44	0.34	3.1
	F 18–64 years	1.63	1.13	3.75	1.67	1.15	3.67
	M & F 65+ years	1.74	0.89	3.32	1.75	0.89	3.3
	F 18–44 years	1.5	1.15	2.79	1.54	1.16	2.8
	All	1.6	1.04		1.63	1.05	
La Rochelle[a]	M 18–64 years	1.39	1.29	3.01	1.42	1.27	3.08
	F 18–64 years	1.59	1.15	3.52	1.65	1.19	3.62
	M & F 65+ years	1.75	1.06	3.58	1.79	1.09	3.81
	F 18–44 years	1.39	0.92	3.03	1.43	0.96	3.09
	All	1.55	1.19		1.59	1.21	
Toulon[a]	M 18–64 years	1.54	1.31	4.73	1.5	1.29	4.09
	F 18–64 years	1.71	1.44	4.11	1.69	1.42	4.43
	M & F 65+ years	1.54	1.13	3.05	1.5	0.8	2.87
	F 18–44 years	1.61	1.27	3.87	1.6	1.29	4.26
	All	1.66	1.38		1.63	1.35	
All[a]	All	1.48	1.2		1.49	1.2	
All[b,c]	M 18–64 years				1.33	1.19	2.83
	F 18–64 years				1.33	1.19	3.86
	M & F 65+ years				1.58	0.98	3.48
	F 18–44 years				1.33	0.92	2.86
	All				1.51	1.17	3.52

F, female; M, male; SD, standard deviation

[a] Leblanc (2006).

[b] Sirot et al. (2008).

[c] Data from a subset of the CALIPSO survey participants.

Table 18. Total mercury exposure from the 2000–2008 Japan TDS[a]

Year	Total diet total mercury exposure (µg/kg bw per day)	Total mercury exposure from fish (µg/kg bw per day)	Total mercury exposure from foods other than fish and shellfish (µg/kg bw per day)
2000	0.14	0.12	0.02
2001	0.14	0.12	0.02
2002	0.18	0.15	0.02
2003	0.16	0.14	0.03
2004	0.17	0.15	0.02
2005	0.19	0.17	0.02
2006	0.15	0.14	0.01
2007	0.15	0.13	0.01
2008	0.17	0.16	0.01

Table 19. Total diet total mercury exposure from the 2003–2004 New Zealand TDS

Population	Mean mercury exposure (µg/kg bw per day)[a]	% from fish and shellfish
Adult males 25–99 years	0.1	NA
Adult males 19–24 years	0.11	74[b]
Males 11–14 years	0.088	NA
Adult females 25–99 years	0.09	NA
Females 11–14 years	0.066	NA
Children 5–6 years	0.1	NA
Children 1–3 years	0.15	65[b]
Infants 6–12 months	0.16	NA

NA, not available

[a] From 2003–2004 New Zealand TDS studies submitted to the GEMS/Food database.

[b] As reported in the 2003–2004 New Zealand TDS.

(i) Republic of Korea

The average total diet total mercury exposure estimate for the population of the Republic of Korea was 0.04 µg/kg bw per day, ranging from 0.03 µg/kg bw per day for adults 65+ years of age to 0.06 µg/kg bw per day for children 3–6 years of age. Total mercury exposure from foods other than fish and shellfish was estimated to be 0.01 µg/kg bw per day (Table 20). The food group contributing most to total

Table 20. Total diet total mercury exposures from the 2005 Republic of Korea TDS

Subpopulation	Total mercury exposure	
	µg/day	µg/kg bw per day
3–6 years	1.2	0.06
7–12 years	1.8	0.05
13–19 years	2.2	0.04
20–29 years	2.7	0.04
30–49 years	2.9	0.05
50–64 years	2.5	0.04
65+ years	1.5	0.03
Males	2.7	0.04
Females	2.1	0.04
All: Total diet	2.4	0.04
All age groups: Foods other than fish and shellfish	0.6	0.01
All age groups: Fish and shellfish	1.8	0.03

Source: Kwon et al. (2009)

mercury exposure from foods other than fish and shellfish is the "vegetables" food group, with a contribution of 36%. However, Kwon et al. (2009) do not clarify how samples with non-detectable total mercury concentrations were treated, so it is not possible to tell if this estimate reflects the true contribution of the "vegetable" food group to total mercury exposure or is an artefact of the high consumption of vegetables in the Republic of Korea and the use of LOD values for samples with non-detectable total mercury concentrations.

(j) Spain

Total diet total mercury exposures in a TDS study conducted in Catalonia, Spain, ranged from 0.26 µg/kg bw per day for adult females and seniors to 0.83 µg/kg bw per day for children. Total mercury exposures from foods other than fish and shellfish ranged from 0.14 to 0.58 µg/kg bw per day (Table 21). The food group contributing most to this exposure is the cereals group. It contributed from 46% (adult females) to 53% (adolescents) of this exposure.

(k) United Kingdom

Average total diet total mercury exposures in the 2006 United Kingdom TDS ranged from 0.02 to 0.04 µg/kg bw per day (free-living elderly) to 0.04–0.12 µg/kg bw per day (children 1.5–4.5 years) (Table 22). Foods other than fish and shellfish contributed 25–92% to the average total mercury exposure (Table 23). The food

Table 21. Total mercury exposure in Catalonia, Spain[a]

Subpopulation	Total diet total mercury exposure (µg/kg bw per day)	Total mercury exposure from fish and shellfish (µg/kg bw per day)	Total mercury exposure from foods other than fish and shellfish (µg/kg bw per day)
Children	0.83	0.25	0.58
Adolescents	0.37	0.12	0.25
Adult males	0.30	0.13	0.18
Adult females	0.26	0.12	0.14
Seniors	0.26	0.12	0.14

[a] Estimates were derived from the µg/day exposure estimates in Llobet et al. (2003) by assuming the following body weight values for children, adolescents, adult males, adult females and seniors: 20 kg, 50 kg, 70 kg, 65 kg and 65 kg, respectively.
Source: Adapted from Llobet et al. (2003)

Table 22. Total diet total mercury exposure in the 2006 United Kingdom TDS

Population	Total mercury exposure (µg/kg bw per day)	
	Mean	High level
Adults	0.01–0.05	0.10–0.13
Toddlers (1.5–4.5 years)	0.04–0.12	0.17–0.26
Young people (4–18 years)	0.03–0.08	0.11–0.18
Elderly (free living)	0.02–0.05	0.09–0.12
Elderly (institutionalized)	0.02–0.04	0.07–0.12
Vegetarians	0.02–0.05	0.12–0.15

Source: UKFSA (2009)

groups contributing most to this exposure are the "beverage" group (when non-detectable concentrations are set at the LOD) and the "other vegetable" group (when non-detectable concentrations are set at zero).

(l) United States of America

Total average total mercury exposures in the USA, based on total mercury concentrations collected between 1991 and 2005, ranged from 0.008 µg/kg bw per day (males 14–16 years) to 0.021 µg/kg bw per day (females 60–65 years) (Table 24). Fish and shellfish contributed more than 96% to total diet total mercury exposures for all subpopulations considered.

Table 23. Contributions to total diet total mercury exposure in the 2006 United Kingdom TDS[a]

Food source	Total mercury exposures (µg/kg bw per day)	
	Assuming LOD for ND concentrations	Assuming 0 for ND concentrations
Fish and shellfish	0.04	0.01
Foods other than fish and shellfish	0.01	<0.01
Total diet	0.05	0.01

ND, non-detected

[a] Estimates derived by combining TDS consumption estimates with concentrations reported in the 2006 United Kingdom TDS (UKFSA, 2009).

Table 24. Total mercury exposure in the 1991–2005 USA TDS[a]

Subpopulation	Total diet total mercury exposure (µg/kg bw per day)
M & F 6–11 months	0.005
M & F 2 years	0.019
M & F 6 years	0.013
M & F 10 years	0.019
F 14–16 years	0.013
M 14–16 years	0.008
F 25–30 years	0.013
M 25–30 years	0.010
F 40–45 years	0.012
M 40–45 years	0.011
F 60–65 years	0.021
M 60–65 years	0.016
F 70 years	0.014
M 70 years	0.017
Total USA	0.016

[a] Estimates derived by combining TDS Version 3 consumption estimates (http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/TotalDietStudy/ucm184232.htm) with concentrations reported in the 1991–2005 TDS (USFDA, 2007).

6.2.2 Methylmercury

Estimates of methylmercury exposures for foods other than fish and shellfish were available from only one study. All other available methylmercury exposure estimates were for fish and shellfish.

(a) Australia

Only fish and shellfish were analysed for methylmercury levels. Table 25 summarizes estimates of total mercury and methylmercury exposures from fish and shellfish. Estimated methylmercury exposures from fish and shellfish are much lower than the estimated total mercury exposures from these foods. It is not clear if there is an error in the concentrations reported for methylmercury in fish and shellfish.

Table 25. Total mercury and methylmercury exposures from fish and shellfish consumption in the 2000–2001 Australian TDS

Subpopulation	Total mercury exposures (ng/kg bw per day)[a]	Methylmercury exposures (ng/kg bw per day)[a]
Adult males (25–34 years)	12	0–0.122
Adult females (25–34 years)	13	0–0.106
Boys (12 years)	11	0–0.112
Girls (12 years)	11	0–0.077
Toddlers (2 years)	15	0–0.143
Infants (9 months)	21	0–0.163

[a] Derived by combining food consumption data and median mercury levels reported in the Food Standards Australia New Zealand 20th Australian TDS report, available at: http://www.foodstandards.gov.au/scienceandeducation/publications/20thaustraliantotaldietsurveyjanuary2003/

(b) China

Estimates of total mercury and methylmercury exposures were available for a subpopulation of fishermen in Zhoushan Island in China and their families (Table 26). Methylmercury constituted most of the mercury exposures for this subpopulation.

(c) France

Estimates of total mercury and methylmercury exposures from fish and shellfish for frequent seafood consumers in France (the CALIPSO study) are summarized in Table 17 above. However, only fish and shellfish were analysed in this study; hence, it is not possible to estimate the fraction of total mercury exposures from total diet that is attributable to methylmercury. However, the results confirm that almost all of the mercury exposure from fish is in the methylmercury form.

Table 26. Total mercury and methylmercury exposures for fishermen and their families in Zhoushan Island, China

Food	Exposure (μg/kg bw per day)		
	Adult males	Adult females	Children
Total mercury[a]			
Total diet	0.92	0.47	0.67
Fish and shellfish	0.87	0.41	0.57
Other foods	0.05	0.06	0.10
Methylmercury			
Total diet	0.88	0.44	0.63
Fish and shellfish	0.84	0.39	0.55
Other foods	0.04	0.05	0.08

[a] Data taken from Table 14.

(d) Spain

Exposures to methylmercury for the Spanish population were estimated by Sahuquillo et al. (2007) to be 46.2 μg/week (6.6 μg/day). However, only fish and shellfish were analysed in this study, and no estimates of exposure to total mercury were provided. Hence, it is not possible to estimate the fraction of total mercury exposure that is attributable to methylmercury or the fraction of methylmercury exposure that is attributable to fish and shellfish.

(e) United States of America

Table 27 summarizes modelled methylmercury exposure estimates for the population of the USA. However, the estimates are for methylmercury exposures from fish only, and no estimates of total mercury exposures were provided. Hence, it is not possible to estimate the fraction of total mercury exposures that is attributable to methylmercury or the fraction of methylmercury exposure that is attributable to fish and shellfish.

6.2.3 Inorganic mercury

IPCS (2003) estimated dietary exposure to inorganic mercury to be approximately 4.3 μg/day—that is, 0.067 μg/kg bw per day for a 64 kg adult.

It is possible to estimate the dietary exposure to inorganic mercury from studies that have provided both total mercury and methylmercury exposure estimates as the difference between total mercury and methylmercury exposures. For countries that have provided only total mercury exposures but have provided separate exposure estimates for fish and shellfish and foods other than fish and

Table 27. Estimated exposure to methylmercury from fish in the USA

Population percentile	Exposure to methylmercury from fish (µg/day)[a]	
	Women of childbearing age	Men aged 16–45 years
Average	1.4 (1.3–1.4)	1.8 (1.7–1.9)
10th percentile	0.0 (0.0–0.1)	0.0 (0.0–0.1)
25th percentile	0.2 (0.1–0.3)	0.3 (0.2–0.4)
Median (50th percentile)	0.7 (0.6–0.7)	0.9 (0.7–1.0)
75th percentile	1.6 (1.5–1.8)	2.1 (1.9–2.3)
90th percentile	3.4 (3.1–3.6)	4.3 (3.9–4.7)
95th percentile	4.9 (4.5–5.5)	6.4 (5.6–7.5)
99th percentile	10.3 (8.1–12.8)	13.4 (10.9–17.3)

[a] Numbers in parentheses are the 5th to 95th uncertainty percentiles.
Source: USFDA (2009)

shellfish, it may be possible to estimate inorganic mercury exposures by applying some default assumptions on the fraction of methylmercury in fish and shellfish and other foods. Based on results summarized above, methylmercury can constitute up to 70–100% of total mercury in fish and up to 50% of total mercury in shellfish. The fraction of total mercury consisting of methylmercury shows a wide variability in bivalve species (20–89%) and canned tuna (30–79%). These percentages can be applied to total mercury exposure estimates associated with these fish and shellfish groups, if available, thus allowing for the estimation of inorganic mercury exposures from fish and shellfish.

6.3 International estimates

Mercury occurrence data were submitted by France and Japan, but were deemed to be not sufficiently representative for use in deriving international estimates of dietary exposure in combination with food consumption data from the GEMS/Food consumption cluster diets. No international estimates of dietary exposure were prepared.

7. DOSE–RESPONSE ANALYSIS AND ESTIMATION OF CARCINOGENIC/ TOXIC RISK

7.1 Identification of key data for risk assessment

In the majority of species tested to date with inorganic mercury compounds, kidney effects (weight changes, histopathology) appear to be consistently observed at relatively similar doses. In the 6-month NTP (1993) study, groups of rats and mice (both sexes, 10 animals per dose group) were treated by gavage with six different

doses of mercury(II) chloride over a 16-fold range. There was no mortality observed, but kidney mercury concentrations and relative kidney weights did increase in a dose-dependent manner. Although minimal nephropathy was a common finding in all male rats, including controls, there was a dose-dependent increase in the incidence of mild nephropathy (defined as dilated tubules with hyaline casts, foci of tubular degeneration and thickened tubular basement membranes) beginning in the second lowest dose group. Relative kidney weight increases were observed in both sexes of rats, with a NOAEL estimated at 0.23 µg/kg bw per day, as mercury (lowest dose tested). While kidney effects were also observed in mice (more prevalent in males), they generally occurred at higher doses (NOAEL of 1.9 mg/kg bw per day, as mercury).

7.1.1 Pivotal data from biochemical and toxicological studies

Toxicological studies in experimental animals available prior to 2000 indicated that the most sensitive adverse effect of exposure to inorganic mercury (mercury(II) chloride) was autoimmune glomerulonephritis. The lowest NOAEL with respect to this end-point was 200 µg/kg bw per day, as mercury, based on short- or medium-term exposure in rats. In the last several years, since the evaluation by the World Health Organization (IPCS, 2003), there have been limited oral toxicological studies in experimental animals with inorganic mercury. Among them, one reproductive study (Khan et al., 2004) and one short-term study (Penna et al., 2009) indicate that at doses lower than the NOAEL of 200 µg/kg bw per day, adverse effects on reproductive performance and on testis can be induced, with LOAELs of 185 µg/kg bw per day, as mercury, in mice and 1.5 µg/kg bw per day, as mercury, in rats, respectively. Another short-term (14-day) study (Kim, Johnson & Sharma, 2003) showed that for the immune system in mice, the LOAEL was 300 µg/kg bw per day, as mercury, but the NOEL was 60 µg/kg bw per day, as mercury (respectively higher and lower than the 200 µg/kg bw per day NOAEL).

Since both the LOAEL of 1.5 µg/kg bw per day, as mercury, in rats and the NOEL of 60 µg/kg bw per day, as mercury, in mice are lower than the current lowest NOAEL of 200 µg/kg bw per day, as mercury, they may be considered relevant for updating the hazard characterizations of inorganic mercury. For the former LOAEL (1.5 µg/kg bw per day, as mercury), although based on morphological alterations in the testis, such as progressive degeneration with spermatogenic arrest at the spermatocyte stage, hypospermatogenesis in seminiferous epithelium and cytoplasmic vacuolation in Leydig cells, no clear dose-dependent response/effects are provided in the original report (Penna et al., 2009). Additional experiments that assessed reproductive performance with significantly higher doses of inorganic mercury (Atkinson et al., 2001; Rao & Sharma, 2001) do support testicular/fertility effects, but the effects seem to be transient, even with continued dosing. In addition, in the NTP (1993) subacute, subchronic and chronic assays, no testicular histopathological effects were reported in rats or mice at mercury(II) chloride doses up to 5 mg/kg bw per day.

In the case of the NOEL of 60 µg/kg bw per day, as mercury, in mice, the data presented appear to demonstrate a pattern of dose-dependent decreases in CD3+, CD4+ and CD8+ lymphocyte populations following 14 days of exposure in

mice, and the effects at the dose of 60 µg/kg bw per day, as mercury, were not statistically different from the controls (Kim, Johnson & Sharma, 2003). In comparison, developmental exposure to a considerably higher dose of mercury(II) chloride (2 mg/kg bw per day) in the same strain of mice for a longer duration produced no effects on thymus or spleen cellularity (Pilones, Tatum & Gavalchin, 2009). Considering the minimal decrease observed in the indicated lymphocyte populations, the toxicological significance of these findings would require additional investigation.

7.1.2 Pivotal data from human clinical/epidemiological studies

While toxicological effects in humans have been induced following exposure through various routes to inorganic mercury compounds, the available data from epidemiological investigations and studies that include biomarkers of exposure or effect were not considered suitable for assessing overall risk.

7.2 General modelling considerations

For the risk assessment of mercury(II) chloride, critical effects observed in the toxicological database included increased relative kidney weight in male and female rats (NTP, 1993). 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 the 95% lower confidence limit of the benchmark dose (BMDL) for the observed increases in relative kidney weights.

7.2.1 Selection of data

The NTP (1993) bioassay in the rat was considered to be the pivotal study for risk assessment because it employed low-dose exposures to mercury(II) chloride by the oral route. The most prominent dose–response effect of mercury(II) chloride in the 6-month NTP (1993) study was increased relative kidney weight in rats. Other end-points from this study were considered (i.e. terminal body weight, alkaline phosphatase, cholinesterase, nephropathy) for benchmark dose (BMD) modelling (data not shown); however, the BMDLs generated were greater than those estimated for increased relative kidney weight. In support of this data set, shorter-term exposure of weanling rats to higher doses of mercury(II) chloride via the diet produced similar effects.

7.2.2 Measure of exposure

Mercury(II) chloride was administered by gavage, 5 days/week, for 6 months to rats in the NTP (1993) bioassay.

7.2.3 Selection of mathematical model

(a) Modelling procedure for continuous data

BMD modelling was conducted using the USEPA's BMD software (BMDS version 2.1.1) with all available continuous models (i.e. exponential, Hill, linear,

polynomial, power). Benchmark responses (BMRs) of one standard deviation of the control mean or 10% extra risk were modelled for comparison purposes. An adequate fit was judged based on the goodness of fit *P*-value ($P > 0.1$), scaled residual closest to the BMR and visual inspection of the model fit. In addition to the three criteria for judging adequate model fit, whether the variance needed to be modelled and, if so, how it was modelled also determined final use of the model results. If a homogenous variance model was recommended based on statistics provided from the BMD model runs, the final BMD results would be estimated from a homogenous variance model. If the test for homogenous variance was negative ($P < 0.1$), the model was run again while applying the power model integrated into the BMDS to account for non-homogenous variance (known as non-homogenous model). If the non-homogenous variance model did not provide an adequate fit to the variance data, the data set would be considered unsuitable for BMD modelling. Models that passed the goodness of fit test ($P > 0.1$) were considered to be acceptable; from these models, the lowest BMDL was selected.

7.3 Potency estimates

7.3.1 BMD analyses for kidney weight

All available continuous models in the BMDS (version 2.1.1) were fit to the relative kidney weight data for male and female F344 rats exposed to mercury(II) chloride by gavage for 6 months in the NTP (1993) bioassay (Table 28). For comparison purposes, BMRs of one standard deviation and 10% of the control mean were used for the BMD modelling. As assessed by the chi-squared goodness-of-fit statistic, the Hill and some exponential models in the BMDS provided adequate fit to the data using a homogenous variance model (Table 29).

Table 28. Relative kidney weights for male and female rats gavaged with mercury(II) chloride for 6 months

Dose (mg/kg bw per day)	*n*	Relative (to body weight) kidney weights ± SE (g)	
		Males	Females
0	10	3.67 ± 0.07	3.80 ± 0.07
0.312	10	4.05 ± 0.06	4.09 ± 0.10
0.625	10	4.34 ± 0.06*	4.29 ± 0.05*
1.25	10	4.34 ± 0.12*	4.46 ± 0.09*
2.5	10	4.38 ± 0.08*	4.57 ± 0.11*
5.0	10	4.17 ± 0.09*	4.62 ± 0.11*

SE, standard error
* $P < 0.01$
Source: NTP (1993)

Table 29. Dose–response modelling of relative kidney weights in male and female F344 rats gavaged with mercury(II) chloride for 6 months[a]

Model	*P*-value	AIC	BMD_{1SD}	$BMDL_{1SD}$	BMD_{10}	$BMDL_{10}$
Males						
Exponential 4[b]	0.1189	93.481	0.136	0.072	0.220	0.112
Exponential 4[c]	0.123	265.555	0.119	0.063	0.221	0.115
Exponential 5[b]	0.127	93.2075	0.275	0.094	0.308	0.148
Exponential 5[c]	0.131	265.271	0.267	0.082	0.307	0.152
Hill[b,d]	0.248	95.2074	0.299	0.131	0.311	0.184
Females						
Exponential 4[b]	0.9926	87.6721	0.291	0.169	0.449	0.250
Exponential 4[c]	0.9898	264.2337	0.253	0.149	0.444	0.258
Exponential 5[b]	0.9926	87.6721	0.291	0.169	0.449	0.250
Hill[b]	0.9993	85.7644	0.291	0.125	0.430	0.193

AIC, Akaike's information criterion; BMD_{1SD}, benchmark dose for a one standard deviation response; BMD_{10}, benchmark dose for a 10% response; $BMDL_{1SD}$, lower limit on the benchmark dose for a one standard deviation response; $BMDL_{10}$, lower limit on the benchmark dose for a 10% response; SD, standard deviation

[a] BMD(L)s have not been corrected for dosing schedule.

[b] Assumes normal distribution.

[c] Assumes lognormal distribution.

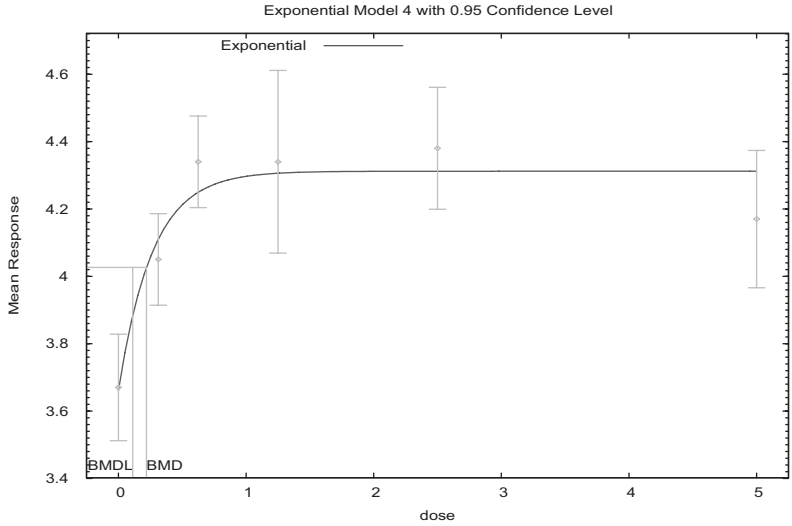
[d] Power parameter was unrestricted.

For both males and females, the BMDLs estimated by the acceptable models were similar; therefore, the more conservative lowest BMD may be selected. As kidney weight changes in male rats appear to be more sensitive than those in female rats, the lowest estimated BMD_{10} and $BMDL_{10}$ for reduced relative kidney weight are 0.220 and 0.112 mg/kg bw per day, respectively. Figure 1 shows the relative kidney weights of male rats fitted to the four-parameter exponential model.

7.3.2 Conclusions from dose–response analysis

In the dose–response analysis, 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_{10} and $BMDL_{10}$ values. This procedure results in a range of BMD_{10} and $BMDL_{10}$ values for each end-point considered (Table 30). The results summarized in Table 30 show that the $BMDL_{10}$s are moderately lower than the BMD_{10}s, indicating that the confidence intervals are fairly narrow.

Figure 1. Exponential four-parameter model of relative kidney weight data in male F344 rats from 6-month NTP (1993) study



Note: BMD(L)s have not been corrected for dosing schedule.

Table 30. Summary of the results of dose–response modelling of relative kidney weights in male rats administered mercury(II) chloride by gavage

End-point	Response	Range of BMD_{10}[a] (mg/kg bw per day)	Range of $BMDL_{10}$[a] (mg/kg bw per day)
Male relative kidney weight	10% extra risk	0.220–0.311	0.112–0.184

[a] Doses have not been adjusted for contribution of Hg^{2+} to mercury(II) chloride or for study dosing schedule.

The range of $BMDL_{10}$s calculated based on the reduction in relative kidney weight in male rats is 59.1–97.1 µg/kg bw per day (adjusted to account for 5 days/week dosing rather than 7 days/week dosing and the fact that Hg^{2+} represents approximately 73.9% of the administered dose of mercury(II) chloride). The more conservative lower end of this range of values is recommended for use in the evaluation.

8. COMMENTS

8.1 Absorption, distribution, metabolism and excretion

Following oral exposure, inorganic mercury salts show limited absorption, which is related to their water solubility. In human volunteers, the average

absorption of a tracer dose of inorganic mercury given as mercury(II) nitrate was 5–10%, whether delivered in a protein-bound matrix or as a solution.

Inorganic mercury compounds are not lipid soluble and do not readily cross the blood–brain barrier or placenta membranes. Ionic species of inorganic mercury readily bind to sulfhydryl groups of various thiol-containing compounds, such as GSH, cysteine and metallothionein. Kidneys exhibit the greatest concentration of mercury following exposure to inorganic mercury compounds. The main pathways of excretion of absorbed inorganic mercury are via the urine and, to a lesser extent, in the faeces. Owing to the poor absorption of orally administered inorganic mercury, the majority of the ingested dose in humans is excreted in the faeces. Inorganic mercury can also be excreted via the breast milk. The half-life for inorganic forms of mercury in humans has been estimated at 1–2 months.

8.2 Toxicological data

Haematological, hepatic and renal effects have been reported in rats or mice administered sublethal single oral doses of mercury(II) chloride. Renal effects usually observed with mercury(II) chloride at doses above 5 mg/kg bw per day include interstitial sclerosis, renal tubular damage and proximal tubular necrosis. Severe gastrointestinal damage, including inflammation and necrosis of the fore-stomach and necrosis of the glandular stomach, can also be induced with high doses of inorganic mercury, in particular for mercury(II) compounds, which are relatively more corrosive than mercury(I) compounds.

Longer-term exposure (subchronic to chronic) to inorganic mercury at doses above 1–5 mg/kg bw per day can induce a variety of effects related to general toxicity (decrease in body weight gain, changes in clinical and haematological parameters), as well as organ-specific effects (increased kidney and adrenal weights, testicular atrophy). Effects associated with relative kidney weight changes include marked thickening of glomerular and tubular basement membranes, degeneration and atrophy of the tubular epithelium and increased severity of nephropathy. Treatment of mice and rats by gavage with mercury(II) chloride at doses ranging from 1.25 to 20 mg/kg bw per day and from 0.312 to 5.0 mg/kg bw per day, respectively, for 6 months produced a variety of renal effects, which occurred with greater frequency and severity in male animals. Unlike organic mercury compounds, neurotoxicity is not usually observed, even at exposure levels that produce frank toxicological effects in other organs.

Reproductive effects induced by inorganic mercury include decreased fertility, reduced implantation efficiency and decreases in both live births and litter sizes. The observed effects seem to involve male-specific end-points (testicular atrophy, androgen decreases, spermatogenesis disruption) more than effects in females. However, inconsistencies have been noted in some experimental responses. A consistent observation in most reproduction studies includes increased relative kidney weights in the offspring.

Inorganic mercury compounds have produced some genotoxic effects in vitro and in vivo, with stronger evidence from in vitro experiments, including single-strand DNA breaks, sister chromatid exchanges and chromosomal aberrations.

However, the mechanisms appear to involve primarily induction of oxidative stress (reactive oxygen species) or disruption of microtubules rather than direct interaction with DNA, including adduct formation, which has not been demonstrated.

Chronic exposure of mice and rats to mercury(II) chloride at doses ranging from 2.5 to 10 mg/kg bw per day has produced some indications of carcinogenicity. The main findings included an increased incidence of forestomach hyperplasia, forestomach squamous cell papillomas and a marginal increase in thyroid follicular cell carcinomas in male rats. In mice, renal tubule tumours were seen only in high-dose males, but the incidence was not statistically significant compared with historical controls. It was concluded by NTP (1993) that there was some evidence of carcinogenic activity of mercury(II) chloride in male F344 rats, based on the increased incidences of squamous cell papillomas of the forestomach and the marginally increased incidence of thyroid follicular cell neoplasias, equivocal evidence in both female rats and male mice, and no evidence in female rats. However, NTP (1993) considered that the forestomach lesions in male rats may have limited relevance, as they did not progress to malignancy (direct tissue irritation effect). Also, as follicular cell carcinomas in rats usually result from increased incidences of hyperplasia and adenomas, it was further noted that the combined incidence of thyroid follicular cell neoplasms (adenomas and carcinomas) was not significantly increased. IARC (1993) considered that there is limited evidence in experimental animals for the carcinogenicity of mercury(II) chloride, based on results from the NTP (1993) bioassay.

8.3 Observations in humans

Human data on the adverse health effects of exposure to inorganic mercury, including renal effects, consist of case reports or case series that do not allow the identification of dose–response relationships. Therefore, they do not provide an adequate basis for deriving a health-based guidance value. They do, however, provide evidence that supports the use of adverse renal effects observed in experimental species as the basis for such a derivation. Nephrotic syndrome, including proliferative or membranous glomerulonephritis, has been associated with the topical use of mercury(II) ammonium chloride creams. Based on the limited number of studies of cancer and the absence of consistent findings, IARC (1993) concluded that there is inadequate evidence in humans for the carcinogenicity of mercury and mercury compounds. As a result, inorganic mercury compounds were not classifiable as to their carcinogenicity in humans (Group 3).

8.4 Analytical methods

Sample handling is generally critical only for water samples. The best materials for water sample storage and processing are polytetrafluoroethylene and fluorinated ethylene-propylene. Fresh samples are usually stored deep-frozen, lyophilized in darkness or sometimes sterilized. It has been reported that methylmercury may be decomposed in some food matrices with repeated freezing and unfreezing (particularly in bivalves). However, relatively little is known about the effect of storage on the stability of methylmercury in food samples.

Following acidic digestion of samples, CV-AAS or CV-AFS has been widely used for the determination of total mercury in several food matrices. An LOQ of about 30 µg/kg dry mass in foods may be obtained by CV-AAS. Further sensitivity enhancement may be obtained by CV-AFS. The main advantage of the cold vapour technique is the separation of the analyte from the potentially interfering sample matrix and its comparatively low cost. However, to avoid interference by CV-AFS, special precautions must be taken to completely remove vapours when nitric acid is used for digestion. With an LOQ of about 10 µg/kg dry mass and greater selectivity, ICP-MS is increasingly being used with an addition of gold chloride to mercury standard solutions to avoid the mercury memory effects. Although the instrumentation is expensive to purchase and to operate, the ability of ICP-MS to provide low LOQs, to provide a wide dynamic linear range and to measure many elements simultaneously can offset these cost factors.

Basically, all the speciation methodology is generally targeted on the separation and determination of methylmercury, and there has been no conclusive identification of other species of mercury.

Extraction of the mercury species from its matrix requires an aggressive treatment, such as acid digestion, distillation or alkaline extraction, with the option of applying ultrasonic or microwave energy to assist in the procedure. Extraction is one of the most critical steps, because two conflicting issues need to be addressed: obtaining high extraction efficiency and preventing losses. In alkaline media, methylmercury appears to be more stable than in acid media, the proteins being easily hydrolysed.

GC has been the most widely used technique for the separation of mercury species, whereas HPLC is increasingly being applied. The detection methods (LOD in parentheses) of CV-AAS (10 µg/kg), CV-AFS (1 µg/kg), MIP-AES or ICP-AES (5 µg/kg), MS (40 µg/kg) and ICP-MS (<3 µg/kg) all have sufficient sensitivity for food samples. The advantage of MS and ICP-MS is their multielement and multi-isotope capabilities that allow for more accurate and precise results by SID-MS, which can also check for species transformations and extraction recoveries. Once in solution, methylmercury may decompose when exposed to light, low pH and high storage temperatures. Other factors, such as the type of storage container, may also affect the stability.

Available certified reference materials and proficiency testing schemes or intercomparison exercises exist for both total mercury and methylmercury to demonstrate and maintain analytical quality assurance. However, there is a current need for fully validated, standardized methods for determination of methylmercury and inorganic mercury.

8.5 Sampling protocols

Some authorities have regulations with regards to specific sampling protocols for mercury and other contaminants. For example, the European Commission has regulated the number and size of incremental samples, size of the aggregate sample and precautions to be taken for control purposes.

8.6 Levels and patterns of contamination in food commodities

At its present meeting, the Committee reviewed data from eight countries on the occurrence of mercury in different food commodities analysed between 1997 and 2009. The total number of analytical results for total mercury was more than 106 740, with 93% coming from Europe (Finland, France, Spain), 5% from Asia (China, Japan), 1% from the Americas (Brazil, Canada) and 1% from Oceania (Australia), for water (85%), fish (6%), shellfish (2%) and other food groups (6%). The 2128 samples analysed for methylmercury were from fish (94%), shellfish (2%) and other products (4%). However, the Committee did not receive any occurrence data on inorganic mercury in foods or water.

Total mercury levels in 98% of 90 545 water samples analysed in France were below the LOQ of 0.02 µg/l, with a maximum of 4.3 µg/l.

Total mercury levels in foods other than fish products were generally low (range 0.0001–0.050 mg/kg), with about 80% of the 6183 samples containing levels below the LOQs. The highest levels were found in fungi. Mean methylmercury levels reported by China in non-fish samples ranged from 0.001 to 0.023 mg/kg, with a maximum concentration found in poultry. No other information on methylmercury in non-fish samples was received from other countries.

Total mercury levels in 1892 shellfish samples (80% above LOQ) ranged from 0.002 to 0.86 mg/kg. No shellfish species contained methylmercury at concentrations greater than 0.5 mg/kg (range 0.002–0.451 mg/kg), with the maximum concentration found in edible crab.

Total mercury levels in 6114 fish samples ranged from 0.001 to 11.4 mg/kg, with the maximum concentration found in marlin.

The proportion of total mercury contributed by methylmercury generally ranged between 30% and 100%, depending on species of fish, size, age and diet. Furthermore, in about 80% of these data, methylmercury accounted for more than 80% of total mercury. However, a few submitted data showed proportions of methylmercury of about 10% or less.

8.7 Food consumption and dietary exposure assessment

8.7.1 National estimates

Most of the available dietary exposure assessments for mercury were from national TDSs. These include the following TDSs: Australia (2000–2001), Canada (1998–2000), China (2007), Czech Republic (2000), France (2001–2002), Japan (2008), New Zealand (2003–2004), the Republic of Korea (2005), the United Kingdom (2006) and the USA (1991–2005). Published data from other studies focusing on special subpopulations were also available. These include TDSs conducted in Chile (Santiago) and Spain (Catalonia) and studies of fishermen and their household members in Zhoushan Island (China), residents of Changchun city in north-east China, secondary-school students in Hong Kong SAR, frequent seafood consumers in France (the CALIPSO study), exposures from fish and shellfish in Spain and modelled exposure estimates for fish consumers in the USA.

In general, most studies available allowed for the estimation of dietary exposure to total mercury from fish and shellfish as well as from other foods. Table 31 summarizes the estimates of mean dietary exposure to total mercury from the total diet, from fish and shellfish, and from other foods extracted from the studies listed above. Estimated mean dietary exposure to total mercury ranged from 0.07 to 5.81 µg/kg bw per week, while the estimated mean dietary exposure to total mercury from fish and shellfish ranged from 0.07 to 1.75 µg/kg bw per week. The estimated mean dietary exposure to total mercury from foods other than fish and shellfish ranged from 0 to 4.06 µg/kg bw per week. The upper limit of that range corresponds to a subpopulation of children. When only total population or subpopulations of adults were considered, the estimated mean dietary exposure to total mercury from foods other than fish and shellfish ranged from <0.01 to 1.01 µg/kg bw per week. The main contributors to this average dietary exposure were breads and cereals.

The studies did not provide 90th-percentile estimates of the dietary exposure to total mercury from foods other than fish and shellfish; hence, the 90th-percentile exposure estimates were derived by multiplying the mean exposure estimates by 2 (WHO, 1985). The resulting 90th-percentile exposure to total mercury from foods other than fish and shellfish was estimated to range from <0.02 to 2.03 µg/kg bw per week for the general population or adult subpopulations and from <0.02 to 8.12 µg/kg bw per week when children subpopulations are included.

The contribution of fish and shellfish to the total dietary exposure ranged from 40% to 100% when samples with non-detectable concentrations were assigned a zero concentration. Estimates of per cent contribution for foods other than fish and shellfish based on dietary exposure estimates derived from concentration data using the LOR or LOR/2 for non-detects are not reliable because they artificially inflate the contribution of these foods, particularly when the LOR is high. Only studies from which it was possible to separately estimate the contribution of fish and shellfish and other foods to total dietary exposure to mercury are presented in Table 31.

It was assumed that the predominant source of inorganic mercury in the diet is foods other than fish and shellfish.

8.7.2 International estimates

The available mercury occurrence data were deemed to be not sufficiently representative for use in deriving international estimates of dietary exposures in combination with food consumption from the GEMS/Food consumption cluster diets. No international estimates of dietary exposure were prepared.

8.8 Dose–response analysis and estimation of carcinogenic/toxic risk

Kidney effects are consistently observed in various experimental species (weight changes, proximal tubule damage and progressive nephropathy). Relative kidney weight increases observed in rats following exposure to mercury(II) chloride are also associated with a dose-dependent increase in renal mercury accumulation and with significant changes in the renal cortex, including increases in both proximal

Table 31. Contribution of fish and shellfish to total dietary exposure to mercury (national estimates)

Country	Average total dietary exposure to mercury (µg/kg bw per day)			% from fish and shellfish
	Total diet	Fish and shellfish	Other foods	
Estimates derived by assigning a zero value to samples with concentrations below the LOD or LOQ				
Australia TDS	0.01–0.02	0.01–0.02	0–0	100–100
Canada TDS (excluding infants)	0.01–0.03	0.01–0.02	<0.01–0.02	51–80
Chile (Santiago)	0.06	0.02	0.03	41
China (Zhoushan Island)	0.47–0.92	0.41–0.87	0.05–0.10	87–95
Japan TDS	0.17	0.16	0.01	92
Republic of Korea TDS	0.04	0.03	0.01	76
United Kingdom TDS[a]	0.02–0.04	—	—	—
USA TDS	0.01–0.02	0.01–0.02	<0.01–<0.01	96–100
Estimates derived by assigning a non-zero value (LOD or LOQ) to samples with concentrations below the LOD or LOQ				
Australia TDS	0.08–0.26	0.01–0.02	0.06–0.24	7–17
Canada TDS (excluding infants)	0.01–0.04	<0.01–0.04	0.01–0.03	40–74
Chile (Santiago)	0.08	0.02	0.06	31
United Kingdom TDS[b]	0.04–0.12	—	—	25
Estimates derived by assigning a non-zero value (LOD/2 or LOQ/2) to samples with concentrations below the LOD or LOQ				
China TDS	0.08	0.01	0.07	13
China (Changchun city)	0.10	0.01	0.09	13
France TDS[c]	0.16–0.26	0.02–0.02	0.15–0.24	9–10
New Zealand TDS	0.11–0.16	0.08–0.10	0.03–0.06	65–74
Spain (Catalonia)	0.28 –0.83	0.12–0.25	0.14–0.58	30–46
Total range (µg/kg bw per day)[d]	**0.01–0.83**	**0.01–0.25**	**0–0.58**	
Total range (µg/kg bw per week)[d]	**0.07–5.81**	**0.07–1.75**	**0–4.06**	

[a] High exposures (97.5th percentile) ranged from 0.07 to 0.17 µg/kg bw per day.
[b] High exposures (97.5th percentile) ranged from 0.12 to 0.26 µg/kg bw per day.
[c] High exposures (95th percentile) ranged from 0.25 to 0.41 µg/kg bw per day.
[d] Excluding the study of fishermen and their families in Zhoushan Island, China.

tubule and glomerular volumes. The Committee therefore considered it appropriate to model kidney weight changes, which generally occurred at doses similar to or lower than other renal effects. Data on relative kidney weight increases were taken from the NTP (1993) study, in which rats and mice of both sexes were exposed by gavage to mercury(II) chloride, 5 days/week for 6 months. Other end-points from this study were considered (i.e. terminal body weight, serum alkaline phosphatase and cholinesterase, incidence of mild nephropathy) for BMD modelling (data not shown); however, the BMDLs generated were greater than those estimated for increased relative kidney weight. Models that passed the goodness-of-fit test ($P > 0.10$) were considered to be acceptable, and the lowest BMDL was selected from these models (Table 32). The 6-month exposure was deemed sufficient to establish a health-based guidance value, because the half-life of mercury(II) chloride in rats is estimated at less than 30 days, steady-state renal mercury concentrations were reached by 4–6 months and exposures in the same dose range for longer durations produced early mortality. The Committee further considered that a 10% change for increased relative kidney weight was appropriate as a BMR to establish a health-based guidance value. This decision was based on the following: the kidney weight data were modelled based on reported mean values, animals in the lowest experimental dose (0.325 mg/kg bw per day) already exhibited a 10% increase in mean relative kidney weight and the severity of nephropathy was significantly increased only at doses greater than or equal to 1.25 mg/kg bw per day.

Table 32. Dose–response modelling[a] for a 10% increase in relative kidney weight for male and female F344 rats gavaged with mercury(II) chloride for 6 months[b]

Sex	BMD_{10} (mg/kg bw per day as mercury(II) chloride)	$BMDL_{10}$ (mg/kg bw per day as mercury (II) chloride)
Males	0.22–0.31	0.11–0.18
Females	0.430–0.45	0.19–0.25

[a] BMDS version 2.1.1.

[b] BMD(L)s have not been adjusted for the dosing schedule of 5 days/week.

9. EVALUATION

The Committee noted that there was a lack of quantitative data on methylmercury in non-fish products and on inorganic mercury in general.

The Committee assumed that the predominant form of mercury in foods other than fish and shellfish is inorganic mercury. While data on speciation of inorganic mercury in foods are limited, the Committee agreed that the toxicological database for mercury(II) chloride was relevant for assessing the health risk of foodborne inorganic mercury. The NTP (1993) bioassay provided limited evidence for carcinogenicity; however, direct reaction of mercury(II) chloride with DNA has

not been demonstrated. Therefore, setting a health-based guidance value was considered appropriate.

The lowest $BMDL_{10}$ for relative kidney weight increase in male rats was calculated to be 0.11 mg/kg bw per day as mercury(II) chloride. This corresponds to 0.06 mg/kg bw per day as mercury, adjusted from a 5 days/week dosing schedule to an average daily dose and for the per cent contribution of inorganic mercury to mercury(II) chloride dose. After application of a 100-fold uncertainty factor, the Committee established a PTWI for inorganic mercury of 4 µg/kg bw (rounded to one significant number).

The previous PTWI of 5 µg/kg bw for total mercury, established at the sixteenth meeting, was withdrawn.

In the absence of evidence to the contrary, the new PTWI for inorganic mercury was considered applicable to dietary exposure to total mercury from foods other than fish and shellfish. The upper limits of estimates of average dietary exposure to total mercury from foods other than fish and shellfish for adults (1 µg/kg bw per week) and for children (4 µg/kg bw per week) were at or below the PTWI.

9.1 Recommendations

There is a need for:

- validated analytical methods for both inorganic mercury and methylmercury applicable in several food matrices;
- more information on the inorganic mercury and methylmercury content of foods as consumed that mainly contribute to overall dietary exposure.

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PERCHLORATE

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1. EXPLANATION

The perchlorate ion (ClO_4^-) is very stable in water, and its salts are highly soluble in water. Perchlorate occurs naturally in the environment, in deposits of nitrate and potash, and can be formed in the atmosphere and precipitate into soil and groundwater (Dasgupta et al., 2005; Rao et al., 2007). It also occurs as an environmental contaminant arising from the use of nitrate fertilizers (Susala et al., 1999) and from the manufacture, use and disposal of ammonium perchlorate (Chemical Abstracts Service [CAS] No. 7790-98-9) used in rocket propellants, explosives, fireworks, flares and air-bag inflators and in other industrial processes. Perchlorate can be formed during the degradation of sodium hypochlorite, used for disinfection of drinking-water and water for food processing, if it is stored for long periods and under less than optimal conditions, including high temperature, storage in the light and storage in containers that contain small amounts of old hypochlorite (Greiner et al., 2008). Water, soil and fertilizers are considered to be potential sources of perchlorate contamination in food. Potassium perchlorate (CAS No. 7778-74-7) has been used as a human therapeutic medicine to treat thyroid disease.

Perchlorate has not been previously evaluated by the Committee. It was referred to the Committee for evaluation on request of the Second Session of the Codex Committee on Contaminants in Food (FAO/WHO, 2008).

The health effects of perchlorate salts are due to the perchlorate ion itself and not the other components.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Rats

Following oral administration of radiolabelled perchlorate to the rat, peak blood concentrations are reached by 3 h. Half-lives in the blood range from less than 8 h to about 20 h. Perchlorate is actively transported into the thyroid gland and concentrated in the lumen of the gland, with a peak uptake at 4–6 h. Its relative distribution between thyroid follicular cell, stroma and lumen is comparable with that obtained for iodine (Wolff, 1998). Nonspecific binding of perchlorate to albumin and prealbumin in plasma is high, but it does not interfere with the binding of thyroid hormones to plasma thyroxine binding globulin (TBG) (Merrill et al., 2003). Nonspecific binding to plasma proteins appears to be saturated at doses of perchlorate between 1.0 and 10.0 mg/kg body weight (bw) per day (Clewell et al., 2003a).

In the rat, clearance of perchlorate from serum is biphasic, with a slow terminal phase. The proportion of a dose of perchlorate that is sequestered in the thyroid over a 4 h time period is inversely proportional to the dose and ranges from 1% for a dose of 10.0 mg/kg bw per day to 11% for a dose of 0.5 mg/kg bw per day (Yu et al., 2002).

Perchlorate crosses the placenta and has been detected in rat maternal milk and in the serum, gastrointestinal contents and skin of neonatal rats (Clewell et al., 2003a,b). Perchlorate is concentrated not only in the thyroid gland, but also in the gastrointestinal contents, skin, mammary gland and milk (Clewell et al., 2004).

(b) Humans

In a study in human volunteers, the serum half-life of perchlorate given at 0.5 mg/kg bw per day was 6.0–9.3 h (average 8.1 h) (Greer et al., 2002). Later, Crump & Gibbs (2005) recalculated the serum half-life in this study as averaging 7.5 h. A study of two workers occupationally exposed to perchlorate indicated elimination half-lives of 7.9 and 8.2 h from measurements of urinary perchlorate (Lamm et al., 1999).

Perchlorate is found in human serum, plasma, urine, saliva and breast milk (Kirk et al., 2005, 2007; Pearce et al., 2007; Dasgupta et al., 2008; Kannan et al.,

2009; Leung et al., 2009; Oldi & Kannan, 2009a,b). Details are given below in section 2.3.1.

2.1.2 Biotransformation

Very little of the perchlorate ion is metabolized in the rat or human, and more than 90% is excreted unchanged in the urine (see reviews by Wolff, 1998; Fisher et al., 2000). In a rat study, more than 99.5% of radiolabelled perchlorate was recovered in the urine over 48 h (Yu et al., 2002).

2.1.3 Effects on enzymes and other biochemical parameters

As the thyroid is the critical target organ for perchlorate toxicity, the effects of perchlorate on hormones released by the hypothalamic–pituitary–thyroid axis, which can be measured in blood, are described in section 2.2, rather than in this section.

2.2 Toxicological studies

2.2.1 Acute toxicity

A dietary concentration of 3.55% (35 500 mg/kg diet, approximately 3621 mg/kg bw per day as perchlorate) was reported as the median lethal dose (LD_{50}) for potassium perchlorate in mice exposed for up to 30 days. The first deaths occurred within 4 days of the start of treatment (Gauss, 1972).

2.2.2 Short-term studies of toxicity

Four groups of rats, each comprising six females weighing 220–240 g at the start of treatment and 280–295 g at the end, were given 1) perchlorate alone for 8 weeks, 2) perchlorate plus 4 µg of thyroxine (T_4) daily by intraperitoneal injection, 3) perchlorate plus 50 µg of estradiol by intramuscular injection for the last 5 days of treatment or 4) perchlorate plus T_4 by intraperitoneal injection and estradiol by intramuscular injection. Perchlorate was administered in the drinking-water at a concentration of 400 mg/100 ml. Assuming that the rats consumed 33–40 ml of drinking-water per day, the daily exposure to perchlorate was equivalent to about 600–750 mg/kg bw per day. Another four control groups, each with six rats per group, were given 1) tap water only, 2) distilled water in combination with T_4, 3) distilled water in combination with estradiol or 4) distilled water in combination with T_4 plus estradiol, administered as above. At the end of the 8-week treatment period, the rats were injected intraperitoneally with radiolabelled iodine 1 h before sacrifice. The mammary glands were examined for iodine uptake and histology. In the rats given perchlorate only, iodine uptake was reduced by 52% compared with controls; the mammary glands were of normal weight but histologically showed mild atrophy, some atypia of the lobular epithelium and scattered foci of hyperplastic activity. Co-administration of T_4 did not mitigate the effects of perchlorate on iodine uptake; if anything, mammary dysplasia was slightly enhanced. Co-administration of estradiol reduced the effect of perchlorate on iodine uptake by the mammary gland and reduced the extent of atypical changes (Eskin et al., 1975).

In a comprehensive 90-day study, Sprague-Dawley rats (20 or 30 of each sex per group) were given ammonium perchlorate in the drinking-water at doses of 0, 0.01, 0.05, 0.2, 1.0 and 10.0 mg/kg bw per day. The animals were approximately 7 weeks of age at the start of treatment. Animals in each group were killed after either 14 or 90 days of treatment; in the 0.05, 1.0 and 10.0 mg/kg bw per day groups, some were killed after a recovery period of 30 days. Analytical chemistry confirmed the absence of nitrate in the drinking-water, the stability of the perchlorate in drinking-water and that the concentrations were within ±10% of the target concentrations. Ten rats of each sex per group were sacrificed at each time point. Body weights, food consumption and water consumption were measured weekly. Estrous cycles were examined in females by daily vaginal smears taken during the 3 weeks before scheduled sacrifice. Sperm samples were taken from males at sacrifice for measurement of sperm count, motility and morphology. Ophthalmology was conducted just prior to sacrifice. Blood samples were taken at sacrifice for haematology and clinical chemistry, thyroid stimulating hormone (TSH), triiodothyronine (T_3) and T_4. All animals were subjected to gross necropsy, and a complete set of tissues and organs was preserved for histological examination. In the control and highest-dose groups, all tissues and organs were examined microscopically, including bone marrow samples for micronucleus formation. Liver, kidneys, lungs, thyroids and gross lesions were examined microscopically in the other four dose groups. There were no effects of treatment on body weight, food consumption, water consumption, survival, clinical observations, haematology, clinical chemistry, ophthalmology, estrous cycles, sperm parameters or bone marrow micronucleus formation. There were no gross or histopathological changes in any tissues or organs, apart from the thyroid. Absolute and relative thyroid weights were significantly increased in the 10.0 mg/kg bw per day group after 14 or 90 days of treatment in males and after 90 days in females, but did not differ from control values in either sex after the 30-day recovery period. Both sexes in the top dose group also showed minimal- to mild-grade increases in thyroid follicular cell hypertrophy, microfollicle formation and colloid depletion at both time points, but the changes reached moderate grade in the males only after 14 days of treatment, and there were no differences from controls after the 30-day recovery period. The authors noted that the histopathological changes observed in the thyroid were similar to those reported by others in rats following the feeding of iodine-deficient diets or inhibition of iodine organification. There were no effects on thyroid weights or histology in any other treatment group. After 14 days of treatment, dose-related increases in TSH concentrations were observed at all dose levels in both males and females; the increases were statistically significant in males from 0.2 mg/kg bw per day and in females from 0.05 mg/kg bw per day. T_4 was significantly reduced in males and females at the top dose of 10.0 mg/kg bw per day, and T_3 was significantly reduced at all doses in males but not at any dose in females. After 90 days of treatment, TSH was significantly increased from 0.2 mg/kg bw per day in males, but only at 10.0 mg/kg bw per day in females. T_3 and T_4 were significantly decreased in a dose-related manner at all doses in both sexes. At the end of the 30-day recovery period, at the doses examined (0, 0.5, 1.0 and 10.0 mg/kg bw per day), TSH and T_3 were unaffected in males, whereas T_4 was significantly reduced at all doses; in the females, TSH was significantly increased at all doses, T_4 was reduced, but only

at 10.0 mg/kg bw per day, and T_3 was unaffected. The authors concluded that 1.0 mg/kg bw per day represented the overall no-observed-adverse-effect level (NOAEL) for the study; they considered that the effects on TSH and thyroid hormones seen at lower doses were not adverse effects, as they were unaccompanied by any changes in thyroid weight or histology, and the changes seen at 10.0 mg/kg bw per day were reversible (Siglin et al., 2000).

It should be noted that this study did not establish a no-observed-effect level (NOEL) for effects of perchlorate on the hypothalamic–pituitary–thyroid axis, as reductions in thyroid hormone concentrations were observed in both sexes at all dose levels after 90 days of treatment. The dose of 0.01 mg/kg bw per day may be a lowest-observed-effect level (LOEL) for thyroid hormone changes. However, no effects on thyroid growth or histopathology were seen at doses less than or equal to 1.0 mg/kg bw per day, whereas the dose of 10.0 mg/kg bw per day increased thyroid weight and caused follicular hypertrophy, microfollicle formation and colloid depletion.

A study in male Fischer 344 rats investigated the effect of ammonium perchlorate administered in the drinking-water on serum TSH, T_4 and T_3. Groups of 10 rats, aged 7 weeks, were exposed in the drinking-water to the nominal concentrations of ammonium perchlorate of 0, 0.1, 1.0 and 10 mg/l for 7 days. From data on body weight and water consumption, these concentrations were calculated to be equivalent to ammonium perchlorate doses of 0, 0.024, 0.205 and 1.170 mg/kg bw per day. There was no effect at any dose on TSH, T_4 or T_3. Histological examination of the thyroid gland showed colloid depletion, hypertrophy and hyperplasia of follicular epithelial cells in the high dose group only (Khan et al., 2005). This study indicates a NOEL of 1.170 mg/kg bw per day for effects on thyroid hormones and a NOEL of 0.205 mg/kg bw per day for effects on thyroid histology following short-term (7 days) dosing of ammonium perchlorate. The observation of effects on thyroid histology, unaccompanied by changes in thyroid hormone levels, is in contrast to the findings in most other studies on perchlorate.

A study in male Sprague-Dawley rats, with the objective of investigating the effects of a combination of perchlorate and a pentachlorobiphenyl (PCB 126) on serum TSH, free T_4, total T_4 and thyroid histology, included groups given perchlorate alone. In a first experiment, groups of 16 rats, weighing an average of 216 g, were given ammonium perchlorate in the drinking-water for 14 days at concentrations of 0, 0.09, 0.9 and 9.0 mg/l, to achieve target perchlorate doses of 0, 0.01, 0.1 and 1.0 mg/kg bw per day. In a second experiment, groups of eight rats, weighing an average of 250 g, were given perchlorate at 0.01 mg/kg bw per day in the drinking-water for 1 or 4 days. Animals were killed at the end of the dosing period. There were no effects on body weight, food consumption or water consumption in either experiment. In the first experiment, there was a dose-related increase in TSH, which was statistically significant at 0.1 and 1.0 mg/kg bw per day. Free T_4 and total T_4 were both significantly reduced at 1.0 mg/kg bw per day. In the second experiment, there were no effects on TSH or thyroid hormones. There were no effects on thyroid weight or histology at any dose in either experiment (McLanahan et al., 2007). This study indicates a NOEL of 0.01 mg/kg bw per day for effects on thyroid hormones following short-term (14 days) dosing of perchlorate.

2.2.3 Long-term studies of toxicity and carcinogenicity

The information on carcinogenicity has been summarized by ATSDR (2008). The original studies were not reviewed by this Committee.

Potassium and sodium perchlorates have been shown to produce thyroid tumours (papillary and/or follicular adenomas and/or carcinomas) in rats and mice with long-term exposure (1–24 months) to 1–1.2% concentrations in the feed (10 000–12 000 mg/kg) or drinking-water (10 000–12 000 mg/l) (Kessler & Kruskemper, 1966; Gauss, 1972; Florencio Vicente, 1990; Fernandez-Rodriguez et al., 1991; Pajer & Kalisnik, 1991; Toro Guillen, 1991). Estimated perchlorate doses in these studies ranged from 928 to 2573 mg/kg bw per day. The cancer effect levels from these studies were around 1000 mg/kg bw per day.

The incidence of Ki-*ras* oncogene mutations was determined in follicular cell carcinomas of the thyroid induced by administration of radiolabelled iodine and potassium perchlorate (1%, or 10 000 mg/l, in drinking-water) for up to 18 months. Direct sequencing showed no mutations in the amplified gene segment of any of the induced thyroid tumours. The results suggested that Ki-*ras* activation via mutations at codons 12 and 13 is neither a constant event nor an early event in the development of rat thyroid follicular cell carcinoma (Fernández-Santos et al., 2004).

Low-level exposure to potassium perchlorate (0.1%, or 1000 mg/kg, in the feed, corresponding to a perchlorate dose of 64 mg/kg bw per day) for 19 weeks was shown to promote the development of thyroid tumours initiated by *N*-bis-(2-hydroxypropyl)nitrosamine (Hiasa et al., 1987).

2.2.4 Genotoxicity

The limited information on genotoxicity has been summarized by ATSDR (2008). The original reports were not available to the Committee for this review.

Magnesium perchlorate was negative in a test for SOS-inducing activity in *Salmonella typhimurium* strain TA1535 (Nakamura & Kosaka, 1989) and in a test for production of deoxyribonucleic acid (DNA)–protein crosslinks in cultured human lymphocytes (Costa et al., 1996). No evidence of mutagenicity for ammonium perchlorate with or without metabolic activation was found in six different *Salmonella* strains (Zeiger, 1998a). Ammonium perchlorate was not mutagenic in the mouse lymphoma assay with or without metabolic activation (San & Clarke, 1999).

No evidence of bone marrow erythrocyte micronucleus formation was found in male or female rats as a result of exposure to ammonium perchlorate in the drinking-water at a perchlorate dose of 8.5 mg/kg bw per day for 90 days (Siglin et al., 2000). No increase was reported in micronucleus formation in bone marrow from mice injected intraperitoneally with ammonium perchlorate at a dose of 500 mg/kg bw per day for 3 consecutive days; higher doses were lethal to the mice (Zeiger, 1998b).

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration reproductive toxicity

In a two-generation study, Sprague-Dawley rats (30 of each sex per group) were given ammonium perchlorate in their drinking-water at target doses of 0, 0.3, 3.0 and 30.0 mg/kg bw per day. Age at commencement of dosing and period of dosing before cohabitation for the parental generations were not stated. Doses exceeded target doses by 33–34% in female parental generations during gestation due to a higher water consumption. The F_1 generation was given the same target doses of ammonium perchlorate as their respective parental (P_1) generation, beginning at weaning and continuing until the day of sacrifice. Adult F_1 animals selected to form the P_2 generation were mated (age at cohabitation not stated), and the P_2 females were continued on ammonium perchlorate until they were sacrificed at the same time as their F_2 offspring on day 21 of lactation. Standard reproductive parameters were evaluated. Blood samples were taken for determination of serum TSH, T_3 and T_4 levels, and histopathological examination was conducted on major tissues, including the thyroid of P_1, F_1 and F_2 animals. The authors reported significant increases in lactation index at 3.0 and 30.0 mg/kg bw per day in the P_1 animals and significant increases in fertility index in all dosed F_1 (P_2) groups compared with controls. There was an increased percentage of stillborn pups at 30.0 mg/kg bw per day in the litters born to the F_1 (P_2) generation (2.5% compared with 0.3% in controls). In F_1 generation adults, relative thyroid weights were significantly increased in all dose groups for female rats and in the 3.0 and 30.0 mg/kg bw per day dose groups for male rats. Histopathological changes in the thyroid in P_1 adults, F_1 pups, F_1 adults and F_2 pups consisted of follicular cell hypertrophy and hyperplasia that increased in incidence and severity in a dose-related manner, reaching statistical significance at 3.0 and 30.0 mg/kg bw per day. TSH and thyroid hormone levels were variable, with some statistically significant changes, not all of which were dose related. TSH levels were clearly and significantly increased at the top dose of 30.0 mg/kg bw per day in the adult P_1 and F_1 animals. The authors concluded that perchlorate is not a reproductive toxicant in rats when administered in the drinking-water at doses up to 30.0 mg/kg bw per day, but it can affect the thyroid at doses greater than or equal to 3.0 mg/kg bw per day. Based on these findings, 0.3 mg/kg bw per day was identified as the NOAEL (York et al., 2001a). It should be noted that this study reported effects on thyroid histology at a dose lower than that affecting serum TSH levels.

In a single-generation study, pregnant Sprague-Dawley rats were given perchlorate at either 0 or 1 mg/kg bw per day in the drinking-water beginning on gestation day (GD) 2 and continuing until lactation day 10. A proportion of the control and exposed dams were sacrificed on GD 20 (number not stated). The remainder were allowed to litter out. Some litters from control and treated dams were cross-fostered immediately after parturition. Other control and exposed dams were allowed to rear their own litters. This resulted in four offspring treatment groups: 1) true control pups born to and reared by their own mothers given untreated water during both gestation and lactation; 2) true exposed pups born to and reared by their own mothers given perchlorate during both gestation and lactation; 3) in utero

exposed pups born to mothers given perchlorate during gestation and reared by foster mothers given untreated water during lactation; and 4) lactational exposed pups born to mothers given untreated water during gestation and reared by foster mothers given perchlorate during lactation. The aim was to achieve target group sizes of eight dams and their litters per treatment group; in practice, because of non-pregnancies, the number was reduced to six or seven. Litters were culled to eight pups: four males and four females. Dams and pups were sacrificed on postnatal day (PND) 10. In both experiments, serum and thyroids were taken from the dams at sacrifice, and serum was taken from fetuses or pups and pooled within litters for analysis of perchlorate, TSH, T_3 and T_4. The calculated average daily doses of perchlorate consumed by the foster and donor dams were 1.09 and 0.97 mg/kg bw per day, respectively. Maternal body weight was not affected by treatment. Dams and fetuses exposed to perchlorate from GD 2 to GD 20 had serum perchlorate levels of 0.71 µg/ml and 0.38 µg/ml, respectively. TSH was significantly increased in perchlorate-treated dams and fetuses compared with controls; T_3 levels were significantly reduced in treated dams, but not in treated fetuses; T_4 levels were significantly reduced in treated dams and fetuses. In the cross-fostering part of the study, maternal body weight was not affected by treatment. Pup body weights were reduced in both in utero and lactational exposure groups that were cross-fostered, compared with true control or true exposure groups reared by their own mothers. Dams in the true exposure group and in the lactational exposure group had serum perchlorate levels of 0.41 µg/ml and 0.51 µg/ml, respectively. Pups in the true exposure group and in the lactational exposure group had similar serum perchlorate levels, averaging 0.50–0.55 µg/ml. TSH levels were significantly increased in both the dams and pups in the true exposure group and in the lactational exposure group, but T_3 levels were not significantly affected. T_4 levels were significantly reduced in female pups exposed to perchlorate only during lactation compared with true control pups and pups exposed to perchlorate only during gestation. T_4 levels in male pups were not affected by perchlorate (Mahle et al., 2003). It should be noted that this study did not control for the procedure of cross-fostering itself; the influence of fostering, aside from any other treatment parameters, can be seen in the reduced body weights of cross-fostered litters.

A study using 64 male and 66 female adult prairie voles and 39 male and 39 female adult deer mice compared the effects of giving perchlorate in water (PCW) with the effects of giving a similar dose of perchlorate in food (PCF). Controls received no perchlorate. For food exposure, soya bean plant matter grown in perchlorate-contaminated irrigation water was incorporated into laboratory chow. Animals were treated for 21 days before pairing, and treatment continued during the period of pairing, pregnancy and lactation. Females producing pups reared their offspring until PND 21, when adults and offspring were sacrificed. Reproductive success, tissue perchlorate concentrations, plasma T_3 and T_4 levels and thyroid histopathology were assessed. The doses of perchlorate received by prairie voles were calculated as follows: before pairing, 0.71 and 0.55 mg/kg bw per day, and during cohabitation, 0.32 and 0.40 mg/kg bw per day, in PCF and PCW groups, respectively. The doses of perchlorate received by deer mice were calculated as follows: before pairing, 1.120 and 0.876 mg/kg bw per day, and during cohabitation, 1.115 and 1.043 mg/kg bw per day, in PCF and PCW groups, respectively. There

were some deaths in both species, attributed to stress. Parental and offspring group sizes for each end-point measured ranged from 13 to 44. Both prairie voles and deer mice receiving perchlorate in water or food tended to have lower reproductive success, in terms of litters born, than controls, with no difference between PCW and PCF groups. Other reproductive end-points, such as number of pups born, birth weight and pup survival to PND 21, showed no significant differences between treated and control groups. Perchlorate was detected in only a few samples of liver and kidney, and the authors considered that their method of analysis was not sensitive enough to detect the likely tissue concentrations. In prairie voles, there were no significant differences in T_3 concentrations between PCW, PCF and control groups; T_4 concentrations were significantly reduced in the PCF group and significantly increased in the PCW group, compared with controls, but only in paired males. In deer mice, T_3 concentrations were significantly decreased compared with controls in PCF unpaired males but not in PCF unpaired females or paired males or in PCW groups; T_4 concentrations were significantly increased in paired PCF males compared with controls, but not in unpaired PCF males or unpaired females and not in PCW groups. Thyroid epithelial cell heights in prairie voles were significantly increased in PCW and PCF parents, both before pairing and after pairing; the offspring were unaffected. There were no effects on colloidal area. In deer mice, there were no effects on thyroid histology. The authors concluded, on the basis of the thyroid histology results in prairie voles, that the trends observed suggest that perchlorate exposure via water may result in slightly greater effects compared with exposure to perchlorate via food (Smith et al., 2006). It should be noted that the effects were slight, and the overall results do not allow a conclusion with respect to perchlorate given in water compared with food.

The effects of in utero and lactational exposure to ammonium perchlorate on developing deer mice were evaluated. Forty breeding pairs were allowed to mate and produce three litters, which were weaned (first and third litters) or killed (second litter) at PND 21. The breeding pairs were dosed continuously with ammonium perchlorate at 0, 1 nmol/l, 1 µmol/l or 1 mmol/l in drinking-water (10 pairs per treatment group), from initial cohabitation until weaning of the pups in the third litter. These concentrations were equivalent to 117 ng/l, 117 µg/l and 117 mg/l, but as neither the amounts of water consumed nor body weights were stated, the dose in relation to body weight cannot be calculated. Pups from the second litter were used for evaluation. Litter size, offspring survival, body weights and organ weights (ovary, testes, kidney, liver, adrenals and heart) were measured. Litter size and percentage survival were not significantly different among treatments, and no other significant differences were observed in any analysis performed when litter means were used in statistical analysis. The authors described a number of significant findings in body weights, liver weights and heart weights when statistical analysis was performed on individual pup data (Thuett et al., 2002a). The Committee notes that the appropriate units for statistical evaluation of reproduction studies are the breeding pair and litter, not individual pups.

A related publication from the same laboratory reported on examinations of thyroid histology and plasma T_3 and T_4 concentrations in the second-litter pups at PND 21 from the study described above. The 1 nmol/l and 1 mmol/l treatment

groups had significantly fewer active follicles per unit area than did controls. The 1 mmol/l treatment group also had significantly fewer active follicles than did the 1 µmol/l and the 1 nmol/l treatment groups. Total T_4 concentrations were significantly increased in the 1 nmol/l and 1 µmol/l groups compared with the controls. No significant difference was observed in total T_3 concentrations. The authors commented that, in contrast to the situation in adult rodents, it appears that ammonium perchlorate increases thyroid hormone production in developing deer mice and produces variable effects with increasing concentrations (Thuett et al., 2002b). It should be noted that as there was no dose–response in either T_4 or thyroid histology changes, an alternative conclusion is that a treatment-related effect has not been established.

(b) Developmental toxicity

A developmental toxicity study was conducted in Sprague-Dawley rats given ammonium perchlorate in the drinking-water at target doses of 0, 0.01, 0.1, 1.0 and 30.0 mg/kg bw per day, beginning 14 days before cohabitation and continuing through sacrifice on GD 21. There were 25 mated rats per group. The actual consumed doses averaged 0, 0.01, 0.08, 0.70 and 21.69 mg/kg bw per day during the 2-week pre-cohabitation period and 0, 0.01, 0.10, 0.96 and 28.27 mg/kg bw per day during the 3-week gestation period. The rats were sacrificed on GD 21, and the fetuses were examined for visceral and skeletal alterations. An additional 16 litters per group were sacrificed on GD 21 for measurement of maternal and fetal serum TSH, T_3 and T_4 levels and thyroid histopathology. There were no effects on maternal body weight, feed consumption or water consumption. There were no treatment-related effects on reproductive or fetal parameters apart from small, but statistically significant, reductions in mean litter size and in ossification of fetal sternebrae and phalanges in the group given 30.0 mg/kg bw per day. Maternal absolute and relative thyroid weights were significantly increased, with decreased colloid and hypertrophy of the thyroid in the 30.0 mg/kg bw per day group. Maternal TSH was significantly increased and T_4 was significantly decreased in a dose-related manner at all dose levels; T_3 was significantly reduced at 30.0 mg/kg bw per day. In the fetuses, decreased colloid was present in male and female thyroids in the 1.0 and 30.0 mg/kg bw per day groups, but there was no evidence of hypertrophy. Fetal TSH was significantly increased at 1.0 and 30.0 mg/kg bw per day, T_4 was significantly reduced at 30.0 mg/kg bw per day and T_3 was significantly decreased in a dose-related manner at all dose levels. The authors concluded that the maternal NOAEL was 1.0 mg/kg bw per day, as thyroid weights and the incidence of histopathological changes were increased at 30.0 mg/kg bw per day, and the developmental NOAEL was 1.0 mg/kg bw per day, based on delays in ossification at 30.0 mg/kg bw per day. The authors considered that colloid depletion in the thyroids and the effects on TSH, T_3 and T_4 levels at lower exposures were adaptive and not adverse (York et al., 2003). A NOEL was not established for effects on thyroid hormones, as these were affected in both dams and fetuses at the lowest dose tested of 0.01 mg/kg bw per day.

A developmental toxicity study was conducted in New Zealand White rabbits given ammonium perchlorate in drinking-water at target doses of 0, 0.1, 1.0, 10.0,

30.0 and 100.0 mg/kg bw per day on GDs 6–28. The actual consumed doses averaged 0, 0.1, 0.9, 10.4, 30.3 and 102.3 mg/kg bw per day. Twenty-five rabbits per group were sacrificed on GD 29, and the fetuses were examined for visceral and skeletal alterations. In addition, blood was collected from does for determination of serum TSH, T_3 and T_4 levels, and the thyroids were examined for histopathological changes. Maternal body weight was not affected by treatment. There were no treatment-related effects on reproductive or fetal parameters at any dose level. A significantly increased incidence of thyroid follicular cell hypertrophy was observed in does treated with doses greater than or equal to 10.0 mg/kg bw per day, and significantly decreased T_4 was observed in does treated with doses greater than or equal to 30.0 mg/kg bw per day. There was no effect on maternal TSH or T_3 levels. Based on these data, the authors concluded that the maternal NOAEL was 1.0 mg/kg bw per day and the developmental NOAEL was 100.0 mg/kg bw per day (York et al., 2001b).

The effect of prenatal and postnatal chronic hypothyroidism induced by iodide-deficient diet and perchlorate in the drinking-water on the formation of the adult-type Leydig cell population was investigated in Wistar rats. Treated females, aged 10 weeks, were put on a diet consisting of an iodide-poor feed supplemented with 0.75% (7500 mg/l) sodium perchlorate in the drinking-water for 2 weeks before mating, during gestation and during lactation. Pups were weaned on PND 28, and the males from treated dams were maintained on iodide-deficient diet plus perchlorate into adulthood. Groups of 6–20 males per group were killed at intervals up to PND 120. Plasma TSH, T_3 and T_4 levels were measured at intervals in dams and offspring. There was no significant effect on maternal body weight. In the dams during treatment, plasma TSH levels were significantly increased, and T_4 and T_3 levels were significantly reduced. In the offspring, plasma TSH levels were increased by 4- to 20-fold from PND 12 to PND 120; T_3 levels were significantly reduced from PND 16 to PND 120; and T_4 levels were severely reduced from PND 16. From PND 12, offspring body weights in the hypothyroid group were significantly reduced by 30–70% compared with controls, the percentage reduction generally increasing with age. In the hypothyroid offspring, eye opening was delayed by 1 day, and body temperature was significantly reduced. Absolute wet testis weights were significantly reduced in hypothyroid males between PND 49 and PND 71, but had caught up with controls by PND 88; from PND 49, relative testis weights (ratio of testis weight to body weight) were significantly increased in the hypothyroid group. Adult-type progenitor Leydig cell formation and proliferation were reduced by 40–60% in hypothyroid males on PND 16 and PND 28, followed by persistence of increased Leydig cell proliferation at later ages than in controls, suggesting a possible slower developmental onset of the adult-type Leydig cell population under hypothyroid conditions. Total testosterone and free testosterone levels were significantly increased 2- to 10-fold in the hypothyroid animals between PND 21 and PND 42, compared with the age-matched controls. The normal peak in luteinizing hormone (LH) levels that occurs between PND 21 and PND 35 was not seen in the hypothyroid males. Prolactin levels in hypothyroid males were significantly decreased from PND 21 onwards. In 84-day-old rats, after correction for body weight to testis weight ratio, plasma insulin-like factor-3 levels were 35% lower in the hypothyroid animals, indicative of a reduced Leydig cell population. This was

confirmed by a 37% reduction in the Sertoli cell to Leydig cell ratio in hypothyroid rats. The authors concluded that dietary-induced hypothyroidism delays, but does not block, the development of the adult-type Leydig cell population (Rijntjes et al., 2009). This study does not allow the effects of perchlorate to be separated from those of the iodide-deficient diet.

The ability of perchlorate to affect the thyroid in a standard Tier 1 male pubertal protocol, developed for the United States Environmental Protection Agency's (USEPA) Endocrine Disrupter Screening Program, was investigated in male Wistar rats. Ammonium perchlorate was administered daily by oral gavage at doses of 62.5, 125, 250 and 500 mg/kg bw for 31 consecutive days, from PND 23 to PND 53, until the day of necropsy. Control animals received vehicle (corn oil) only. At necropsy, blood was taken for hormone measurements, reproductive organs were weighed and thyroids were weighed and examined histologically. There were no effects on body weight or signs of toxicity in treated males. There were no effects on age of onset of preputial separation or on ventral prostate, lateral prostate, seminal vesicle, epididymal or testis weights. Doses of 125–500 mg/kg bw per day significantly increased TSH and decreased T_4 levels in a dose-dependent manner, whereas the T_3 level was unchanged at all doses. Thyroid histology was significantly altered at all four doses, with a clear dose-dependent decrease in colloid area and increase in follicular cell height (Stoker et al., 2006). This study, using relatively high doses of perchlorate, showed that exposure during the period of sexual maturation in juvenile male rats did not alter any of the reproductive developmental end-points, but had marked effects on thyroid structure and function. A NOAEL for effects on the thyroid was not established.

(c) Developmental neurotoxicity

Groups of 24 Sprague-Dawley rats were exposed to ammonium perchlorate in the drinking-water at a target dose of 0, 0.1, 1.0, 3.0 or 10.0 mg/kg bw per day from GD 0 (day of mating) throughout gestation and the first 10 days of lactation. The actual consumed doses averaged 0, 0.1, 1.1, 3.5 and 11.6 mg/kg bw per day during the 3-week gestation period and 0, 0.2, 1.6, 4.7 and 15.4 mg/kg bw per day during the 10-day lactation exposure period. Litters were culled to eight pups on PND 5 and weaned on PND 21. Offspring examinations were carried out on 20 litters per group. One pup of each sex per litter per group was assigned to one of four examinations: 1) juvenile brain weights, morphometry and neuropathology; 2) passive avoidance and water maze testing; 3) motor activity and auditory startle habituation; and 4) adult regional brain weights, morphometry and neuropathology. Thyroid histopathology and serum TSH, T_3 and T_4 levels were examined in culled pups (PND 5) and dams (day after pups weaned). There were no effects on maternal body weight, feed consumption or water consumption. There were no effects on reproductive or litter parameters, including offspring weights and sexual maturation. There were no behavioural effects in the offspring in any dose group as evaluated by passive avoidance, swimming water maze, motor activity and auditory startle. There was an apparent increase in the thickness of the corpus callosum of the 10.0 mg/kg bw per day group pups on PND 12. There were no effects on maternal thyroid weight or histology in dams killed when their pups were weaned (12 days

after cessation of treatment). In pups culled on PND 5, an increase in hypertrophy of the thyroid follicular epithelium and a decrease in the thyroid follicle size were observed in females in the 3.0 and 10.0 mg/kg bw per day groups and in males in the 10.0 mg/kg bw per day group. The exposure level for effects on T_3, T_4 and TSH levels in pooled serum from pups culled on PND 5 were 1.0, 3.0 and 10.0 mg/kg bw per day, respectively. The NOAEL for maternal toxicity was greater than 10.0 mg/kg bw per day. Based on the thyroid histopathology and reduced thyroid hormones, the authors concluded that the NOAEL for pups was 0.1 mg/kg bw per day (York et al., 2004).

Another study from the same group further investigated the potential effects of maternal exposure to ammonium perchlorate during gestation and lactation on offspring development and thyroid function. Groups of 25 female Sprague-Dawley rats were given continual access to ammonium perchlorate at 0, 0.01, 0.1, 1.0 and 30.0 mg/kg bw per day in drinking-water beginning 2 weeks prior to cohabitation through to PND 10. The actual consumed doses for the female rats averaged as follows: during pre-cohabitation, 0, 0.01, 0.09, 0.77 and 23.80 mg/kg bw per day; during gestation, 0, 0.01, 0.09, 0.97 and 28.34 mg/kg bw per day; and during lactation, 0, 0.01, 0.12, 1.26 and 38.32 mg/kg bw per day. Maternal, fetal and pup serum TSH, T_3 and T_4 levels and thyroid histopathology were evaluated on GD 21, PND 5, PND 10 and PND 22. There were no effects on maternal body weight, feed consumption or water consumption. There were no effects on reproductive or litter parameters. Thyroid weights and histopathological changes (decreased colloid and follicular hypertrophy) were significantly increased in dams sacrificed on GD 21, PND 10 and PND 22 at 30.0 mg/kg bw per day. Increases in follicular hyperplasia were also evident at 1.0 and 30.0 mg/kg bw per day, but significance levels were not indicated. Maternal TSH levels were significantly increased at all doses on GD 21 and PND 10 and still elevated at doses greater than or equal to 0.1 mg/kg bw per day on PND 22, 12 days after cessation of exposure. Maternal T_4 level was significantly reduced at all doses on GD 21 and at 30.0 mg/kg bw per day on PND 10 and PND 22. Maternal T_3 level was significantly reduced only at 30.0 mg/kg bw per day on GD 21. In the pups, significantly increased thyroid weights in males were observed on PND 5 in the 30.0 mg/kg bw per day group, on PND 10 in all dose groups and on PND 22 in the 1.0 and 30.0 mg/kg bw per day groups; in female pups, significantly increased thyroid weights were observed on PND 10 in the 30.0 mg/kg bw per day group and on PND 22 in the 1.0 and 30.0 mg/kg bw per day groups. Histopathological changes in male and female pup thyroids (decreased colloid) were significantly increased at 1.0 mg/kg bw per day. Pup TSH levels were not significantly increased on PND 5, but were significantly increased at 30.0 mg/kg bw per day on PND 10 and at all doses on PND 22. Pup T_4 levels were significantly reduced at 1.0 and 30.0 mg/kg bw per day on PND 5, not significantly altered at PND 10 but significantly reduced at all dose levels at PND 22. Pup T_3 levels were significantly reduced at 30.0 mg/kg bw per day on PND 5 and at 1.0 and 30.0 mg/kg bw per day on PND 10 and PND 22. The authors concluded that the maternal NOEL was 0.1 mg/kg bw per day, based on follicular cell hyperplasia at 1.0 and 30.0 mg/kg bw per day. A developmental NOEL was not established, as thyroid weights in male pups were increased at all doses on PND 10 (York et al., 2005a).

Another study from the same group generated additional data on developmental neurotoxicity in offspring from in utero and lactational exposure to ammonium perchlorate. Groups of 23–25 female Sprague-Dawley rats were given continuous access to ammonium perchlorate at 1) 0, 0.1, 1.0, 3.0 and 10.0 mg/kg bw per day in the drinking-water beginning 2 weeks prior to mating and continuing through to PND 10 for the behavioural function assessment or 2) 0, 0.1, 1.0, 3.0 and 30.0 mg/kg bw per day beginning on GD 0 and continuing through day 10 of lactation for neurodevelopmental assessments. Motor activity was investigated on PND 14, PND 18 and PND 22, and juvenile brain weights were measured, neurohistopathological examinations were conducted and regional brain morphometry was assessed on PND 10 and PND 22. There were no effects on maternal body weight, feed consumption or water consumption. There were no effects on reproductive or litter parameters. The mean values for the linear dimensions of a number of brain regions in male pups on both PND 10 and PND 22 were found to be significantly greater than for the controls, whereas in female pups at the same ages, some regions were significantly smaller than for the controls; for the majority of these measurements, however, there was no clear dose–response. Some brain regions were larger in perchlorate-treated male offspring than in comparable controls, but there was no evidence of any obvious exposure-related effects on male rat brain weights or neuropathology. Behavioural testing did not show any effect on the development of gross motor movements in the pups (York et al., 2005b).

Groups of 30 pregnant Long-Evans rats were exposed to ammonium perchlorate in drinking-water at 0, 30, 300 or 1000 mg/l from GD 6 until the pups were weaned at PND 30. Actual consumed doses averaged 0, 4.5, 44.2 and 140.3 mg/kg bw per day. A proportion of pups were sacrificed at PND 4, PND 14 and PND 21 for hormone measurements. Adult male offspring were evaluated on a series of behavioural tasks and neurophysiological measures of synaptic function in the hippocampus. There were no effects on maternal body weight. In dams sacrificed on PND 30, TSH levels were significantly increased at 1000 mg/l, T_3 levels were unaffected, but T_4 levels were significantly reduced compared with controls by 16%, 28% and 60% at 30, 300 and 1000 mg/l, respectively. In pups, at PND 4, there were no significant effects on hormone levels; at PND 14, T_3 and T_4 levels were unaffected, whereas TSH levels were significantly increased in the 30 and 300 mg/l groups, but not in the 1000 mg/l group; at PND 21, TSH levels were unaffected, but T_3 and T_4 levels were significantly reduced at 300 and 1000 mg/l. There were no effects on offspring body weight, time of eye opening, brain weight or hippocampal weight. Perchlorate did not impair motor activity at 13 months of age, spatial learning in a Morris water maze at 3 months of age or fear conditioning at 7–8 months of age. However, deficits were observed in inhibitory and excitatory synaptic function in the hippocampus of the offspring tested at 5–9 months of age. Significant reductions in baseline synaptic transmission were observed in hippocampal field potentials at all three dose levels. Reductions in inhibitory function were evident at 300 and 1000 mg/l, and augmentations in long-term potentiation were observed in the population spike measure at 1000 mg/l. The authors concluded that dose-dependent deficits in hippocampal synaptic function were detectable following relatively minor perturbations of the thyroid axis, indicative of an irreversible impairment in synaptic transmission in adults in response to

developmental exposure to perchlorate (Gilbert & Sui, 2008). A NOAEL was not established, as effects on the hippocampus were seen at the lowest dose tested, equivalent to 4.5 mg/kg bw per day. It should be noted that the middle and high doses used in this study (44.2 and 140.3 mg/kg bw per day, respectively) had considerably less marked effects on maternal TSH and T_4 levels than have been observed in other studies, reviewed above, that used lower doses of perchlorate administered via drinking-water (e.g. 30.0 mg/kg bw per day). In addition, at the lowest dose, only baseline synaptic transmission in the hippocampus was affected, and no behavioural measures were affected in any dose group in tests that were anticipated to be sensitive to hippocampal disturbances.

The behavioural effects of perinatal and chronic hypothyroidism induced by iodide-deficient diet and perchlorate in the drinking-water were assessed during development in male and female offspring of Wistar rats. To induce hypothyroidism, six dams were fed an iodide-poor diet and drinking-water containing 0.75% (7500 mg/l) sodium perchlorate from 2 weeks prior to mating. Six control dams were given normal diet and untreated drinking-water. Four days after birth, the litters were sexed and divided between six foster mothers (four hypothyroid and two control), with four males and four females allocated per litter. From PND 14, two of the hypothyroid foster mothers received control diet and no further perchlorate ("hypo-normal" group). Pups were weaned from the dams at PND 29. Treatment of pups continued either until the day of killing on PND 76 (chronic hypothyroidism) or only until weaning (perinatal hypothyroidism) to test for reversibility of any effects observed. Neuromotor competence, locomotor activity and cognitive function were monitored in the offspring until PND 71 and were compared with age-matched control rats. Body weights of the hypothyroid offspring were significantly reduced compared with hypo-normal or control offspring and were only 37% of control values by PND 75. Early neuromotor competence, as assessed in the grip test and balance beam test, was impaired by both chronic and perinatal hypothyroidism. The open field test, assessing locomotor activity, revealed hyperactive locomotor behavioural patterns in chronic hypothyroid offspring only. The Morris water maze test, used to assess cognitive performance, showed that chronic hypothyroidism adversely affected spatial memory. In contrast, perinatal hypothyroidism was found to impair spatial memory in female rats only (van Wijk, Rijntjes & van de Heijning, 2008). It should be noted that only small numbers of animals were used in this study, each treatment group comprising two dams and eight pups per dam, and that the statistical analysis is limited, as individual pup numbers were used as the value for *n*, which would not control for litter effects. This study does not allow any firm conclusions to be drawn and does not allow the effects of perchlorate to be separated from those of the iodide-deficient diet.

2.2.6 Special studies

(a) Physiologically based pharmacokinetic modelling

Physiologically based pharmacokinetic (PBPK) models for prediction of perchlorate distribution and its effects on iodide uptake in the thyroid have been developed for the adult male rat, the lactating rat and the neonatal rat (Clewell et

al., 2001, 2003a,b; Merrill et al., 2003). The models describe the simultaneous kinetics for iodide and perchlorate, their interaction at the sodium–iodide symporter (NIS) and the inhibition of iodide uptake by perchlorate. The predictions of the models were checked against experimental data from studies using intravenous infusions of iodide and perchlorate or perchlorate alone or administration of perchlorate in drinking-water, covering doses ranging from 0.01 to 30.0 mg/kg bw per day. The predictions were successful for both acute intravenous kinetics and subchronic oral kinetics. Perchlorate has a higher affinity for the NIS than does iodide, with a Michaelis-Menten constant (K_m) approximately 10 times lower than that of iodide. Tissues with active uptake were the thyroid, stomach, skin, placenta and mammary gland. The compartments of kidney, liver, plasma, fat and milk were described by passive diffusion. The gestational model suggested significant fetal exposure to perchlorate in late gestation (up to 82% of maternal dose). A comparison of model-predicted internal dosimetrics in the adult male, pregnant female and fetal rat indicated that the fetal thyroid is more sensitive to inhibition than that of the adult. Model simulations predicted a transfer to maternal milk of between 50% and 6% of the maternal dose in the dose range 0.01–10.0 mg/kg bw per day. Comparison of predicted dosimetrics across life stages in the rat indicated that inhibition of the uptake of iodine by perchlorate in the neonate (PND 10) is similar to that in the adult and approximately 10-fold less than that for the fetus. The authors concluded that these rat models can be used to extrapolate to humans, provided a longer time course is included to account for the longer lag time in humans for onset of upregulation of activity of the inhibited thyroid following exposure to perchlorate. Whereas humans show inhibition of iodine uptake into the thyroid by perchlorate similar to that in the rat for equal perchlorate doses, upregulation, in terms of number of NIS and activity of NIS, occurs in the rat within 12 h of commencement of treatment, but is still not evident in humans after 2 weeks of exposure. This may reflect the greater thyroid hormone storage capacity and greater serum binding of thyroid hormones to TBG rather than to albumin and transthyretin in the human compared with the rat. The binding of thyroid hormones to TBG is very strong, which protects them from degradation in the circulation. The level or duration of perchlorate exposure required to induce upregulation in the human is not known.

Data on perchlorate from the published literature were used to compare species differences in perturbation of thyroid homeostasis by perchlorate. Dose–response data for changes in serum T_3, T_4 and TSH concentrations following perchlorate administration in humans, rats, rabbits and mice were analysed. Although the data indicated that humans and rats exhibit similar dose–response relationships in terms of acute inhibition of thyroidal iodide uptake by perchlorate, the two species exhibit notable differences in terms of thyroid hormone response, with the rat thyroid being much more sensitive to perchlorate than any of the other species. Rats exhibited an increase in serum TSH level at 0.1 mg/kg bw per day, whereas other species remained unresponsive even at doses of 10 mg/kg bw per day. Less pronounced but consistent effects were seen with serum T_3 and T_4 levels (Lewandowski, Seeley & Beck, 2004).

(b) Mode of action studies

There has been a debate about whether the mode of action of perchlorate is simply to block the NIS or whether perchlorate is actually a substrate for the NIS and is actively transported into the thyroid follicular cell. The proposal that perchlorate is actively transported into the thyroid follicular cell by the NIS was challenged using an indirect electrochemical technique to infer movement of perchlorate. The authors concluded that perchlorate was not taken up into the thyroid gland, because, in contrast to iodide uptake, an electrical gradient was not created (Riedel et al., 2001).

A subsequent study in rats demonstrated transport of perchlorate into the thyroid. The effect of ammonium perchlorate on the uptake of radiolabelled iodide into the thyroid was also studied. Perchlorate was administered orally in the drinking-water to adult male Sprague-Dawley rats at target doses of 0, 1.0, 3.0 and 10.0 mg/kg bw per day for 1, 5 or 14 days, with six rats per dose per time point, or as single intravenous injections, at doses ranging from 0.01 to 3.0 mg/kg bw. There was a dose-dependent inhibition of uptake of radiolabelled iodide at all doses by both routes of administration and at all time points. The inhibition occurred quickly and was closely related to serum perchlorate concentrations. Other groups of eight rats were given perchlorate in drinking-water at 0, 0.1, 1.0, 3.0 or 10.0 mg/kg bw per day, and serum TSH and thyroid hormone responses were measured on days 1, 5 and 14 of perchlorate treatment. TSH was significantly increased in a dose-related manner at all doses and time points. After 1 day of treatment, T_4 levels were decreased in all dose groups except the lowest. By 14 days of perchlorate treatment, the T_4 values in the 0.1 and 1.0 mg/kg bw per day groups had returned to control values, but T_4 values in the 3.0 and 10.0 mg/kg bw per day groups were still reduced. After 1 day of treatment, free T_4 levels were significantly increased in all dose groups. On day 5 of treatment, free T_4 levels were similar to control values, but by day 14, free T_4 levels in all dose groups were increased again, except in the 0.1 mg/kg bw per day group. T_3 levels declined throughout the perchlorate treatment period for all dose groups. However, the T_3 levels in each dose group were not statistically different from controls. There were no effects on body weight at any dose (Yu et al., 2002). A NOEL for effects on iodide uptake or on TSH or serum thyroid hormones was not established.

Active transport of perchlorate into maternal milk by the NIS in the mammary gland has also been demonstrated using both in vivo and in vitro rat models (Dohán et al., 2007). The same group also showed that the process of active transport of perchlorate in vitro is not electrogenic and that perchlorate ion is transported stoichiometrically with sodium ion in a ratio of 1:1, whereas iodide is transported by the NIS in a ratio of two sodium ions to each iodine ion. This would explain the earlier findings of Riedel et al. (2001), mentioned above, and why those authors had misinterpreted their findings. Dose-related competitive inhibition of iodide uptake by perchlorate has also been confirmed in vitro in rat FRTL-5 cells, which maintain functional NIS (Tran et al., 2008).

Using a simpler PBPK model than that of Merrill et al. (2003) and comparison with previously published experimental data on administration of perchlorate in

drinking-water in the rat, the assumption that the mode of action of perchlorate is solely competitive inhibition of iodide uptake by the NIS was tested. The rapid decrease in serum total T_4 levels and the corresponding increase in serum TSH levels after 1 day of treatment were considered to be inconsistent with a mode of action related solely to competitive inhibition at the level of the NIS and appeared consistent with a reduction in thyroid hormone production or secretion. The authors concluded that perchlorate is translocated into the thyroid gland, where it may also act directly or indirectly on thyroid hormone synthesis or secretion in the rat (McLanahan et al., 2009).

These studies, together with other data reviewed by Clewell et al. (2004), provide strong evidence that the mode of action of perchlorate involves competitive inhibition of iodide transport into the thyroid follicle, transport of perchlorate into the thyroid follicle against a concentration gradient, further transport into the thyroid lumen (where it may again interfere with iodide transport) and, finally, passive diffusion back into the blood.

The affinity of the NIS for perchlorate has been shown to be 30-fold greater than its affinity for iodide in Chinese hamster ovary cells stably transfected with human NIS (Tonacchera et al., 2004). However, in contrast to the rat, in which the NIS is expressed in thyroid follicular cells at a high density, in humans, NIS expression in thyroid follicular cells is "patchy" (Josefsson et al., 2002). The NIS is expressed in human placenta, lactating breast, salivary gland and gastric mucosa (Clewell et al., 2007; Tran et al., 2008) and transports perchlorate across these tissues, resulting in exposure of the developing fetus, neonate and breastfed infant to perchlorate (Kirk et al., 2005, 2007; Blount et al., 2006b; Dasgupta et al., 2008).

2.3 Observations in humans

2.3.1 Biomarkers of exposure

Exposure to perchlorate was assessed in a nationally representative population of 2820 residents of the USA, aged 6 years and older, during 2001 and 2002 as part of the National Health and Nutrition Examination Survey (NHANES). Detectable levels of perchlorate (>0.05 µg/l) were found in all 2820 urine samples tested, indicating widespread human exposure. Urinary perchlorate levels were lognormally distributed, with a median level of 3.6 µg/l (3.38 µg/g creatinine) and a 95th-percentile level of 14 µg/l (12.7 µg/g creatinine). When geometric means of urinary perchlorate levels were adjusted for age, fasting, sex and race or ethnicity, significantly higher levels of urinary perchlorate were found in children compared with adolescents and adults. Total daily perchlorate exposures were estimated for each adult aged 20 years and older, based on urinary perchlorate, urinary creatinine concentration and physiological parameters predictive of creatinine excretion rate. The 95th percentile of the distribution of estimated daily perchlorate doses in the adult population was 0.234 µg/kg bw per day (95% confidence interval [CI] 0.202–0.268 µg/kg bw per day), which is below the USEPA reference dose (RfD) of 0.7 µg/kg bw per day (USEPA, 2005b; Blount et al., 2006a).

In the most recent NHANES data set on 2522 residents of the USA from the period 2003–2004, geometric mean values for urinary perchlorate concentrations in all age, sex and race or ethnicity groups were slightly lower than the corresponding values for 2001–2001. The overall geometric means were 3.54 µg/l (95% CI 3.29–3.81 µg/l) for 2001–2002 and 3.22 µg/l (2.93–3.55 µg/l) for 2003–2004. The 95th-percentile geometric mean values were 14 µg/l (11–17 µg/l) for 2001–2002 and 13 µg/l (12–15 µg/l) for 2003–2004 (CDC, 2009).

A number of studies have investigated perchlorate in human breast milk. They demonstrate that breast milk is a significant route of excretion of perchlorate. It should be noted that studies that have attempted to correlate levels of perchlorate in drinking-water with levels in breast milk may be confounded by the contribution from the diet. It should also be noted that there are large temporal variations in urinary iodine levels within individuals, which may also be reflected in breast milk; therefore, studies using spot samples—that is, the majority of those described below, except Kirk et al. (2007) and Dasgupta et al. (2008)—may not be representative of longer-term breast milk iodine levels. There has been concern that exposure of infants via breast milk or possibly infant formula may lead to exceedance of health-based guidance values (Baier-Anderson et al., 2006; Schier et al., 2010).

Thirty-six breast milk samples from women in 18 states in the USA were analysed, and perchlorate was found in all samples at concentrations ranging from 1.4 to 92.2 µg/l, with a mean of 10.5 µg/l. In this study, in the six breast milk samples with a perchlorate content greater than 10 µg/l, the iodide content was linearly correlated with the inverse of the perchlorate concentration, with an r^2 of >0.9 (Kirk et al., 2005).

In a smaller study involving 10 women, from whom about six samples were collected on each of 3 days, making a total of 147 samples, half the women were from Texas, and the others were from five other states in the USA. There was significant variation in all samples, with a concentration range of 0.5–39.5 µg/l and mean and median perchlorate concentrations of 5.8 µg/l and 4.0 µg/l, respectively. Samples collected in the morning before any food or liquid was consumed had significantly lower amounts of perchlorate than matched samples collected in the evening. Three of the 10 subjects had average perchlorate concentrations in the breast milk above 7 µg/l; thus, for the infants of these mothers, the USEPA RfD for perchlorate of 0.7 µg/kg bw per day (USEPA, 2005b) could have been exceeded, assuming the infants were older than 12 months of age and had an average milk consumption of 100 ml/kg bw per day (Kirk et al., 2007).

Analysis of 55 breast milk samples from a study in three cities in Chile with differing perchlorate levels in the drinking-water indicated that perchlorate levels were highly variable, and no significant correlations could be established between breast milk perchlorate concentrations and either urinary perchlorate concentrations or breast milk iodide concentrations for the individuals evaluated in this study (Téllez Téllez et al., 2005).

Measurements of perchlorate in breast milk obtained from 49 healthy volunteers from the Boston area in Massachusetts, USA, between 10 and 250 days

postpartum found perchlorate in all samples at concentrations ranging from 1.3 to 411 µg/l, with a median concentration of 9.1 µg/l and a mean concentration of 33 µg/l. No correlation was found between perchlorate and iodine concentrations in breast milk, including in a subset of 27 women with breast milk perchlorate concentrations above 10 µg/l (Pearce et al., 2007).

Breast milk samples and 24 h urine samples collected over 9 consecutive days from 13 lactating women from Texas in the USA were analysed for perchlorate and iodine. Breast milk perchlorate concentrations ranged from 0.01 to 48 µg/l, with a median concentration of 7.3 µg/l and a mean concentration of 9.3 µg/l (457 samples in total). The authors calculated that 9 out of 13 infants receiving this milk would have received exposures greater than the proposed National Research Council RfD of 0.7 µg/kg bw per day (NRC, 2005). Iodine concentrations in breast milk ranged from 1 to 1200 µg/l, with a median concentration of 43 µg/l and a mean concentration of 120 µg/l (447 samples in total). Urinary perchlorate concentrations ranged from 0.6 to 80 µg/l, with a median concentration of 3.2 µg/l and a mean concentration of 4.0 µg/l (110 samples in total). Iodine concentrations in urine ranged from 26 to 630 µg/l, with a median concentration of 110 µg/l and a mean concentration of 140 µg/l (117 samples in total) (Dasgupta et al., 2008).

Single spot collections of colostrums from 97 healthy women from the Boston area in the USA were obtained within 60 h postpartum. The median colostrum iodine content in 61 samples was 51.4 µmol/l (range 21.3–304.2 µmol/l). The median urinary iodine concentration in 97 samples was 82.2 µmol/l (range 0.3–417.1 µmol/l). Perchlorate was detectable in 43 of 46 colostrum samples (median 2.5 µmol/l; range <0.05–188.9 µmol/l) and in all 97 urine samples (median 2.6 µmol/l; range 0.2–160.6 µmol/l). There were no significant correlations between perchlorate concentrations and iodine concentrations in urine or colostrum (Leung et al., 2009).

Levels of three physiologically relevant NIS inhibitors (perchlorate, nitrate and thiocyanate) and iodide in maternal and fetal fluids collected during caesarean section surgeries on 150 women in the USA have been measured. Geometric means of perchlorate, thiocyanate and nitrate levels in maternal urine (2.90 µg/l, 947 µg/l and 47 900 µg/l, respectively) were similar to previously published results, whereas urinary iodide levels (1420 µg/l) were significantly higher, likely because of prevalent prenatal vitamin use in the study population (74%). Perchlorate was detected in most samples: urine (100%), maternal serum (94%), cord serum (67%) and amniotic fluid (97%). Maternal urinary perchlorate levels were positively correlated with perchlorate levels in amniotic fluid (r = 0.57). Maternal serum perchlorate was generally higher than cord serum perchlorate (median ratio 2.4:1 for paired samples), and maternal urinary perchlorate was always higher than fetal amniotic fluid perchlorate levels (mean ratio 22:1). Iodide levels were typically higher in fetal fluids than in maternal fluids. There was no association between cord blood levels of these anions and newborn weight, length or head circumference (Blount et al., 2009).

2.3.2 Clinical observations and biomarkers of effect

On a population basis, there are wide variations in circulating levels of T_3 and T_4 among humans, and levels also fluctuate considerably within individuals, reflecting short-term pulsatile secretion and diurnal variation. Therefore, TSH measurements are considered to be critical for reliable diagnostic information on thyroid function (Braverman & Utiger, 2005). For studies involving neonatal TSH measurements, it should be noted that there is a postnatal surge of TSH immediately after birth, which peaks around 12 h of age and then declines during the first 24 h of life. Thus, results obtained during the first 18–24 h of life are not necessarily informative about subsequent thyroid function (Braverman & Utiger, 2005; Buffler et al., 2006).

(a) Healthy, non-pregnant adults

A study was carried out in nine healthy male volunteers, aged 22–30 years, to investigate the effects of short-term ingestion of perchlorate on thyroid hormones. Perchlorate providing a daily dose of 10 mg was ingested in spring water consumed throughout the day for 14 days. Assuming a body weight of 70 kg, an exposure of 10 mg/day would equate to a dose of approximately 0.14 mg/kg bw per day. TSH, free T_4, total T_3, uptake of radiolabelled iodide by the thyroid and 24 h urinary iodine excretion were measured at baseline before perchlorate administration, on days 7 and 14 during perchlorate administration and 14 days after cessation of perchlorate administration. Urinary and serum perchlorate concentrations were elevated in all subjects. By day 14, the 4, 8 and 24 h thyroid radioiodide uptakes were reduced in all subjects by an average of 38% below baseline; uptake rebounded to 25% above baseline 14 days after cessation of perchlorate administration. There was no effect at any time point on TSH, thyroid hormones or urinary iodine excretion (Lawrence et al., 2000).

In a follow-up study, the same group investigated the effects of ingestion of a dose of 3 mg of perchlorate per day for 14 days in eight healthy male volunteers. Perchlorate was again given in spring water consumed throughout the day. Assuming a body weight of 70 kg, an exposure of 3 mg/day would equate to a dose of about 0.04 mg/kg bw per day. Uptake of radiolabelled iodide by the thyroid and other thyroid function parameters were measured at baseline, after 14 days of perchlorate administration and 14 days after cessation of perchlorate administration. The 8 and 24 h values for thyroid uptake of radiolabelled iodide were slightly reduced by about 10% during perchlorate administration, but the reductions were not statistically significant compared with baseline values; 8 and 24 h values for uptake rebounded to 22% and 18%, respectively, above baseline at 14 days after cessation of perchlorate administration. TSH and thyroid hormones were unaffected (Lawrence, Lamm & Braverman, 2001). This study suggests that short-term administration of perchlorate at a dose of 3 mg/day, equivalent to about 0.04 mg/kg bw per day, is around the LOEL for effects of perchlorate on iodine uptake in the thyroid.

A key study in 16 male and 21 female volunteers investigated the time course and dose–response for perchlorate inhibition of iodine uptake by the thyroid. The

volunteers were screened to exclude the possibility of thyroid disease. Perchlorate was given in drinking-water at doses of 0.007, 0.02, 0.1 or 0.5 mg/kg bw per day for 14 days. The daily dose was consumed as four divided doses of 100 ml ingested at 08:00, 12:00, 16:00 and 20:00. The uptake of radiolabelled iodine into the thyroid was measured at baseline before administration of perchlorate, on days 2 and 14 of administration and 15 days after cessation of perchlorate administration. The radiolabelled iodine was given at 09:00 on each day of measurement, and uptake was measured at 8 and 24 h after administration. The uptake of iodide was linearly related to the logarithm of the perchlorate dose and did not differ between day 2 and day 14 of perchlorate administration, indicating that more or less steady-state inhibition was achieved by day 2. There was no difference between the sexes. At 15 days after cessation of perchlorate administration, uptake of iodide had returned to baseline values. Inhibition of iodide uptake on day 14 compared with baseline was 1.8%, 17%, 44% and 67% in the 0.007, 0.02, 0.1 and 0.5 mg/kg bw per day groups, respectively. The inhibition was not significantly different from baseline at the lowest dose of 0.007 mg/kg bw per day, but was significantly increased in all other dose groups. The authors estimated, using their terminology, a "true no-effect level" by extrapolation from the regression relationships for relative uptakes at 8 and 24 h on day 14; the true no-effect levels were 5.2 and 6.4 µg/kg bw per day, respectively. Based on the variability observed in the subjects, the authors estimated that there is a 95% probability that thyroidal iodide uptake will be inhibited by no more than 8.3–9.5% at a dose of 5.2–6.4 µg/kg bw per day. Given default body weight and exposure assumptions, the authors calculated that these no-effect level doses would be ingested by an adult if the drinking-water supply contained perchlorate at concentrations of approximately 180 and 220 µg/l, respectively. Serum total T_4, free T_4, total T_3 and TSH levels were measured in 24 of the subjects on 16 occasions throughout the study. There were no effects at any dose on total T_4, free T_4 or total T_3 levels. A significant effect on TSH levels was observed only in the 0.5 mg/kg bw per day group, which showed marginally decreased TSH concentrations in morning blood draws during perchlorate administration, with recovery by 15 days after cessation of perchlorate administration; this was the opposite of what would be expected from exposure to a thyroid inhibitor. The NOEL from this study for effects of perchlorate on uptake of iodine by the thyroid was 0.007 mg/kg bw per day, and the NOEL for effects on levels of thyroid hormones and TSH was the highest dose tested, 0.5 mg/kg bw per day (Greer et al., 2002).

It should be noted that the dose–response relationships from the other human clinical volunteer studies (Lawrence et al., 2000; Lawrence, Lamm & Braverman, 2001) are essentially in agreement with the results of Greer et al. (2002) and that benchmark dose (BMD) calculations (see section 2.3.2(b) below) from two occupational studies (Lamm et al., 1999; Braverman et al., 2005) are within the range of the Greer et al. (2002) study and in agreement with the NOEL from that study.

An occupational study of employees working in perchlorate production at a manufacturing plant in Utah, USA, was conducted to assess whether exposure to ammonium perchlorate affected thyroid function. Thirty-seven employees (35 males and 2 females) were studied, 40% of whom had been employed for more than

5 years. Workers typically worked three 12 h shifts followed by 3 days off. Results were compared with those from 21 employees (19 males and 2 females, 50% employed for more than 5 years) in the same industrial facility but working in sodium azide production. Exposure to perchlorate was assessed by measurement of ambient air concentrations of total and respirable perchlorate particles, and systemic absorption was assessed by measurement of urinary perchlorate excretion pre- and post-shift. Airborne exposures ranged from 0.004 to 167 mg of total particulate perchlorate per day. Urinary perchlorate measurements demonstrated that exposure to the airborne particulate perchlorate resulted in systemic absorption that is related to airborne concentrations. Workers were grouped into four exposure categories, with mean absorbed perchlorate dosages of 1, 4, 11 and 34 mg/day. Thyroid function was assessed by measurement of serum TSH, T_4 and T_3 levels, free T_4 index, thyroid hormone binding ratio and thyroid peroxidase antibodies and by clinical examination. No differences in thyroid function parameters were found between the four groups of workers across approximately 3 orders of magnitude of exposure and of dose. In addition, no clinical evidence of thyroid abnormalities was found in any exposure group. The authors concluded that a mean absorption of 34 mg/day from occupational airborne exposure to perchlorate demonstrated a NOAEL that can assist in the evaluation of human health risks from environmental perchlorate contamination (Lamm et al., 1999).

The effects of occupational exposure to perchlorate on thyroid function were studied in workers employed for at least 1.7 years (50% over 5.9 years) in the same ammonium perchlorate production plant in Utah as studied by Lamm et al. (1999). The following parameters were assessed in 29 workers after 3 days off and during the last of three 12 h night shifts in the plant and in 12 volunteers (controls) not working in the plant: serum perchlorate, thiocyanate and nitrate; serum T_4, free T_4 index, total T_3, thyroglobulin and TSH; 14 h radiolabelled iodine uptake by the thyroid; and urinary iodine and perchlorate. Estimated exposures to perchlorate during the study, from urinary and serum perchlorate concentrations, ranged from 0.01 to 3 mg/kg bw per shift. Over the year preceding this study, the median absorbed dose was estimated to be 0.33 mg/kg bw per shift. Serum thiocyanate and nitrate concentrations were similar in all groups. Serum and urinary perchlorate were not detected in the control group. In workers after 3 days off, urinary perchlorate was not detected in 12 of 29 workers and was a mean value of 0.27 mg/g creatinine in the remaining 17 workers; serum perchlorate was not detected in 27 of 29 workers. Serum and urinary perchlorate were markedly elevated to 868 µg/l and 43 mg/g creatinine, respectively, during perchlorate exposure. Radiolabelled iodine uptakes by the thyroid were significantly decreased by 38% in workers during three 12 night shifts compared with workers after 3 days off (13.5% versus 21.5%) and were associated with a significant increase in urinary iodine excretion (230 µg of iodine per gram creatinine versus 148 µg of iodine per gram creatinine), but were not significantly different from those in the control group (14.4%). Serum TSH and thyroglobulin concentrations were normal and similar in the three groups. Serum T_4 (8.3 µg/dl versus 7.7 µg/dl), free T_4 index (2.4 versus 2.2) and total T_3 (147 ng/dl versus 134 ng/dl) were slightly but significantly increased in the workers during 12 h night shifts compared with the workers after 3 days off. Thyroid volumes and patterns by ultrasound were similar in the 29 workers and 12 community volunteers.

The authors concluded that high perchlorate absorption during 3 nights of work exposure decreased the 14 h radiolabelled iodine uptakes by the thyroid by 38% compared with those in workers after 3 days off. However, serum TSH and thyroglobulin concentrations and thyroid volume by ultrasound were not affected by perchlorate, suggesting that long-term, intermittent, high exposure to perchlorate does not induce hypothyroidism or goitre in adults (Braverman et al., 2005).

The same group conducted a clinical study in volunteers that was designed to assess the effects of long-term exposure to perchlorate and to counter criticism (Braverman, 2007) that the workers in the study described above were not exposed continuously to perchlorate. The effect of 6 months of administration of perchlorate on thyroid function was studied in a prospective, double-blind, randomized trial. The study population consisted of 13 healthy volunteers (9 women, 4 men), and responses in individuals on placebo (4 subjects) were compared with those administered 0.5 mg (5 subjects) or 3.0 mg (4 subjects) of potassium perchlorate daily. Perchlorate and placebo were administered orally once daily by capsule. Assuming an average daily water ingestion of 2 litres, the 3.0 mg dose corresponded to a perchlorate concentration of approximately 1500 µg/l and the 0.5 mg dose to 250 µg/l. Assuming a body weight of 70 kg, these doses are equivalent to 0.04 and 0.007 mg/kg bw per day as potassium perchlorate. Serum thyroid function tests, 24 h radiolabelled iodide uptake, serum thyroglobulin, urinary iodine and perchlorate, and serum perchlorate were measured. Serum perchlorate was not detected in baseline samples or in those subjects receiving placebo. Serum perchlorate was detected in subjects receiving perchlorate, averaging, over the 6 months, 24.5 ± 16 µg/l in the 0.5 mg perchlorate group and 77.9 ± 18.2 µg/l in the 3.0 mg group. Urinary perchlorate was detected at low levels at baseline in all 13 subjects, averaging 9.2 ± 5.7 µg per 24 h or 7.3 ± 5.5 µg/g creatinine. It remained low in the subjects receiving placebo. The mean urinary perchlorate values during ingestion of 0.5 mg perchlorate daily were 332.7 ± 66.1 µg per 24 h or 248.5 ± 64.5 µg/g creatinine; during ingestion of 3.0 mg perchlorate daily, the values were 2079.5 ± 430.0 µg per 24 h or 1941.7 ± 138.5 µg/g creatinine. There was no significant change in the thyroid radioiodide uptakes during perchlorate administration. There were no significant changes in serum T_3, free T_4 index, TSH or thyroglobulin concentrations during the exposure period, compared with baseline or post-exposure values. Urinary iodine values for the 3.0 mg perchlorate group were higher, but not significantly so, at baseline than during perchlorate exposure. The authors acknowledged the small sample size (further recruitment to the study had been prevented by adverse publicity), concluding that a 6-month exposure to perchlorate at doses up to 3.0 mg/day was without any effect on thyroid function (Braverman et al., 2006). It should be noted that the serum perchlorate values in this volunteer clinical study were considerably lower than those obtained in the worker exposure study by the same group described previously (Braverman et al., 2005) and, in contrast to that study, were not sufficient to affect radiolabelled iodine uptake by the thyroid.

The potential relationship between urinary levels of perchlorate and serum levels of TSH and total T_4 in men and women 12 years of age and older participating in NHANES during 2001–2002 was evaluated. Multiple regression models that

included perchlorate and covariates known to be or likely to be associated with T_4 or TSH levels were used (i.e. age, race or ethnicity, body mass index, estrogen use, menopausal status, pregnancy status, premenarche status, serum C-reactive protein, serum albumin, serum cotinine, hours of fasting, urinary thiocyanate, urinary nitrate and selected medication groups). Perchlorate was not a significant predictor of T_4 or TSH levels in men, and the main analysis presented was for the 1111 women in the survey. The geometric mean value for urinary perchlorate concentration in the women was 2.84 µg/l (95% CI 2.54–3.18 µg/l). For women overall, perchlorate was a significant predictor of both T_4 and TSH. Urinary perchlorate was associated with an increased TSH and decreased total T_4 in women 12 years of age and older in the general population in the USA with urinary iodine levels below 100 µg/l (a potentially susceptible subpopulation). For women with urinary iodine levels at or above 100 µg/l, urinary perchlorate was a significant predictor of TSH but not T_4. The authors concluded that these effects of perchlorate on T_4 and TSH were coherent in direction and independent of other variables known to affect thyroid function, but were found at lower perchlorate exposure levels than were anticipated, based on previous studies (Blount et al., 2006b).

Others have pointed out that analysis of women of reproductive age from the same NHANES data set, but using creatinine-adjusted urinary iodide concentrations, found no association between T_4 and urinary perchlorate concentrations (Lamm et al., 2007). Similarly, a survey from Europe also found no correlation between urinary perchlorate concentrations and TSH or free T_4 among pregnant women with urinary iodide concentrations below 100 µg/l (Pearce, Lazarus & Smythe, 2007), whereas analysis of a cohort of women from Chile also found no significant associations between serum free T_4 or TSH concentrations and urinary perchlorate, whether expressed as micrograms per litre or as micrograms per gram of creatinine (Gibbs & Van Landingham, 2008).

In a further analysis of data from the NHANES 2001–2002 population, the impact of smoking and thiocyanate on the relationship between urinary perchlorate and serum T_4 and TSH was assessed. In women with urinary iodine levels below 100 µg/l, the association between the logarithm of urinary perchlorate concentration and decreased T_4 was greater in smokers (regression coefficient = −1.66, $P = 0.0005$) than in non-smokers (−0.54, $P = 0.04$). In subjects with high, medium and low cotinine levels, these regression coefficients were −1.47 ($P = 0.0002$), −0.57 ($P = 0.03$) and −0.16 ($P = 0.59$). For high, medium and low thiocyanate tertiles, they were −1.67 ($P = 0.0009$), −0.68 ($P = 0.09$) and −0.49 ($P = 0.11$). Clear interactions between perchlorate and smoking were not seen with TSH or with T_4 in women with urinary iodine levels at or above 100 µg/l or in men. The authors concluded that these results suggest that thiocyanate in tobacco smoke and perchlorate interact in affecting thyroid function, and this effect can take place at commonly occurring perchlorate exposures (Steinmaus, Miller & Howd, 2007).

The potential effects of environmental inhibitors of iodide uptake by the NIS, other than perchlorate, have also been considered from the basis of a literature search performed on articles published up to early 2006 on perchlorate, iodide, nitrate and thiocyanate. Using robust data from Tonacchera et al. (2004) on the relative potencies of perchlorate, nitrate and thiocyanate with respect to inhibition

of iodide uptake and the maximum contaminant levels allowed in the USA for nitrate and cyanide in drinking-water, the authors concluded that perchlorate levels in drinking-water would account for only 10% of the resulting iodine uptake inhibition from the presence of these three inhibitors in drinking-water and a negligible proportion with respect to exposure from food (De Groef et al., 2006).

(b) Benchmark dose calculations from data on non-pregnant adults

BMDs for perchlorate effects on TSH and T_4 have been calculated from three of the studies described above—the two occupational cohorts with long-term exposure to perchlorate (Lamm et al., 1999; Braverman et al., 2005) and the clinical study of volunteers exposed to perchlorate for 2 weeks (Greer et al., 2002). Two serum indicators, TSH and free T_4 index, were used in the calculation of BMDs. The BMD for each serum indicator was defined as the dose predicted to cause an additional 5% or 10% of persons to have a serum measurement outside of the normal range. Using the data from the clinical study, the half-life of perchlorate in serum was estimated to be 7.5 h and the volume of distribution 0.34 l/kg. Using these estimates and measurements of perchlorate in serum or urine, doses in the occupational cohorts were estimated and used in benchmark calculations using a non-linear model. Because none of the three studies found a significant effect of perchlorate on TSH or free T_4, all of the BMD estimates were indistinguishable from infinity. The lower 95% statistical confidence limit on the benchmark dose (BMDL) estimated from a combined analysis of the two occupational studies ranged from 24 to 50 mg/day, corresponding to 0.21–0.56 mg/kg bw per day, for free T_4 index and from 57 to 83 mg/day, corresponding to 0.36–0.92 mg/kg bw per day, for TSH. Corresponding estimates from the short-term clinical study were within these ranges (Crump & Gibbs, 2005). Crump & Gibbs (2005) argued that although none of the three studies they utilized found evidence of an effect of perchlorate on thyroid function, BMDLs calculated from such negative results nevertheless represent valid statistical lower bounds on the dose that accounts for a potential, but unobserved, effect of perchlorate. They did acknowledge, however, that BMDLs based on negative data could possibly be highly conservative.

Another BMD analysis based on the Greer et al. (2002) study was conducted by ICF Consulting (2004). BMDLs were calculated from data on free T_4, total T_4 and T_3. The thyroidal measurements for an individual at each of three measurement times (morning, midday and afternoon) on day 14 minus the average of his or her measurements on 2 pre-exposure days and 1 post-exposure day were modelled. For each of these nine cases, BMDLs were calculated corresponding to 5%, 10% or 20% change in response. This resulted in 27 BMDLs ranging from 7 to 89 mg/day. The nine BMDLs calculated for free T_4 correspond to a range of 8–76 mg/day, which contains the range of BMDLs for free T_4 obtained in the analysis by Crump & Gibbs (2005). Crump & Gibbs (2005) considered that the differences between the ICF Consulting (2004) results and their analysis for free T_4 were likely due largely to the decisions by ICF Consulting (2004) to segment the data by time of day (compared with their approach of using all the data and controlling for time of day) and to exclude the data from the uptake study. The ICF Consulting (2004) analysis also employed a different definition of the BMD than was used in the Crump

& Gibbs (2005) analysis and employed a linear model as opposed to the non-linear model employed by Crump & Gibbs (2005).

Crump & Gibbs (2005) also discussed other BMD analyses that have been published for perchlorate based on human data, all of which used the Greer et al. (2002) study of perchlorate consumption by volunteers. The analysis performed by the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (CalEPA, 2004; Ting et al., 2006) modelled the ratio of the radiolabelled iodine uptake after perchlorate exposure to the baseline value and defined the BMD as the dose corresponding to a 5% change in this ratio. CalEPA (2004) calculated a BMDL of 0.0037 mg/kg bw per day, which, in a 70 kg individual, is equivalent to 0.26 mg/day. This value is much lower than the BMDLs obtained in the analysis by Crump & Gibbs (2005), either from the Greer et al. (2002) study or from the occupational studies (Lamm et al., 1999; Braverman et al., 2005); however, this is not surprising, given that the former were based on inhibition of iodine uptake by the thyroid, whereas the latter were based on TSH and T_4. Crump & Gibbs (2005) commented that the main reason for their different approach is that, unlike CalEPA (2004) and the USEPA, they did not consider a reduction in uptake of radiolabelled iodine, without accompanying changes in TSH and T_4, to be an adverse response.

(c) Pregnant adults, fetuses and neonates

In 1997, perchlorate was detected in the drinking-water supplies of six counties in California, USA, and one in Nevada, USA, at concentrations of 4–16 µg/l. In a series of ecological investigations, the incidence of congenital hypothyroidism and thyroid function (blood T_4 and TSH) in newborns and school performance and the incidence of neurobehavioural diseases in children were assessed in exposed or unexposed populations from these two states in the USA. The incidence of thyroid diseases in exposed and unexposed populations in Nevada was also investigated.

In the first part of the investigation, data on nearly 700 000 newborns from the seven counties in California and Nevada in which perchlorate was detected in the drinking-water were obtained from the neonatal screening programmes of the state health departments during 1996 and 1997. They were analysed for any increased incidence of congenital hypothyroidism in those counties compared with that expected for the whole state. In all, 249 cases were identified, compared with 243 expected, giving an overall risk ratio of 1.0 (95% CI 0.9–1.2). The risk ratios for the individual counties ranged between 0.6 and 1.1. The data in this analysis did not indicate an increase in the incidence of congenital hypothyroidism associated with the reported perchlorate levels (Lamm & Doemland, 1999).

In the second part of the investigation, neonatal blood T_4 levels on days 1–4 of life during the period April 1998 through June 1999 were compared for 17 308 newborns from the city of Las Vegas, Nevada, which had perchlorate in its drinking-water, and 5882 newborns from the city of Reno, Nevada, which did not (detection limit 4 µg/l). Perchlorate levels in drinking-water in Las Vegas measured monthly during this study period ranged from non-detectable for 8 months to levels of 9–15 µg/l for 7 months. No significant difference was found in the mean T_4 levels of the

newborns from these two cities (crude analysis: 17.11 µg/dl for Las Vegas versus 17.22 µg/dl for Reno). The authors noted that the study was sufficiently sensitive to detect the effects of sex, birth weight and the day of life on which the blood sample was taken on the neonatal T_4 level, but it detected no effect from environmental exposures to perchlorate that ranged up to 15 µg/l (Z. Li et al., 2000).

In the third part of the investigation, neonatal blood TSH levels were studied in the same two populations of Las Vegas and Reno over a similar time period, from December 1998 to October 1999. The blood samples from neonates within the birth weight range 2500–4500 g were taken during the first month of life, excluding the first day of life. No significant difference was found in TSH levels between 540 newborns from Las Vegas (geometric mean ± standard deviation [SD] 11.5 ± 1.3 micro-international units [IU] per millilitre) and 130 newborns from Reno (12.5 ± 1.3 µIU/ml), analysed by multiple linear regression on log-transformed data, controlling for the potential confounders of sex and age in days. As TSH levels were measured in neonates only in the lowest 10% of the distribution of blood T_4 levels, this analysis was considered to represent a sensitive subpopulation with respect to the possibility of raised TSH levels (F.X. Li et al., 2000).

In the fourth part of the investigation, school performance and the incidence of attention deficit hyperactivity disorder and autism were assessed in children from exposed and unexposed populations in Nevada. The exposed population of 39 084 children from Clark County, Nevada, which includes the city of Las Vegas, was compared with 8471 unexposed urban controls from Washoe County, which includes the city of Reno, and 7859 rural controls from the rest of the state. Drinking-water supplied to Clark County between July 1997 and May 2002 had a mean perchlorate level of 10.9 µg/l (10th to 90th percentile range 7–15 µg/l). Nevada Medicaid records for children under 18 years of age for the years 1996–2000 were accessed. School performance in fourth graders was ascertained from the results of standardized tests that were administered nationwide. There were no differences between the exposed population and the urban or rural control populations in the incidence of neurobehavioural diseases or in school performance (Chang et al., 2003). Assuming a daily ingestion of water of 2 litres for adults and 1.5 litres for schoolchildren, exposures for the exposed populations in the above investigations were estimated to be 10–32 µg/day for adults and 7.5–24 µg/day for schoolchildren.

In the fifth part of the investigation, an analysis of all users of the Medicaid programme in Nevada was undertaken to determine whether there was an increase in the prevalence of any thyroid disease associated with a perchlorate level of 4–24 µg/l in the water supply of Clark County (including Las Vegas), Nevada. The overall prevalence of thyroid disease, of specific thyroid diseases (goitre, nodule, thyrotoxicosis, congenital hypothyroidism, acquired hypothyroidism, thyroiditis and other thyroid disorders) and thyroid cancer among the Medicaid-eligible population of each county was calculated for the 2-year period 1997–1998. Data from residents in Clark County (population 122 519 exposed to perchlorate) were compared with those from residents in Washoe County (population 29 622 not exposed to perchlorate), the second most populous county in the state, and with those for the rest of the state (also not exposed to perchlorate). There was no evidence of any increase in rate for overall thyroid disease or of any specific thyroid disease, including thyroid cancer, associated with perchlorate exposure (Li, Squartsoff & Lamm, 2001).

Studies were conducted using data on congenital hypothyroidism and neonatal blood TSH levels from the California Newborn Screening Program from 1983 to 1997. Neonates from an exposed population in Redlands (15 090 newborns) were compared with those from unexposed populations in San Bernadino and Riverside counties (685 161 newborns). The adjusted prevalence ratio for congenital hypothyroidism in the study community of Redlands compared with San Bernardino and Riverside counties combined was 0.45 (95% CI 0.06–1.64). The odds ratios for elevated TSH concentration for Redlands compared with San Bernardino and Riverside were 1.24 (95% CI 0.89–1.68) among all newborns screened and 0.69 (95% CI 0.27–1.45) for newborns whose age at screening was 18 h or greater. There were no differences between the study population and control populations in congenital hypothyroidism or TSH levels (Kelsh et al., 2003).

The above study was expanded using data from the California Newborn Screening Program for 1998 to examine the prevalence of congenital hypothyroidism and TSH levels for all California newborns whose mothers resided in communities where water supplies were tested for perchlorate in 1997 and 1998. Thyroid function results for newborns from 24 communities with average perchlorate concentrations in drinking-water above 5 µg/l ($n = 50\,326$) were compared with those for newborns from 287 communities with average concentrations at or below 5 µg/l ($n = 291\,931$). All adjusted odds ratios were controlled for sex, ethnicity, birth weight and multiple birth status. Fifteen cases of congenital hypothyroidism from communities with average concentrations above 5 µg/l were observed, with 20.4 expected (adjusted prevalence odds ratio [POR] = 0.71; 95% CI 0.40–1.19). For newborns screened at or after 24 h, the adjusted POR for high TSH was 0.73 (95% CI 0.40–1.23). This study did not find an association between estimated average perchlorate concentrations above 5 µg/l in drinking-water supplies and the prevalence of clinically diagnosed congenital hypothyroidism or high TSH concentrations (Buffler et al., 2006).

Another study examined whether exposure to drinking-water contaminated by perchlorate affected neonatal TSH levels, using data from the Arizona Newborn Screening Program between October 1994 and the end of 1997. The exposed population comprising 1099 newborns was from Yuma, where drinking-water, supplied from the Colorado River below Lake Mead, is contaminated by ammonium perchlorate. The unexposed population comprising 433 newborns was from Flagstaff, which was supplied by drinking-water that had not been contaminated by perchlorate. Data on perchlorate levels in drinking-water during the study period itself were not available, but concentrations of 6 µg/l were measured in water supplied to Yuma in 1999, whereas perchlorate was undetectable in water supplied to Flagstaff. TSH levels in newborns from Yuma, after adjusting for age in days at measurement and for race or ethnicity, were significantly higher than levels in newborns from Flagstaff (Yuma median 19.9 mIU/l, interquartile range 12.5–28.3 mIU/l; Flagstaff median 13.4 mIU/l, interquartile range 8.8–18.9 mIU/l). This ecological study demonstrated a statistically significant association between perchlorate exposure and newborn TSH levels. The authors concluded that it suggests that even low-level perchlorate contamination of drinking-water may be associated with adverse health effects in neonates (Brechner et al., 2000).

This study is notable in that it is the only study to report an effect of low-level exposure to perchlorate on thyroid function in newborns. Others have commented on these findings, pointing out that there were marked imbalances in age at sampling and suggesting that they could have resulted from variation in medical practices, demographic factors or other geographic factors, rather than differences in perchlorate exposures between the two cities of Flagstaff and Yuma (Goodman, 2001; Lamm, 2003).

A study was conducted in three coastal cities in Chile to investigate whether perchlorate in drinking-water affected thyroid function in newborns and school-aged children. Drinking-water in the city of Taltal had a mean perchlorate concentration of 112 µg/l (range 100–120 µg/l). In the city of Chanaral, perchlorate was not detected (limit of detection [LOD] 4 µg/l) in 4 out of 25 samples, and concentrations in the other 21 samples averaged 6.2 µg/l (range 5.3–6.7 µg/l). In the city of Antofagasta, perchlorate was undetectable. Neonatal screening records were obtained for a total of 9784 neonates born in the three cities between February 1996 and January 1999. A total of 162 children 6–8 years of age were studied; of these, 127 had lived in their city since birth, and their mothers had lived in their city for the year preceding their birth. The study found no evidence that perchlorate in drinking-water affected thyroid function in newborns, adjusted for age, or in school-aged children, as assessed by TSH or free T_4 levels. No cases of congenital hypothyroidism were detected in the cities with perchlorate in the drinking-water, and the prevalence of goitre among the school-aged children was similar in the three cities (Crump et al., 2000).

In a subsequent longitudinal study conducted between November 2002 and April 2004 in the same three cities in Chile, thyroglobulin, urinary perchlorate, TSH and free T_4 were measured in women during early pregnancy (16.1 ± 4.1 weeks) and late pregnancy (32.4 ± 3.0 weeks) and in cord serum at birth. The mean perchlorate levels were similar to those reported in their previous study (Crump et al., 2000), with mean values of 113.9 µg/l for Taltal, 5.82 µg/l for Chanaral and non-detectable for Antofagasta. Exposure from tap water was calculated from self-reported tap water consumption, which was around 1 litre per day, and additional exposure from the diet was calculated by subtracting this amount from the measured urinary perchlorate levels in micrograms per gram creatinine and an assumed creatinine excretion for pregnant women of 1.08 g/day. Total calculated median perchlorate excretion values were 22.1, 40.0 and 118.8 µg/day for Antofagasta, Chanaral and Taltal, respectively, indicating that 21.7, 33.8 and 25.3 µg/day in the three respective cities came from the diet. Perchlorate was detectable in maternal and cord serum in subjects from Taltal, but was non-detectable in serum samples from the other two cities. The incidence of goitre was higher, ranging up to 36.4%, than the incidence in the USA, which the authors suggested was possibly due to higher iodine levels in the past in Chile. The incidence of goitre in subjects from Chanaral was higher than that in those from Antofagasta or Taltal, but the authors considered this finding unexplainable. There were no significant differences in any thyroid parameters or in neonatal birth weight, head circumference or length between the three cities (Téllez Téllez et al., 2005).

A study in Ramat Hasharon, Israel, in 2004 assessed the effect of gestational perchlorate exposure through drinking-water on neonatal T_4 by comparing newborns from mothers residing in suburbs where drinking-water contained differing levels of perchlorate: ≤340 µg/l (very high exposure, *n* = 97), 42–94 µg/l (high exposure, *n* = 216) and <3 µg/l (low exposure, *n* = 843). In the very high and high exposure areas, T_4 values in newborns whose mothers drank tap water exclusively (as determined by a telephone interview) were analysed as a subset. Serum perchlorate levels in blood from donors residing in the area in 2003–2004 were used as proxy indicators of exposure. Neonatal T_4 values (mean ± SD) in the very high, high and low exposure groups were 13.9 ± 3.8 µg/dl, 13.9 ± 3.4 µg/dl and 14.0 ± 3.5 µg/dl, respectively. Serum perchlorate concentrations in blood from donors residing in areas corresponding to these groups were 5.99 ± 3.89 µg/l, 1.19 ± 1.37 µg/l and 0.44 ± 0.55 µg/l, respectively. This study found no change in neonatal T_4 levels despite maternal consumption of drinking-water that contained perchlorate at levels in excess of the USEPA drinking-water equivalent level (DWEL) of 24.5 µg/l based on the RfD of 0.7 µg/kg bw per day (USEPA, 2005b; Amitai et al., 2007).

2.3.3 Physiologically based pharmacokinetic modelling

The work of Clewell and co-workers (Clewell, Merrill & Robinson, 2001; Clewell et al., 2003a,b, 2004) in animals on PBPK modelling, described in section 2.2.6, laid the foundation for development of a human PBPK model for different life stages in humans. A model developed for adult humans describes the kinetics and distribution of radiolabelled iodide and perchlorate in healthy subjects and simulates the subsequent inhibition of thyroid uptake of iodide by perchlorate. The model was checked against data from published studies on human serum and urinary levels of perchlorate and perchlorate inhibition of uptake of iodide in the thyroid following administration in drinking-water. The model incorporates uptake of iodide and perchlorate into human skin, which serves as a large pool for storage and release of the anions, and from where it is assumed to slowly diffuse back into the systemic circulation. However, the authors concluded that the model was not yet sufficiently developed to predict effects of perchlorate on thyroid hormones (Merrill et al., 2005).

In a subsequent study, the same group modelled iodide and perchlorate kinetics at various life stages: pregnant and lactating woman, fetus and nursing infant. This would allow estimation of dose delivery to critical targets, such as the developing thyroid. They modelled iodide uptake inhibition levels for external (i.e. ingested) doses of perchlorate of 1, 10, 100 and 1000 µg/kg bw per day. At the two lower modelled doses of 1 and 10 µg/kg bw per day, the pregnant and lactating woman, fetus and nursing infant were predicted to have higher blood perchlorate concentrations (by 4- to 5-fold) and greater inhibition of iodide uptake by the thyroid at a given drinking-water concentration of perchlorate than either the non-pregnant adult or the older child. Smaller relative differences were predicted at external doses of 100 and 1000 µg/kg bw per day. The authors attributed this change to saturation of uptake mechanisms. The fetus was predicted to receive the greatest dose (per kilogram body weight) due to several factors, including placental NIS activity and reduced maternal urinary clearance of perchlorate. The model predicted a minimal effect of perchlorate on iodide uptake inhibition in all groups of 1.1% or less at an

external dose of 1 µg/kg bw per day, which is about 1–1.5 times the RfD of 0.7 µg/kg bw per day (USEPA, 2005b). Inhibition was predicted to be 10% or less in all groups at an external dose of 10 µg/kg bw per day, which is about 14 times the RfD (Clewell et al., 2007).

2.3.4 Existing reference doses and drinking-water standards

(a) United States National Academy of Sciences and Environmental Protection Agency

In 1998, the USEPA issued a preliminary risk assessment for perchlorate (USEPA, 1998) and, after revision, issued guidance in 1999 on perchlorate, recommending continued use of a provisional reference dose range of 7–35 µg/day for a 70 kg adult or 0.1–0.5 µg/kg bw per day (USEPA, 1999a). In 2002, the USEPA published a revised assessment for comment, which proposed an RfD of 0.03 µg/kg bw per day (USEPA, 2002). This was based on a weight of evidence approach, in which data from rat developmental neurobehavioural studies provided the critical effect of alteration of offspring brain morphometry (York et al., 2004, 2005a,b). The RfD was derived by application of an uncertainty factor of 300 to the human exposure equivalent of 0.01 mg/kg bw per day obtained using PBPK modelling of the BMD from the developmental neurobehavioural studies. At that time, the USEPA considered that the observational epidemiological and human clinical studies had scientific and technical limitations that precluded their use for derivation of an RfD (USEPA, 2002).

In 2003, the United States National Academy of Sciences (NAS) reviewed the USEPA's 2002 draft health assessment for perchlorate. The report of the NAS was published in 2005 (NRC, 2005). Concerning specific health outcomes, the NAS concluded the following, on the basis of the then available evidence:

- *Congenital hypothyroidism:* All studies of a causal association with perchlorate exposure were negative.
- *Changes in thyroid function in newborns:* The evidence was not consistent with a causal association between perchlorate exposure during gestation to concentrations of perchlorate in drinking-water up to 120 µg/l and effects on TSH or T_4 in newborns. However, it was noted that no data were available on the association of perchlorate exposure with thyroid dysfunction in groups of greatest concern, low birth weight or preterm newborns, offspring of mothers who had iodide deficiency during gestation or offspring of hypothyroid mothers.
- *Neurodevelopmental outcomes:* The evidence is inadequate.
- *Hypothyroidism and other thyroid disorders in adults:* The evidence is not consistent with a causal association between perchlorate exposure and thyroid diseases.
- *Thyroid cancer in adults:* The evidence is insufficient, but the committee questioned the plausibility of thyroid cancer as a likely outcome of perchlorate exposure.

Concerning the derivation of an RfD, the NAS concluded the following: After reviewing the human and laboratory animal data, the committee found that the

human data provided a more reliable point of departure for the risk assessment than the laboratory animal data. The committee recommended using clinical data collected in a controlled setting with the relevant route of exposure to derive the RfD, as it considered that the epidemiological studies, while informative, were limited for the purposes of quantitative risk assessment. The committee also noted that the epidemiological studies typically focused on changes in serum thyroid hormones and TSH concentrations or clinical manifestations of the changes, not on inhibition of iodide uptake by the thyroid, which the committee considered to be the key precursor event to the adverse effect of hypothyroidism and the only effect that has been consistently demonstrated in humans exposed to perchlorate. The committee recommended using the Greer et al. (2002) study, in which groups of healthy men and women were administered perchlorate at 0.007–0.5 mg/kg bw per day for 14 days, and which identified a NOEL for inhibition of iodide uptake by the thyroid of 0.007 mg/kg bw per day, noting that this NOEL was consistent with other clinical studies that have investigated iodide uptake inhibition by perchlorate. The committee considered that using the NOEL value from Greer et al. (2002) would be a health-protective and conservative point of departure that was supported by the results of a 6-month study of a small group of healthy subjects exposed to 0.007 mg/kg bw per day, a 4-week study of healthy subjects exposed to higher doses, the studies of perchlorate treatment of patients with hyperthyroidism, and extensive human and laboratory animal data that demonstrated that there will be no progression to adverse effects if no inhibition of iodide uptake occurs. The committee recommended using a total uncertainty factor of 10, comprising a full intraspecies factor of 10 to protect the most sensitive population—the fetuses of pregnant women who might have hypothyroidism or iodide deficiency—which would lead to an RfD of 0.0007 mg/kg bw per day (i.e. 0.7 µg/kg bw per day). No additional factors were needed for duration or database uncertainties. If inhibition of iodide uptake by the thyroid was used, chronic exposure would have no greater effect than that resulting from short-term exposure (it may even have less effect because of the capacity of the pituitary–thyroid system to compensate for iodide deficiency by increasing iodide uptake). The committee considered the database sufficient, given that the point of departure selected was based on inhibition of iodide uptake by the thyroid. The committee recognized that the use of a non-adverse effect upstream in the pathway of events following exposure to perchlorate to derive an RfD differed from the traditional approach of using an adverse effect, but that such an approach would be conservative and protective of health (NRC, 2005).

Some subsequently commented that the NAS RfD, based on data from iodine-replete, healthy adults, was not sufficiently conservative, arguing that inhibition of iodine uptake should be considered as adverse and that a higher overall uncertainty factor should have been used because of remaining uncertainties in the database, particularly those relating to iodine-deficient, pregnant women and lactational exposure of newborns (Ginsberg & Rice, 2005a,b; Zoeller, 2006). Conversely, others argued that the RfD was overly conservative (Strawson, Dourson & Zhao, 2005). Members of the NAS committee on perchlorate and others responded to these comments and criticisms, emphasizing that the committee's recommended RfD of 0.0007 mg/kg bw per day provided a wide margin of safety for all subjects of all ages (Gibbs, Engel & Lamm, 2005; Johnston et al., 2005).

Following completion of the NAS review (NRC, 2005), the USEPA adopted the NAS-recommended RfD of 0.0007 mg/kg bw per day for perchlorate (USEPA, 2005b). A DWEL of 24.5 µg/l for perchlorate was then set, based on the RfD (USEPA, 2005a,b). This level corresponds to the amount that would deliver the RfD of 0.0007 mg/kg bw per day for a 70 kg adult ingesting 2 litres of water a day.

This same DWEL was also used as a groundwater preliminary remediation goal (PRG) set by the USEPA's Office of Solid Waste and Emergency Response in 2006 (USEPA, 2006). Crawford-Brown, Raucher & Harrod (2006), after applying probabilistic risk analysis to varying intakes of perchlorate in drinking-water of between 1 and 50 µg/l, concluded that maximum contaminant levels of up to 24.5 µg/l should pose little or no incremental risk to the large majority of individuals in the most sensitive subpopulations (pregnant women) exposed in the USA at current levels of perchlorate in water. Ginsberg et al. (2007), however, commented that this PRG is not necessarily protective for nursing and bottle-fed infants who consume more liquid per unit body weight than adults, and it does not include the relative source contribution, a factor normally used to lower the PRG due to non-water exposures. Monte Carlo analysis was used to simulate nursing infant exposures associated with the Office of Solid Waste and Emergency Response PRG when combined with background perchlorate, and these were compared with the RfD. It was estimated that the PRG could lead to a 7-fold increase in breast milk concentration, causing 90% of nursing infants to exceed the RfD by an average 2.8-fold, and that drinking-water perchlorate must be below 6.9 µg/l and 1.3 µg/l to keep the median and the 90th-percentile nursing infant exposures, respectively, below the RfD. The authors also analysed data on urinary excretion of perchlorate from women in the NHANES 2001–2002 survey (Blount et al., 2006a) to assess the dietary (non-water) contribution to perchlorate exposure, and this suggested a relative source contribution of 0.7 for pregnant women and of 0.2 for nursing infants.

In December 2008, the USEPA revised its advice to take account of the contribution to overall perchlorate intake from food (USEPA, 2008a). Using data on urinary perchlorate excretion generated in the NHANES database and data on perchlorate intake from food generated in the United States Food and Drug Administration's (USFDA) Total Diet Study (TDS) (Murray et al., 2008), the 90th-percentile intake from food was calculated to be 38% for pregnant women, leaving 62% for the contribution from drinking-water. Accordingly, the USEPA issued an interim drinking water health advisory of 15 µg/l, which covers pregnant women, infants and developing children (USEPA, 2008a). At the same time, the USEPA announced it was seeking further advice from the NAS, including an evaluation of the interim health advisory value of 15 µg/l, the use of modelling to evaluate impacts on infants and young children, and the implications of recent biomonitoring studies, before making a final determination on whether to issue a national regulation for perchlorate in drinking-water (USEPA, 2009).

(b) Others

Strawson, Zhao & Dourson (2004) considered that the most relevant data for developing an RfD for perchlorate exposure come from human epidemiological and clinical studies, supplemented with available and extensive information on

experimental animals. Based on mode of action analysis and the evidence provided by rodent studies on perchlorate, these authors considered a decrease in serum T_4 to be the critical effect of perchlorate, but noted that no T_4 decreases have been observed in human populations following perchlorate exposure at non-therapeutic doses. They identified a freestanding NOAEL of 0.006 mg/kg bw per day for T_4 decrease in children from the epidemiological study of Crump et al. (2000), supported by the threshold for inhibition of iodine uptake of 0.006 mg/kg bw per day from the study of Greer et al. (2002) as a measurable surrogate for the critical effect of T_4 decrease in humans. They selected an overall uncertainty factor of 3-fold, based on expected differences in toxicokinetics and toxicodynamics between children and pregnant women and their fetuses, to derive an RfD of 0.002 mg/kg bw per day (Strawson, Zhao & Dourson, 2004).

Zoeller & Rice (2004), in commenting on the RfD derived by Strawson, Zhao & Dourson (2004), noted that there were uncertainties in applying a NOAEL obtained in human adults to human neonates, who might be more sensitive to perchlorate because of much lower levels of stored hormones in the neonatal thyroid gland and their requirement for thyroid hormones for normal neurobehavioural development.

Srinivasan & Viraraghavan (2009) summarized drinking-water guidance values and advisory action levels proposed by bodies other than the USEPA, including 10 individual states in the USA and Health Canada. The values range from 1 to 18 µg/l.

2.3.5 Cancer

In response to concerns about the possibility of cancer stemming from drinking-water contaminated with ammonium perchlorate (and trichloroethene), the observed and expected numbers of new cancer cases for all sites combined and 16 individual cancer types in the California (USA) community of Redlands, San Bernardino County, were assessed from 1988 to 1998. Cancer cases in the area of the city of Redlands, which had a population of 54 500–66 400 during the study period, were identified from one of the regional cancer registries in California. Expected numbers of cases were obtained from the overall data in the California Cancer Registry, which gathers data from the five regional cancer registries, including the region in which Redlands is located. Contamination of well water was considered likely from 1980 onwards, but testing for perchlorate in one of the areas covered by the study (Redlands) accompanied improved technology and began only in 1997. Levels of perchlorate in Redlands wells in 2001 ranged from 5 to 98 µg/l, although the city of Redlands did not deliver water to customers containing perchlorate at concentrations in excess of 18 µg/l since testing began. Cancer rates were expressed as standardized incidence ratios (SIRs: observed cases divided by expected cases) and 99% confidence intervals. There was a total of 3098 cancer cases in Redlands during the decade studied. No significant differences were found between observed and expected numbers for all cancers (SIR 0.97; 99% CI 0.93–1.02), thyroid cancer (SIR 1.00; 99% CI 0.63–1.47) or 11 other cancer types. Significantly fewer cases were observed than expected for cancer of the lung and bronchus (SIR 0.71; 99% CI 0.61–0.81) and the colon and rectum (SIR 0.86; 99%

CI 0.74–0.99), whereas more cases were observed for uterine cancer (SIR 1.35; 99% CI 1.06–1.70) and skin melanoma (SIR 1.42; 99% CI 1.13–1.77). The findings in this community did not identify a generalized cancer excess or thyroid cancer excess (Morgan & Cassady, 2002).

The analysis of the Medicaid database from Nevada, USA, described previously found no evidence of any increased incidence of thyroid cancer associated with perchlorate exposure from drinking-water (Li et al., 2001).

3. ANALYTICAL METHODS

3.1 Chemistry

A combination of human activity and natural sources has led to the widespread presence of perchlorate in the environment. Perchlorate is highly soluble and very stable in water. The order of solubility of the common perchlorate salts is sodium > lithium > ammonium > potassium (Mendiratta et al., 1996). Although perchlorate has excellent oxidizing ability, the activation energy required to initiate the chemical reaction is very high. The high activation energy and solubility of the salts lead to perchlorate's stability and mobility in the environment.

3.2 Description of analytical methods

Perchlorate is soluble in water and polar organic solvents and is easily extracted from foods using either water or water–acetonitrile mixtures. Water samples are analysed directly, whereas food extracts are subjected to solid-phase extraction (SPE) cleanup prior to determination. Analytical methods used for the detection and determination of perchlorate levels in water and foods include ion chromatography (IC) with conductivity detector, IC coupled to mass spectrometry (IC-MS or IC-MS/MS) and liquid chromatography (LC) coupled to mass spectrometry (LC-MS or LC-MS/MS). IC-MS/MS has significantly improved sensitivity, and the LODs are reported to be in the range of 0.5–5 ng/l for water and 1–15 ng/g for various food matrices.

3.2.1 Screening tests

Development of screening tests for the detection of trace levels of perchlorate in foods has been rather limited. Before 1997, the detection methods for perchlorate were mainly gravimetric and spectrophotometric methods. The LOD for these methods was higher than 100 µg/l, and trace amounts of perchlorate in groundwater were undetectable (Zhou, 2007). Other methods, such as capillary electrophoresis (Ellington et al., 2001), colorimetry (Thorne, 2004) and use of biological sensors (Okeke et al., 2007), have sensitivity limitations. The perchlorate-selective electrode developed using a submicrometre-thick plastic membrane coated on a poly(3-octylthiophene)-modified gold electrode gave an LOD in the range of 20–50 ng/l for purified water, bottled water and tap water. Surface-enhanced Raman scattering was used in a method by Gu, Ruan & Wang (2009) to measure perchlorate at an LOD of 0.1 µg/l. When coupled to a portable Raman spectrometer, this technique has the potential to be used as an in situ, rapid

screening tool for perchlorate in the environment. However, method validation has not been carried out.

3.2.2 Quantitative methods

Analysis of perchlorate at trace and ultra-trace levels in complex food matrices has become possible with the development of chromatographic and mass spectrometric techniques. At present, IC with conductivity detector or IC coupled to MS is commonly used for the determination of perchlorate in drinking-water. Tandem mass spectrometers (MS/MS) coupled to either IC or LC offer lower LODs for the analysis of complex food matrices. Stable isotope–labelled perchlorate ([^{18}O]perchlorate) is used as an internal standard to enhance the accuracy of the method (USFDA, 2005; Oldi & Kannan, 2009b).

Water samples are directly analysed after centrifugation or filtration (USFDA, 2005). Fruits and vegetables are chopped and freeze-dried, then extracted with water using an ultrasonic bath or under boiling conditions. A 1% acetic acid solution is also used to prevent perchlorate retention on graphitized carbon during the SPE cleanup. After centrifugation, the supernatant is cleaned on SPE (C8, C18, RP, Oasis HLB or graphitized-carbon black cartridge), and metal ions are removed by ion exchange or silicon, aluminium or silver adsorbents (USFDA, 2005; Li & George, 2006; Zhang et al., 2007a). An accelerated solvent extraction (ASE) method has been developed for extraction of perchlorate. Grains such as wheat and corn are powdered, homogenized and extracted with water, and acetonitrile is added to precipitate protein.

An IC-MS method has been developed for the determination of perchlorate in milk based on precipitation of proteins with acetonitrile, resulting in satisfactory recoveries at different spiking levels (Zhang et al., 2007b). Frozen ethanol is also used in the extraction of perchlorate from milk, followed by SPE cleanup (Kirk et al., 2003, 2005). The interferences in milk can also be removed by centrifuging multiple times without adding any solvent (Dyke et al., 2006); however, the procedure is time-consuming.

Analytical methods for the determination of perchlorate in animal tissues are rather limited. Anderson & Wu (2002) evaluated a method in which homogenized samples are fortified with an internal standard, dried at 65 °C, extracted with water using ASE and recovered after cleanup on different SPE cartridges. Perchlorate in whole fish, fish heads and fillets was detected following this extraction method and using the Alumina-B and Bakerbond silica gel SPE cartridges for the further cleanup (Theodorakis et al., 2006).

An IC method using conductivity detector has been adopted as the standard method for the determination of perchlorate in water (EPA Methods 314.0 and 314.1; USEPA, 1999b, 2005c). The LOD of EPA Method 314.0 is 0.53 µg/l, and the method reporting limit is 4.0 µg/l (USEPA, 1999b). EPA Method 314.1 is suitable for the determination of perchlorate in waters with a wide range of total dissolved solids, and method reporting limits are in the range of 0.5–1 µg/l. Owing to the nonspecific property of conductivity detectors, false positives may occur if perchlorate is not properly separated (Mathew, Gandhi & Hedrick, 2005). Two-dimensional IC enhanced perchlorate separation, and an anion-suppressed conductivity detection gave an LOD of 55 ng/l (EPA Method 314.2; USEPA, 2008b).

In IC-MS (and MS/MS) methods, predominant ions detected are $^{35}ClO_4^-$ and $^{37}ClO_4^-$ (*m/z* 99 and 101, respectively), as well as the ^{18}O isotope–labelled internal standard ($Cl^{18}O_4^-$). Ion suppression is a common problem in electrospray ionization (ESI) or atmospheric pressure ionization (API). Mobile phases consisting of non-volatile buffers are usually avoided, or suppressors unique to IC are used to avoid the problem. LODs are in the range of 50–100 ng/l (Mathew, Gandhi & Hedrick, 2005). To avoid inorganic salt buildup, an anion self-regeneration is employed (Mathew, Gandhi & Hedrick, 2005; Mou, Liu & Ding, 2005). Perchlorate in water samples is determined using IC-MS coupled with suppressor (Tan et al., 2004). Valve switching is also used to reduce interferences introduced into the MS detector. An LOD of 0.1 µg/l has been achieved for milk using IC-MS or IC-MS/MS (ITRC, 2005). Ion transition in the tandem MS (99>83, 101>85, 107>89 and 109>91) significantly improves sensitivity and selectivity. Derivation of perchlorate with 1,12-bis(trimethylammonium) dodecane (DQ) difluoride offers higher molecular weight ions ([$DQ^{35}Cl^{16}O_4^+$, *m/z* 384.8], [$DQ^{37}Cl^{16}O_4^+$, *m/z* 386.8], [$DQ^{35}Cl^{18}O_4^+$, *m/z* 393.0], [$DQ^{37}Cl^{18}O_4^+$, *m/z* 395.0]), which helps in eliminating background interferences and improving sensitivity (Martinelango et al., 2005; Martinelango, Gumus & Dasgupta, 2006).

Currently, high-performance liquid chromatography (HPLC) coupled to tandem MS has become the main method for perchlorate analysis. Specific IC or C18 columns are used to detect perchlorate (Li & George, 2006). A rapid, sensitive and specific LC-MS/MS using an IC column has been developed for the determination of perchlorate in selected foods, such as lettuce, milk and bottled water (Alexander, Richard & David, 2004; USFDA, 2005). Perchlorate was detected under ESI negative mode, while an internal standard ($Cl^{18}O_4^-$) was used to correct the matrix effects. This method has a wide range of LODs in different matrices. LODs of LC-MS (selected ion mode) and LC-MS/MS (multiple ion reaction mode), using IonPac AS21 columns, were found to be 0.005 µg/l and 0.008 µg/l, respectively (EPA Method 331.0; USEPA, 2005d). LC-ESI-MS/MS has also been used for the determination of perchlorate in water (Soukup-Hein et al., 2007).

3.2.3 Summary

IC-MS and IC-MS/MS methods are used in most surveys conducted for the determination of perchlorate levels in foods and drinking-water. Stable isotope–labelled derivatives are used as internal standards. Currently, certified reference materials are not available, and collaborative method validation studies have not been conducted. A proficiency testing programme is available for potable water only (Massachusetts Department of Environmental Protection, 2004). Other methods, such as spectrophotometry, capillary electrophoresis and ion-selective electrode–based potentiometric methods, lack in sensitivity and may not be suitable for detection at lower levels in foods and water. Rapid screening methods have not yet been developed.

4. SAMPLING PROTOCOLS

Parallel experiments conducted in foods with six individual samples and one composite sample have shown a correlation between the mean value for the six

individual food samples and that of the composite samples, which indicates that common sampling protocols using representative samples appear to be adequate (Wang et al., 2009). This approach has been adopted for analysis of perchlorate in the TDSs in the USA and China and for analysis of human breast milk samples.

5. EFFECTS OF PROCESSING

Perchlorate is stable in the environment, for at least 300 days in groundwater and 90 days in surface water samples. Limited data are available on the fate of perchlorate during food processing. However, perchlorate is stable during whipping, grinding, heating and cooking processes.

6. PREVENTION AND CONTROL

Recent studies have shown that plant species are able to absorb perchlorate from soil and irrigation water. Elevated levels of perchlorate have been found in food and crops (Sanchez et al., 2006, 2008), including lettuce (Sanchez et al., 2005a,b), fish (Park et al., 2005), seaweed (Martinelango, Tian & Dasgupta, 2006; Snyder et al., 2006) and some beverages (Aribi et al., 2006). Perchlorate accumulation in leafy vegetables was found to be higher than that in root vegetables (Tan et al., 2004; Jackson et al., 2005). Hutchinson (2004) found perchlorate accumulated in lettuce during early growth stages under glasshouse conditions. Another potential source of perchlorate in water supplies is the degradation of sodium hypochlorite, used for disinfection of water, if it is stored for long periods and under less than optimal conditions, including high temperature, storage in the light and storage in containers that contain small amounts of old hypochlorite (Greiner et al., 2008). Perchlorate in drinking-water cannot be removed by conventional water treatment processes (Richardson, 2007).

Reduction of perchlorate in foods and water relies mainly on the control of contamination in fertilizers, irrigation systems and water used in food processing and preparation. However, washing can reduce surface contamination in vegetables and fruits.

7. LEVELS AND PATTERNS OF CONTAMINATION IN FOOD COMMODITIES

7.1 Surveillance data

The Committee, at its present meeting, reviewed data from six countries on the occurrence of perchlorate in water and various foods analysed between 2004 and 2009 (Tables 1 and 2). To harmonize the national occurrence data, all data below the limit of reporting (LOR) (below the LOD or limit of quantification [LOQ] where the percentage of non-quantified values was less than 60% for major contributing foods) have been assigned as follows: data below LOD = ½ LOD and data below LOQ = ½ LOQ, following the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) guidelines (GEMS/Food-EURO, 1995).

Table 1. Summary of perchlorate occurrence data from various countries, 2004–2009

Region	Country	Food		Water	
		Number of analytical results	% of values below LOR	Number of analytical results	% of values below LOR
Asia	China[a]	92	0	83	40
	India[b]	—	—	66	24
	Japan[c]	209	7	50	28
	Republic of Korea[d]	—	—	146	0
North America	Canada[e]	500	26	—	—
	USA[f]	1 065	41	34 728	98
Total		**1 866**	**—**	**35 073**	**—**

[a] China submitted data from pooled samples. Human milk data from China have not been included in the table.
[b] Data for India are from the literature; LOR for drinking-water is 0.02 µg/l.
[c] Japan submitted data on food; data on water were both from submitted information and from the literature.
[d] Data for the Republic of Korea are from the literature; LOR for drinking-water is 0.05 µg/l.
[e] Canada submitted 150 data on food, and a further 350 data were taken from the literature.
[f] USA food data are from exploratory surveys (2004–2005) and TDS (2005–2006); LOR for drinking-water is 4 µg/l.

7.2 National occurrence data

7.2.1 Canada

Canada submitted the results of a survey conducted in 2006 to establish the prevalence of perchlorate in vegetables and fruits, including lettuce, carrot and apple (Health Canada, 2009; Wang et al., 2009). Perchlorate concentrations in 150 samples collected from retail outlets as well as fast food outlets in Ottawa were analysed using the IC-MS/MS technique (LOD and LOQ were 0.3 µg/kg and 3 µg/kg, respectively), and 26% of values were below the LOR. Perchlorate levels were found to be in the range from less than the LOD to 536 µg/kg. Perchlorate levels (mean ± SD) were reported in cantaloupes from Guatemala (156 ± 232 µg/kg), spinach from the USA (133 ± 24.9 µg/kg), green grapes from Chile (45.5 ± 13.3 µg/kg) and romaine lettuce from the USA (29.1 ± 10.5 µg/kg). Perchlorate levels appeared to vary with the type of commodity and its country of origin (Wang et al., 2009). In another study, perchlorate levels were found in over 350 samples of foods and beverages, with concentrations ranging from not detected (ND) to 5.098 µg/l in water samples, from 0.047 to 463.5 µg/kg in food products, from 0.013 to 50.25 µg/l in wine samples, from 0.005 to 21.096 µg/l in beer as well as from ND to 4.795 µg/l in beverage products (Aribi et al., 2006).

Table 2. Occurrence of perchlorate in foods and water from different countries

Description	Country, sample	Number of samples	Number of samples with concentrations higher than LOD	Mean (range) (µg/l or µg/g)	Test method	LOD[a]	Reference
Water	Canada			<1	NA	NA	Health Canada (2008)
	China	54	36	0.2–6.8	IC	0.20 µg/l	Liu et al. (2004)
	China, bottled water	29	14	0.25 (0.037–2.013)	IC-MS/MS	2.0 ng/l (MDL)	Shi & Zhang (2007)
	India	66		<0.02–6.9	LC-MS/MS	0.006 µg/l	Kannan et al. (2009)
	Japan	20	6	0.14–0.35	IC-MS/MS	0.03 ng/g	Japan Ministry of Health, Labour and Welfare (2009)
	Japan, tap water samples	30	30	0.06–37	IC-MS/MS	0.05 µg/l (MRL)	Kosaka et al. (2007)
	Republic of Korea, tap water	146	146	0.15–35	IC; LC-MS/MS	5.0 µg/l; 0.05 µg/l (MRL)	Quiñones et al. (2007)
	United Kingdom	20 sites		Low levels	NA	NA	Blake et al. (2009)
Vegetables Lettuce	Canada	18	18	11.5 (1.27–46.7)	IC-MS/MS	~0.5 µg/kg	Health Canada (2009); Wang et al. (2009)
	Japan	29	29	3 (0.3–21.1)	IC-MS/MS	0.1 µg/kg	Japan Ministry of Health, Labour and Welfare (2009)

Table 2 (contd)

Description	Country, sample	Number of samples	Number of samples with concentrations higher than LOD	Mean (range) (µg/l or µg/g)	Test method	LOD[a]	Reference
	USA	137		10.3 (0.3–129)	IC-MS/MS	1 µg/kg	US FDA (2007b)
	USA	63		7.4	NA	NA	Sanchez et al. (2005a)
	USA	24		8	NA	NA	Sanchez et al. (2005b)
	USA	69		16.5	NA	NA	Sanchez et al. (2005a)
	USA	24		33	NA	NA	Sanchez et al. (2005b)
	USA	8		2.1	NA	NA	USFDA (2009)
			Weighted mean	11.6			
Spinach	Canada	18	18	133 (99–175)	IC-MS/MS	~0.5 µg/kg	Wang et al. (2009)
	Japan	13	13	5.3 (0.6–20.5)	IC-MS/MS	0.1 µg/kg	Japan Ministry of Health, Labour and Welfare (2009)
	USA	36		115 (5.94–927)	IC-MS/MS	1 µg/kg	USFDA (2007b)
	USA	10		85.1	IC-MS/MS	1 µg/kg	Sanchez et al. (2005a)
			Weighted mean	111 (<1–927) (Japan not included)			

Table 2 (contd)

Description	Country, sample	Number of samples	Number of samples with concentrations higher than LOD	Mean (range) (µg/l or µg/g)	Test method	LOD[a]	Reference
Potato	Canada	24	7	1 (<0.4–2.2)	IC-MS/MS	0.39 ± 0.28 µg/kg	Health Canada (2009); Wang et al. (2009)
	USA	60		7.52	IC-MS/MS	1 µg/kg	USFDA (2009)
	USA	6		0.15 (½ LOD)	IC-MS/MS	1 µg/kg	USFDA (2007a)
			Weighted mean	4.8			
Squash	USA	30		56	IC-MS/MS	1 µg/kg	USFDA (2009)
Tomato	Canada	18	11	0.3 (0.2–1.6)	IC-MS/MS	0.39 ± 0.28 µg/kg	Health Canada (2009); Wang et al. (2009)
	USA	73	54	13.7 (<0.3–268)	IC-MS/MS	1 µg/kg	USFDA (2007b)
	USA	4		78	IC-MS/MS	1 µg/kg	USFDA (2009)
			Weighted mean	14			
Carrot	Canada	12	10	1.31 (0.2–3.1)	IC-MS/MS	0.39 ± 0.28 µg/kg	Health Canada (2009); Wang et al. (2009)
	USA	59	50	15.8 (0.3–111)	IC-MS/MS	1 µg/kg	USFDA (2007b)
	USA	49		6.6	IC-MS/MS	1 µg/kg	USFDA (2009)
			Weighted mean	10.5			

Table 2 (contd)

Description		Country, sample	Number of samples	Number of samples with concentrations higher than LOD	Mean (range) (μg/l or μg/g)	Test method	LOD[a]	Reference
	Eggplant	USA	60		78	IC-MS/MS	1 μg/kg	USFDA (2009)
	Broccoli	USA	55		23.8 (3.5–107)	IC-MS/MS	2–3 μg/kg	Sanchez et al. (2007)
		USA	60		3.34	IC-MS/MS	1 μg/kg	USFDA (2009)
				Weighted mean	19			
	Cauliflower	USA	38	38	12.8 (4.2–40.7)	IC-MS/MS	2–3 μg/kg	Sanchez et al. (2007)
		USA	30		1.1	IC-MS/MS	1 μg/kg	USFDA (2009)
				Weighted mean	7			
	Cabbage	Japan	12	12	2 (0.5–6.3)	IC-MS/MS	0.1 μg/kg	Japan Ministry of Health, Labour and Welfare (2009)
		USA	19	19	18.5 (4.6–63.2)	IC-MS/MS	2–3 μg/kg	Sanchez et al. (2007)
				Weighted mean	10			
	Komatsunn and 17 other species of vegetables	Japan	40	39	0.1–29.7	IC-MS/MS	0.1 μg/kg	Japan Ministry of Health, Labour and Welfare (2009)

Table 2 (contd)

Description		Country, sample	Number of samples	Number of samples with concentrations higher than LOD	Mean (range) (µg/l or µg/g)	Test method	LOD[a]	Reference
Fruits	Apple	Canada	24	19	0.48 (0.1–1.60)	IC-MS/MS	0.39 ± 0.28 µg/kg	Health Canada (2009); Wang et al. (2009)
		USA	9	0	0.15 (½ LOD)	NA	0.3 µg/kg	USFDA (2007b)
				Weighted mean	0.5	NA	NA	
	Grape	Canada	12	12	27.7 (1.96–62.05)	IC-MS/MS	0.39 ± 0.28 µg/kg	Health Canada (2009); Wang et al. (2009)
		USA	15		3.3	IC-MS/MS	NA	Sanchez et al. (2006)
		USA	12		8.58 (<0.3–38.6)	IC-MS/MS	NA	USFDA (2007b)
				Weighted mean	28	NA	NA	
	Orange	Canada	12	10	0.5 (0.2–2.89)	IC-MS/MS	0.39 ± 0.28 µg/kg	Health Canada (2009); Wang et al. (2009)
		USA	10	5	3.4 (0.3–23.3)	IC-MS/MS	NA	USFDA (2007b)
		USA	28		7.4	IC-MS/MS	0.39 ± 0.28 µg/kg	
				Weighted mean	5			

Table 2 (contd)

Description		Country, sample	Number of samples	Number of samples with concentrations higher than LOD	Mean (range) (μg/l or μg/g)	Test method	LOD[a]	Reference
	Melon	Canada	18	17	56.1 (0.1–536.4)	IC-MS/MS	0.39 ± 0.28 μg/kg	Health Canada (2009); Wang et al. (2009)
		USA	48	48	28.6 (0.52–713)	IC-MS/MS	0.3 μg/kg	USFDA (2007b)
		USA	32		1.7	NA	NA	
				Weighted mean	19			
	Vegetable and fruit juice	Canada	53	53	25.81 (0.047–463.5) Median, 0.43	IC-MS/MS	0.5 ng/l for deionized water	Aribi et al. (2006)
	Honeydew	Canada	6	6	0.3 (0.1–0.66)	IC-MS/MS	0.39 ± 0.28 μg/kg	Health Canada (2009); Wang et al. (2009)
Whole wheat flour		USA	38		3.5	IC-MS/MS	NA	USFDA (2009)
Rice		China	65	65	0.83 (0.16–4.88)	IC-MS/MS	0.04 μg/kg (MDL)	Shi & Zhang (2007)
		Japan	10	6	(>0.3–0.73)	IC-MS/MS	0.3 ng/g	Japan Ministry of Health, Labour and Welfare (2009)
		USA	19		0.50 (average)	IC-MS/MS	NA	USFDA (2007b)
				Weighted mean	1			

Table 2 (contd)

Description	Country, sample	Number of samples	Number of samples with concentrations higher than LOD	Mean (range) (µg/l or µg/g)	Test method	LOD[a]	Reference
Milk	China	17	17	3.9 (0.30–9.1)	IC-MS/MS	10 ng/l (MDL)	Shi & Zhang (2007)
	Japan	54	54	9.39 (5.67–16.4)	IC-MS	NA	Dyke et al. (2007)
	Japan	25		6.5 (2.0–10.6)	IC-MS/MS	0.1 ng/g	Japan Ministry of Health, Labour and Welfare (2009)
	USA	125		5.8 (1.9–11.3)	IC-MS/MS	1.0 µg/l	USFDA (2007a,b)
			Weighted mean	6.8			
Breast milk	China	24 (1200)	24	19.7 (2.12–136.1)	IC-MS/MS	0.4 µg/l	Chinese CDC (2009)
	USA	147	147	5.8 (0.5–39.5)	IC-MS	0.07 µg/l	Kirk et al. (2007)
	USA	49		33 (1.3–411)	IC-MS		Pearce, Lazarus & Smythe (2007)
	USA	457	457	9.3 (0.01–48)	IC-MS/MS	0.005 µg/l	Dasgupta et al. (2008)
			Weighted mean	16.4			
Infant formula (powder)	China	10	10	22.8 (12.3–43.1)	IC-MS/MS	2 µg/l	Chinese CDC (2009)
	Japan	10	10	7.5 (2.5–35.3)	IC-MS/MS	0.7 ng/g	Japan Ministry of Health, Labour and Welfare (2009)
			Weighted mean	10			

Table 2 (contd)

Description	Country, sample	Number of samples	Number of samples with concentrations higher than LOD	Mean (range) (µg/l or µg/g)	Test method	LOD[a]	Reference
Fish and fishery products	Japan	20	1	<0.8 (0.32–71.6)	IC-MS/MS	0.2 ng/g	Japan Ministry of Health, Labour and Welfare (2009)
	USA	166	50	14.2–1593	IC-CD	2.5 µg/l	Theodorakis et al. (2006)
Wine	Canada	77	77	5.00 (0.013–50.25) Median, 1.54	IC-MS/MS	0.5 ng/l for deionized water	Aribi et al. (2006)
	Japan	27	27	9.4 (0.2–56.9)	IC-MS/MS	0.1	Japan Ministry of Health, Labour and Welfare (2009)
			Weighted mean	6			
Beer	Canada	144	144	1.04 (0.005–21.10) Median, 0.34	IC-MS/MS	0.5 ng/l for deionized water	Aribi et al. (2006)

CD, conductivity detector; MDL, method detection limit; MRL, minimum reporting limit; NA, not available

[a] This column presents the LOD of the analytical method unless stated otherwise in parentheses. For example, for tap water in the Republic of Korea, "5.0 µg/l; 0.05 µg/l (MRL)" means that the LOD of the IC method and the MRL of the LC-MS/MS method were 5.0 µg/l and 0.05 µg/l, respectively (line 8 in this table).

7.2.2 China

China submitted data on perchlorate levels in rice, milk, infant formula, human milk and drinking-water (Chinese CDC, 2009). There were 92 data points from composite samples analysed using the IC-ESI-MS/MS technique, with LORs of 0.1–2.2 µg/kg, depending on the food or matrix. The samples of infant formula and of human breast milk were from a Chinese TDS conducted in 2007. Twenty-four composite samples of breast milk were collected from the urban and rural areas of the 12 provincial sampling sites, and each composite sample consisted of milk from 50 mothers (total of about 1200 subjects). The concentration of perchlorate in breast milk from mothers from urban and rural areas ranged from 2.12 to 136 µg/l and from 2.18 to 93 µg/l, respectively. In infant formula, perchlorate levels ranged from 12.3 to 43.1 µg/kg, with an average of 22.8 µg/kg. The levels of perchlorate in rice, bottled water and milk samples were 0.16–4.88 µg/kg, 0.04–2.01 µg/l and 0.30–9.1 µg/l, respectively (Shi & Zhang, 2007). Perchlorate was found in six of nine samples of finished water from drinking-water plants at average concentrations of 0.5–2.4 µg/l (Liu et al., 2004). Perchlorate was detected in tap water (3.16 µg/l) in Macao Special Administrative Region.

7.2.3 India

In India, 66 samples of tap water from restaurants and homes, groundwater (or well water), bottled water, surface water (rivers and lakes) and rainwater were collected from 13 locations in six states in 2008. The samples were analysed using LC-MS/MS (Kannan et al., 2009). Twenty-four per cent of the samples were below the LOQ (0.02 µg/l). Perchlorate levels ranged from less than 0.02 to 6.9 µg/l (mean 0.42 ± 1.1 µg/l, median 0.07 µg/l). Mean concentrations in drinking-water, groundwater, bottled water, surface water and rainwater were 0.1 µg/l, 1.0 µg/l, <0.02 µg/l, 0.05 µg/l and <0.02 µg/l, respectively. Out of a total of 66 water samples analysed, only 3 groundwater samples contained perchlorate levels above 1 µg/l.

7.2.4 Japan

Japan submitted aggregated results on the occurrence of perchlorate in foods obtained from 209 food samples and 20 bottled water samples (Japan Ministry of Health, Labour and Welfare, 2009). Samples were analysed using the IC-ESI-MS/MS technique. The LOQs ranged from 0.1 to 2.2 µg/kg for different food matrices. The perchlorate concentration in 14 samples was below the LOR. Perchlorate was detected at concentrations ranging from less than 0.1 to 29.7 µg/kg in vegetables (n = 82), from 2.0 to 35.3 µg/kg in milk and milk products, including infant formula (n = 50), from less than 0.2 to 71.6 µg/kg in fishery foods (n = 20), from 0.2 to 56.9 µg/kg in wine (n = 27), from less than 0.3 to 0.73 µg/kg in rice (n = 10) and from less than 0.03 to 0.35 µg/kg in bottled water (n = 20). Perchlorate was detected in powdered infant formula in the concentration range of 2.5–35.3 µg/kg, with an average concentration of 7.4 µg/kg. Dyke et al. (2007) reported that the average concentration of perchlorate in 54 Japanese milk samples was above the LOQ, at 9.39 µg/l, with a range of 5.47–16.40 µg/l. Perchlorate levels in 30 tap water samples were up to 37 µg/l (Kosaka et al., 2007).

7.2.5 Republic of Korea

Perchlorate levels in tap water samples from the Nakdong River basin collected in two investigations ranged from 18.7 to 95.6 µg/l and from 4.0 to 25.6 µg/l (Kim, Kim & Lee, 2009). Among other sampling sites, one at Daegu showed the highest perchlorate level, at 22.3 µg/l.

7.2.6 USA

Perchlorate was detected in public water supplies in 26 states as well as Puerto Rico and the Mariana Islands and was typically present at concentrations below 12 µg/l (Brandhuber, Clark & Morley, 2009). In a USEPA drinking-water survey, only 160 of 3870 water supplies (4%) had perchlorate levels above the LOR (4 µg/l), in the range of 4–420 µg/l, with a mean concentration of 9.9 µg/l. The mean was the average of the concentrations in the samples in which perchlorate was detected. Less than 1% of public water supplies had perchlorate levels of 20 µg/l or higher (NRC, 2005).

The USA submitted data on the occurrence of perchlorate in foods from 2004–2005 USFDA exploratory surveys (USFDA, 2007b) and the 2005–2006 TDS survey (USFDA, 2009). In a study on perchlorate levels in freshwater fish, high perchlorate concentrations of 2092 µg/kg, 2740 µg/kg and 1593 µg/kg were found in whole fish, fish heads and fillets, respectively (Theodorakis et al., 2006). In the USFDA exploratory survey (USFDA, 2007b), perchlorate analysis was targeted on 28 types of foods and beverages, consisting of bottled water, milk, fruits and fruit juices, vegetables, grain products and seafood. The TDS covered a broader range of foods. Of the 1065 samples from the 285 kinds of TDS foods, 41% samples were below the LOR, and perchlorate was not detected in 26% of the samples (Murray et al., 2008). Out of the 104 milk samples analysed, 3 samples were found to contain perchlorate at levels below the LOR. The mean perchlorate levels were found to be in the range of 3.16–11.3 µg/l, with a mean concentration of 5.76 µg/l. Perchlorate levels were found in 81% of infant and baby foods on a ready-to-serve basis, with levels ranging from 1.1 to 1.3 µg/l. In a United States Centers for Disease Control and Prevention study on 15 brands of infant formula from the market, perchlorate levels ranged from 0.03 to 5.05 µg/l in reconstituted samples (Schier et al., 2010). In two studies involving 652 mothers, perchlorate levels in breast milk were found to be in the range of 0.01–411 µg/l, with a mean concentration of 9.3 µg/l (Kirk et al., 2005, 2007).

7.3 Summary of national occurrence data

The Committee, at its present meeting, reviewed data from six countries on the occurrence of perchlorate in water and different foods analysed between 2004 and 2009 (Table 1). Analytical data on 35 073 samples of groundwater and drinking-water indicated that perchlorate levels in 98% of samples were below the LOR. In drinking-water, perchlorate levels exceeded 20 µg/l in only 1% of the samples. The USA reported that only 160 out of 3870 water supplies (4%) had perchlorate levels above the LOR (4 µg/l), in the range of 4–420 µg/l, with a mean concentration of 9.9 µg/l. Analytical data from 1866 samples (vegetables, fruits, rice, milk, infant

formula, fish and fish products, beverages such as juices, beer and wine) were reviewed, and perchlorate levels in 33% of samples were found to be below the LOR. Weighted mean (mean of reported means weighted by the number of samples) perchlorate levels in vegetables were in the range 4.8–110 µg/kg (potato, 4.8 µg/kg; carrot, 6.6 µg/kg; spinach, 110 µg/kg; lettuce, 11.6 µg/kg; tomato, 14 µg/kg; squash, 75 µg/kg; eggplant, 78 µg/kg; broccoli, 19 µg/kg; cauliflower, 7 µg/kg; cabbage, 10 µg/kg). In fruits, the weighted means were in the range 0.5–28 µg/kg (oranges, 5 µg/kg; apples, 0.5 µg/kg; grapes, 28 µg/kg; melons, 19 µg/kg). The weighted mean perchlorate levels for other foods were as follows: rice, 1 µg/kg; whole wheat flour, 3.5 µg/kg; milk, 6.8 µg/kg; beer, 1 µg/kg; and wine, 6 µg/kg. The mean perchlorate levels in human milk in China (n = 24 composite samples) and the USA (n = 652) were found to be 19.7 µg/l (range 2.1–136 µg/l) and 9.3 µg/l (range 0.01–411 µg/l), respectively. Limited data show that the weighted mean perchlorate level in infant formula was 10 µg/kg. Sampling and analysis were carried out on targeted foods. However, general surveys involving a broader range of foods in different countries were lacking. The widespread presence of perchlorate in the environment has been shown in a few countries, but in view of its uses and natural occurrence, it is probable that it will be found more widely in drinking-water and in food.

8. FOOD CONSUMPTION AND DIETARY EXPOSURE ESTIMATES

The Committee evaluated occurrence and dietary exposure data for perchlorate from submissions from China, Japan, Canada and the USA and from the literature. The occurrence data have been summarized in section 7.

Dietary exposure to perchlorate is primarily from vegetable and fruit crops that have been irrigated with water containing perchlorate, from milk and other beverages made from crops that may contain perchlorate, and from drinking-water. Infants are exposed to perchlorate in breast milk and infant formula made with contaminated drinking-water, in addition to any foods consumed.

Perchlorate exposure from drinking-water will be considered separately from dietary exposure.

8.1 International estimates of dietary exposure

The Committee used perchlorate values from the literature as well as submitted information from member countries to arrive at “mean” contamination concentrations for use with the per capita estimates of commodity consumption in the GEMS/Food consumption cluster diets (Table 3). The Committee notes that there has not been a broad examination of foods that might be contaminated and that many foods have not been explored at all. Additionally, the foods that have been examined are in most cases those thought most likely to be contaminated. It is thus not clear whether the derived estimates of exposure are complete or representative, and consequently they should be viewed as preliminary. The national estimates presented in the next section will serve as better indicators of perchlorate dietary exposure, at least for those countries.

Table 3. Food consumption from GEMS/Food consumption cluster diets

Food	Per capita food consumption (g/day)												
	A	B	C	D	E	F	G	H	I	J	K	L	M
Rice	91.0	31.6	94.6	33.2	12.7	12.7	376.9	64.3	38.0	74.3	238.4	381.3	34.6
Potato	19.1	160.8	61.2	243.6	230.1	204.7	52.7	57.1	50.1	4.3	54.7	41.0	168.0
Carrot	0.6	15.1	8.1	13.9	27.1	28.4	5.4	7.9	2.5	3.5	4.1	8.6	19.4
Spinach	0.0	5.0	1.1	0.1	2.6	0.1	9.4	0.4	0.0	0.0	0.2	4.3	2.0
Lettuce	0.2	23.8	3.6	0.6	11.9	18.0	7.1	7.0	0.6	1.9	2.0	7.1	30.6
Tomato	11.8	185.0	118.0	60.7	31.6	40.9	23.5	31.7	15.0	16.2	35.6	9.9	103.0
Squash, pumpkin, gourd	16.3	12.3	14.4	21.9	3.2	1.0	7.1	4.6	11.3	3.0	7.0	6.7	7.6
Eggplant	1.7	17.5	12.3	1.7	0.8	0.4	20.1	0.1	0.6	6.3	0.5	6.3	0.7
Orange	4.2	57.9	38.0	14.4	29.4	41.2	7.4	118.9	8.3	2.4	203.0	12.0	102.4
Apple	0.3	60.5	18.5	39.9	50.8	39.4	14.4	10.1	2.2	0.0	9.8	17.9	36.3
Grape	3.7	128.5	27.1	33.1	107.5	44.0	2.6	4.8	11.7	0.3	6.8	10.9	58.8
Melon	3.6	26.7	22.6	11.5	5.6	2.0	7.5	6.1	0.7	1.4	2.5	6.9	12.4
Cauliflower	0.1	5.2	1.2	0.1	1.7	0.1	3.2	0.1	0.3	0.1	0.6	0.4	1.4
Broccoli	0.0	0.7	1.2	0.1	4.2	4.0	3.2	7.8	0.0	0.0	0.3	0.4	6.6
Cabbage	2.1	19.8	8.3	43.9	29.9	28.0	23.6	5.0	12.0	1.9	3.8	55.5	18.9

Table 3 (contd)

Food	Per capita food consumption (g/day)												
	A	B	C	D	E	F	G	H	I	J	K	L	M
Wheat flour	63.4	296.3	327.5	300.0	181.6	166.2	133.0	60.1	52.4	32.2	87.7	79.6	180.1
Milk of cattle, goat, sheep	52.7	189.5	65.7	295.6	179.6	237.9	48.0	121.1	80.8	94.7	207.7	56.1	287.9
Beer	18.3	84.1	4.1	66	243.1	161.3	21.9	102.7	29.5	12.6	100.9	82.2	218.8
Wine	1.3	76.8	1.1	15.4	68.8	25.6	1.0	0.9	6.8	0.1	3.4	3.6	31

For this analysis, the Committee chose the food perchlorate concentrations set out in section 7.3 above. These figures were approximations derived primarily from three sources: exploratory data posted on the USFDA web site (USFDA, 2007b) and submitted to this meeting of the Committee, Canadian data published in the literature (Wang et al., 2009) and Chinese data submitted to this meeting of the Committee. If the food was mentioned in only one of the sources, the highest levels were approximated. If the food was mentioned in more than one source, then a rough average, towards the higher end, was chosen. Weighted average levels were calculated where feasible. There were data available on more vegetable types from the USFDA, but these vegetables had low levels of perchlorate and are not commonly consumed in large amounts (e.g. kale, turnip greens). Additionally, a number of foods where no quantifiable perchlorate was found have been excluded (e.g. peaches, plums). Because of the reporting regimen for the Food and Agriculture Organization of the United Nations balance sheets that feed into the GEMS/Food consumption cluster diets, wine consumption may cause a double counting, as it is also included under "grapes" from which it is produced. Any double counting would be insignificant in this exercise, as wine does not contribute more than 5% of the total perchlorate exposure for any of the consumption cluster diet totals (cluster E, highest).

The Committee noted that the range of estimated perchlorate dietary exposure (Table 4) is relatively narrow, 0.03–0.22 µg/kg bw per day, when milk consumption is included. Milk consumption provides a large proportion of the perchlorate exposure for most clusters, ranging from 7% (clusters C and G) to 42% (cluster J) of the total.

8.2 National estimates of dietary exposure

National dietary exposure estimates from Canada and the USA were evaluated by the Committee. The Canadian estimates were published by Wang et al. (2009), whereas three different published estimates were available from the USA. One estimate was derived from urinary biomarkers of perchlorate exposure (Blount et al., 2007), one was taken from the USA TDA (Murray et al., 2008) and the third is available on the USFDA web site (USFDA, 2007a). Additionally, a supplement to the USA estimate available on the USFDA web site was submitted to the Committee.

The Canadian estimates were made using data taken from foods available in a public market and included imported fruits and vegetables (Wang et al., 2009). A deterministic approach was used. Perchlorate concentrations were combined with food consumption data available from the Nutrition Canada Food Consumption Survey (<18 years old) and the Nova Scotia Provincial Nutrition Survey (adults >18 years old, including women of childbearing age). Mean dietary exposures to perchlorate were higher for children than for adults. The mean dietary exposure was approximately 0.04 µg/kg bw per day for children 1–11 years of age, compared with 0.03 µg/kg bw per day for adults, including women of childbearing age. The researchers noted that these estimates were made with a limited number of data available from only a few foods and should be considered preliminary. They are in general agreement, however, with the international estimates described above and the estimates from the USA discussed below.

Table 4. International estimates of perchlorate dietary exposure using GEMS/Food consumption cluster diets

Food	Dietary exposure (ng/day)												
	A	B	C	D	E	F	G	H	I	J	K	L	M
Rice	91	32	95	33	13	13	377	64	38	74	238	381	35
Potato	76	643	245	974	920	819	211	228	200	17	219	164	672
Carrot	6	151	81	139	271	284	54	79	25	35	41	86	194
Spinach	0	625	138	13	325	13	1175	50	0	0	25	538	250
Lettuce	2	262	40	7	131	198	78	77	7	21	22	78	337
Tomato	165	2590	1652	850	442	573	329	444	210	227	498	139	1442
Squash, pumpkin, gourd	913	689	806	1226	179	56	398	258	633	168	392	375	426
Eggplant	46	473	332	46	22	11	543	3	16	170	14	170	19
Orange	21	290	190	72	147	206	37	595	42	12	1015	60	512
Apple	0	30	9	20	25	20	7	5	1	0	5	9	18
Grape	104	3598	759	927	3010	1232	73	134	328	8	190	305	1646
Melon	68	507	429	219	106	38	143	116	13	27	48	131	236
Cauliflower	1	68	16	1	22	1	42	1	4	1	8	5	18
Broccoli	0	17	29	2	101	96	77	187	0	0	7	10	158
Cabbage	40	376	158	834	568	532	448	95	228	36	72	1055	359
Wheat flour	222	1037	1146	1050	636	582	466	210	183	113	307	279	630

Table 4. International estimates of perchlorate dietary exposure using GEMS/Food consumption cluster diets

Food	Dietary exposure (ng/day)												
	A	B	C	D	E	F	G	H	I	J	K	L	M
Milk of cattle, goat, sheep	369	1327	460	2069	1257	1665	336	848	566	663	1454	393	2015
Beer	18	84	4	66	243	161	22	103	30	13	101	82	219
Wine	7	384	6	77	344	128	5	5	34	1	17	18	155
Total non-milk (μg/day)	1.8	11.9	6.1	6.6	7.5	5.0	4.5	2.7	2.0	0.9	3.2	3.9	7.3
Total with milk (μg/day)	2.1	13.2	6.6	8.6	8.8	6.6	4.8	3.5	2.6	1.6	4.7	4.3	9.3
Total non-milk (μg/kg bw per day)	0.03	0.20	0.10	0.11	0.13	0.08	0.07	0.04	0.03	0.02	0.05	0.06	0.12
Total with milk (μg/kg bw per day)	0.04	0.22	0.11	0.14	0.15	0.11	0.08	0.06	0.04	0.03	0.08	0.07	0.16
% from milk	17	10	7	24	14	25	7	24	22	42	31	9	22

For the USA, an estimate of dietary exposure to perchlorate was made using urinary biomarker concentrations of perchlorate, available from the 2001–2002 NHANES (Blount et al., 2007). The dietary exposure (which would include exposure from water) was estimated using the ratio of the perchlorate to creatinine concentrations in urine, assuming that 100% of perchlorate in the diet is absorbed and excreted unmetabolized. The 50th-percentile estimate for the total adult population was 0.06 µg/kg bw per day, whereas the 95th-percentile estimate was 0.23 µg/kg bw per day. Dietary exposure for males was slightly higher than that for females. These estimates are also in general agreement with the international estimates. Perchlorate was detected in all of the urine samples examined, confirming the widespread nature of environmental perchlorate contamination.

The USA estimate available on the USFDA web site (USFDA, 2007a) is listed as a preliminary estimate for the reasons already noted: too few foods and a limited number of samples for each. It was also made using perchlorate concentrations measured for the raw commodities and the food consumptions for each, available from the 1994–1996, 1998 Continuing Survey of Food Intake by Individuals (http://www.ars.usda.gov/Services/docs.htm?docid=14531). Food consumption based on United States Department of Agriculture (USDA) food commodity codes (ingredient use) was used for those foods primarily consumed as an ingredient in a mixed food dish: lettuce, tomatoes, spinach, carrots, apples, cantaloupes, grapes, cucumbers, onions, potatoes, rice, oatmeal, wheat flour and corn meal. Consumption of fruit juices was also calculated based on ingredient use. Food consumption based on USDA food codes (consumption as a whole food) was used for oranges, strawberries, watermelon, broccoli, cabbage, greens, green beans, sweet potatoes, milk, catfish, salmon and shrimp. The foods were determined to be consumed primarily as an ingredient or as a whole food so as to maximize the reported intake of the food (i.e. the approach that resulted in the higher exposure to perchlorate was used). For example, because spinach is consumed in higher amounts as an ingredient than as a whole food, it was categorized as an ingredient, which resulted in higher perchlorate intake. The mean estimate of dietary perchlorate exposure from food for the total population was 0.05 µg/kg bw per day, whereas the 90th-percentile exposure was 0.12 µg/kg bw per day. The estimates for children 2–5 years old were 0.17 and 0.34 µg/kg bw per day at the mean and 90th percentiles of exposure, respectively.

The final USA estimate is from the USFDA's TDS. This type of survey provides a good estimate of dietary exposure, as all of the foods in the model diets are prepared for consumption prior to analysis for the substance under investigation. USA model diet "market basket" surveys from 2003 to 2008 were used to make estimates. The 2003–2006 period is published (Murray et al., 2008); an additional estimate from the 2005–2008 market basket surveys was submitted to this meeting of the Committee. The mean estimates for age groups 14 and over were all about 0.1 µg/kg bw per day, with 90th-percentile exposures of approximately 0.23 µg/kg bw per day or less. Dietary exposure was greatest for the 2-year-olds examined through the 2005–2008 market basket surveys. The mean exposure for this group was 0.44 µg/kg bw per day, with a 90th-percentile exposure of 0.734 µg/kg bw per day.

The Committee concluded that the estimates from the USA TDS provide the best indication of dietary exposure to perchlorate, as they do not depend on large numbers of analyses of individual foods or commodities and include water and beverages made from water that might be contaminated with perchlorate. Although all of the national estimates are in general agreement, the Committee noted that the highest estimates were from the USFDA's TDS, confirming the suggestion that the other estimates (and the international estimates prepared by the Committee) are preliminary and do not include all food sources of perchlorate.

9. DOSE–RESPONSE ANALYSIS

The pivotal study for dose–response analysis was the human clinical study on 16 male and 21 female healthy adult volunteers published by Greer et al. (2002), in which perchlorate was given in drinking-water at doses of 0.007, 0.02, 0.1 or 0.5 mg/kg bw per day for 14 days. The uptake of iodide into the thyroid was measured at baseline before administration of perchlorate and on days 2 and 14 of administration at both 8 and 24 h after administration of radiolabelled iodine. These data were used for modelling.

The Committee selected a critical effect size of 50% inhibition of iodide uptake as the benchmark response. This choice was made because human clinical data from healthy adults following both short-term and chronic exposure to perchlorate have shown that such a level of inhibition is not associated with any changes in TSH or thyroid hormone levels. The Committee noted that a benchmark response of 50% inhibition was within the observed range of the study.

PROAST software (version 23.0) for analysis of continuous data was used. In the clinical study, each subject served as his or her own control, with baseline values measured 1 day before the start of the 14-day perchlorate exposure period. In the BMD analysis, the baseline value for iodide uptake in each subject was set at 100%. Analysis of the post-treatment values for iodide uptake at 8 and 24 h and the values from exposure days 2 and 14 as covariates showed that these factors did not have any significant impact. Therefore, the two models used for continuous data—the exponential and Hill models—were fitted to all the data combined.

The plots obtained using the two models were very similar. That obtained using the Hill model is shown as illustration in Figure 1.

Similar values for the BMD_{50} and $BMDL_{50}$ (the BMD and BMDL, respectively, for a 50% response) were obtained from the two models, as shown in Table 5. The lower of the two $BMDL_{50}$ values (rounded to two significant figures) of 0.11 mg/kg bw per day was used as the point of departure.

Figure 1. Graphical plot of the data on inhibition of uptake of radiolabelled iodine by the thyroid in humans exposed to perchlorate for 14 days (Greer et al., 2002)

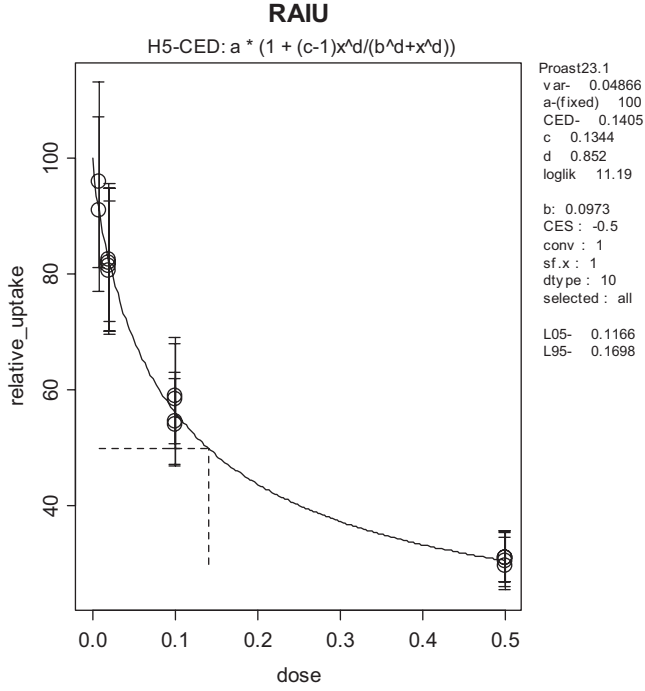

Table 5. BMD and BMDL values for 50% inhibition of uptake of radiolabelled iodine by the thyroid in humans exposed to perchlorate for 14 days

Model	Number of regression parameters	BMD_{50} (mg/kg bw per day)	$BMDL_{50}$ (mg/kg bw per day)
Exponential model	4	0.137	0.114
Hill model	4	0.141	0.117

Source: Greer et al. (2002)

10. COMMENTS

10.1 Absorption, distribution, metabolism and excretion

Perchlorate is rapidly absorbed following ingestion and rapidly excreted unchanged, mostly in the urine. It crosses the placenta and is also found in breast milk.

10.2 Relevance of animal data for human risk assessment

The key toxicological issue for perchlorate is its ability to competitively inhibit uptake of iodide by the thyroid gland, potentially causing hypothyroidism. The inhibition is at the level of the NIS, a glycoprotein expressed in the outer, basolateral membrane of the thyroid follicular cell, which actively transports iodide (and perchlorate) from the blood into the thyroid gland. Inhibition of iodide uptake by perchlorate reduces the amount of iodide available for the synthesis of thyroid hormones, resulting in reductions in the concentrations of circulating T_4 and the more biologically active hormone, T_3. In a negative feedback loop, reductions in the concentrations of T_4 and T_3 reaching the brain trigger the release of thyrotropin-releasing hormone in the hypothalamus, which in turn causes the release of thyrotropin, also known as TSH, from the anterior pituitary gland. TSH initiates the events in the thyroid that result in NIS transport of iodide into the thyroid and synthesis of T_3 and T_4. If there is a sustained reduction in iodide uptake by the thyroid, hypothyroidism may occur. Hypothyroidism has adverse implications for structural and functional brain development in the fetus, infant and child and for metabolism and the functioning of cardiovascular, gastrointestinal, skeletal, neuromuscular and reproductive systems in adults (Wolff, 1998; Braverman & Utiger, 2005; Kirk, 2006).

There is general agreement that for substances known to affect the thyroid and having a mode of action involving inhibition of the uptake of iodide, the rat is not a good model for humans. This applies not only to the likelihood of thyroid cancer (Capen et al., 1999; NRC, 2005; USEPA, 2008a) but also to other perturbations of thyroid physiology and pathology in response to thyroid toxicants (Capen, 1997). The reasons for this are several. There is a difference between rat and human in the proteins to which the thyroid hormones, T_3 and T_4, are bound and their relative binding affinity to those proteins. In humans, thyroid hormones bind mainly to TBG with high affinity, which reduces their rate of hepatic metabolism and hence clearance from blood. In contrast, in the rat, thyroid hormones bind to albumin and transthyretin with affinities that are much lower, by around 100-fold or more, than those for TBG (Connors, 1997). This results in rapid clearance of thyroid hormones in the rat, with serum half-lives of 0.5–1 day and 0.25 day for T_4 and T_3, respectively. This compares with a much slower clearance in humans, with serum half-lives of 5–9 days and 1 day for T_4 and T_3, respectively (Choksi et al., 2003). The rapid clearance in the rat creates a demand for greater synthesis of thyroid hormones, requiring increases in secretion of TSH. The demand for fresh synthesis of thyroid hormones in the rat is further exacerbated because there is much less storage of hormones in thyroidal colloid; the amounts stored in rats are sufficient for a few days, compared with several months in humans (Greer et al., 2002; Braverman & Utiger, 2005). The resulting increases in serum TSH in the rat following exposure to anti-thyroid agents are greater than those seen in humans and, if sustained, will cause increased thyroid growth and pathological changes in the thyroid. For these reasons, the laboratory animal data reviewed by the Committee, which comprised mostly data from rats, while qualitatively supportive of the human data, were not further considered for deriving a health-based guidance value for perchlorate.

10.3 Observations in humans

Human observations include clinical studies on thyroid function in healthy adult volunteers given perchlorate in drinking-water, clinical studies on thyroid function in workers occupationally exposed to perchlorate for several years, population-based epidemiological studies and ecological studies comparing populations living in areas with differing concentrations of perchlorate in the drinking-water. Some of the ecological studies included pregnant women, newborns and children and, in the case of newborns, took advantage of neonatal screening programmes that included measurements of serum TSH and/or thyroid hormones. In the various studies, outcome measures investigated included one or more of the following: radioactive iodide uptake by the thyroid, serum TSH and thyroid hormone concentrations, urinary perchlorate and iodide concentrations, incidence of thyroid diseases, neonatal birth weight, head circumference and length, incidence of congenital hypothyroidism and neurobehavioural measures in children. There are also historical clinical data from past use of perchlorate as a treatment for hyperthyroidism.

None of the ecological studies showed any relationship between perchlorate concentrations in drinking-water and the incidence of thyroid diseases, including congenital hypothyroidism and thyroid cancer. With respect to TSH and thyroid hormones, one study reported a significant association between elevated newborn TSH levels and concentrations of perchlorate in drinking-water. However, it is notable that the concentrations of perchlorate in drinking-water in this study were not as high as in other, negative studies on newborns.

The clinical studies in healthy adult volunteers and workers did not show any significant effects on TSH or thyroid hormone concentrations at daily exposures of up to 0.5 mg/kg bw per day. The NAS in the USA has estimated, from consideration of the human clinical studies in healthy subjects and from studies of long-term treatment of patients with hyperthyroidism, that a sustained exposure to perchlorate of more than 0.4 mg/kg bw per day would probably be necessary in order to trigger hypothyroidism in normal adults. The NAS also commented that in pregnant women, infants, children and people with low iodide intake or pre-existing thyroid dysfunction, the dose of perchlorate required to cause hypothyroidism may be lower (NRC, 2005). Despite lack of effects on TSH and thyroid hormones, the studies in healthy adult volunteers did show clear, dose-related effects on radiolabelled iodide uptake by the thyroid.

A key issue for human risk assessment is whether a point of departure for deriving a health-based guidance value for perchlorate should be based on the end-point of inhibition of thyroidal uptake of iodide or on end-point(s) with clearer implications for adversity, such as increases in TSH, reductions in circulating thyroid hormone levels and clinical hypothyroidism. Inhibition of iodide uptake by the thyroid is clearly a precursor event in the chain of events that ultimately can lead to adverse effects on thyroid function, but by itself cannot be considered adverse, as there will not be adverse effects from iodide uptake inhibition provided circulating thyroid hormone levels remain unchanged. The NAS review also expressed the view that in adults with a normal dietary iodine intake, uptake of iodide by the thyroid would

need to be inhibited by at least 75% for several months or longer in order to cause declines in thyroid hormone production that would have adverse health effects. The available human data do, however, provide some support for such a statement. For example, the key 14-day clinical study by Greer et al. (2002) in healthy adults found no effect on TSH or thyroid hormones at the highest perchlorate dose tested of 0.5 mg/kg bw per day, despite the observation that the highest dose caused a 67% inhibition of iodide uptake by the thyroid. There is also support from the two occupational studies in which there was chronic exposure; in one study (Lamm et al., 1999), perchlorate exposure equivalent to about 0.5 mg/kg bw per day was not associated with any change in TSH or thyroid hormones, and in the other study (Braverman et al., 2005), in which uptake of radiolabelled iodide by the thyroid was measured, a 38% reduction in iodide uptake by the thyroid was not associated with any effect on TSH or thyroid hormones. A further practical constraint in selecting the end-point is that, with the single exception of one study (Blount et al., 2006b), none of the human clinical and epidemiological studies on perchlorate actually identified any significant association between perchlorate exposure and changes in TSH and thyroid hormones that could be used for the point of departure.

A second key issue is to what extent a point of departure derived from studies in healthy adults relates to potentially more vulnerable groups in the population. Given the critical role of thyroid hormones in brain development, it is widely considered that the probability of a permanent adverse effect on neurodevelopment from thyroid disruption, including transient disruption, would be greatest during early life. Consideration needs to be given to the differing thyroid physiology in the fetus and neonate compared with that of children and adults; for example, the amount of thyroid hormone stored in the colloid in late-gestation fetuses and neonates is estimated to be sufficient for less than 1 day only, compared with several months for adults (Scinicariello et al., 2005). The uncertainties surrounding this issue cannot currently be addressed by using data from pregnant women, neonates and young infants, as there are no good quantitative data relating perchlorate intake to thyroidal iodide uptake, TSH or thyroid hormones in these groups. It is also unclear whether nursing infants may be additionally at risk if perchlorate were to reduce the passage of iodine into breast milk. At present, there are very few data on this, and what data are available are contradictory.

As perchlorate competitively inhibits iodide uptake by the thyroid, another issue for consideration is whether populations living in parts of the world where the diet is deficient in iodine might be more susceptible to perchlorate than iodine-replete individuals. WHO (1999) has estimated that 2.2 billion people from 130 countries are at risk from iodine deficiency, living in areas where soil and therefore the crops grown are deficient in iodine. In many countries, the risks from iodine deficiency have been greatly reduced by the provision of iodized salt, but many still remain at risk. The data on this aspect in relation to perchlorate are sparse. In a review, Charnley (2008) pointed out that a study in 398 European women with urinary iodine concentrations below 100 µg/l during the first trimester of pregnancy, who were exposed to perchlorate at levels similar to those in the USA, found no effects on maternal thyroid function (Pearce, Lazarus & Smythe, 2007). However, these findings have been published as an abstract only. WHO (2001) guidance

classifies urinary iodine concentrations in the range 99–50 µg/l as mild deficiency, 49–20 µg/l as moderate deficiency and below 20 µg/l as severe deficiency. NHANES data from 2001–2002 indicate that about 7% of pregnant women in the USA have urinary iodine concentrations below 50 µg/l (Caldwell, Jones & Hollowell, 2005). However, NHANES data from 1988–1994 showed no differences in TSH or T_4 concentrations when women, including pregnant women, with urinary iodine concentrations below 50 µg/l were compared with women with higher urinary iodine concentrations (Soldin, Tractenberg & Pezzullo, 2005). These data collectively suggest that iodine deficiency may not pose an additional risk, at least during gestation. Only one study has indicated a relationship between low urinary iodine (<100 µg/l) and serum TSH increases and T_4 decreases (Blount et al., 2006b). However, a reanalysis of the data on T_4 from that study, adjusting urinary iodine concentrations for creatinine to better reflect 24 h urinary excretion of iodine, showed that the relationship between perchlorate and T_4 disappeared (Lamm et al., 2007). Charnley (2008) also pointed out that the reported effects on TSH in the NHANES data could be due to co-exposure in the diet to other ubiquitous anti-thyroid substances, either with the same mode of action, such as nitrate or thiocyanate, or with differing modes of action, such as organochlorines. Alternatively, the reported effects could be statistical artefacts, because perchlorate naturally co-occurs with iodine, and there is an association between iodine and TSH in the NHANES data.

A further consideration is whether individuals, in particular pregnant women, who already have the condition of hypothyroidism or subclinical hypothyroidism might be additionally affected by low-level perchlorate exposure. These subgroups of the population are numerically significant; in women, including pregnant women, in the general population, estimates of the prevalence of subclinical hypothyroidism range from 2.3% to 10%, and those for overt hypothyroidism, from 0.2% to 5% (Charnley, 2008).

Given the mode of action of perchlorate and the groups most likely to show enhanced susceptibility to perchlorate, the key vulnerable groups are likely to be pregnant women, fetuses, newborns, young infants, those with hypothyroidism and possibly those with iodine-deficient diets. As there are no adequate data on perchlorate exposure and inhibition of thyroidal iodide uptake in these key groups that are comparable to the quantitative data obtained in the study by Greer et al. (2002), these represent significant data gaps and are the major sources of uncertainty in any population-wide risk assessment. The uncertainties discussed above need to be weighed alongside the fact that the point of departure for the risk assessment can be based on inhibition of iodide uptake by the thyroid, a precursor event that, at least at low to moderate levels of inhibition, appears to be non-adverse.

10.4 Analytical methods

Perchlorate is soluble in water and polar organic solvents and is easily extracted from foods using either water or water–acetonitrile mixtures. Water samples are analysed directly, whereas food extracts are subjected to SPE cleanup prior to determination. Analytical methods used for the detection and determination

of perchlorate levels in water and foods include IC with conductivity detector, IC-MS or IC-MS/MS and LC-MS or LC-MS/MS. IC-MS/MS and LC-MS/MS methods offer lower LODs and can be used for the determination of perchlorate in foods. Stable isotope–labelled perchlorates are used as internal standards. Currently, certified reference materials are not available, and collaborative method validation studies have not been conducted. Other methods, such as spectrophotometry, capillary electrophoresis and ion-selective electrode–based potentiometric methods, lack in sensitivity and may not be suitable for detection at lower levels in foods and water. Rapid screening methods have not yet been developed. Most survey data in foods have been obtained using the IC-MS/MS method.

10.5 Effects of processing

Limited data are available on the fate of perchlorate during food processing. However, perchlorate is generally stable at temperatures used in food processing.

10.6 Prevention and control

Reduction of perchlorate in foods and water relies mainly on the control of contamination in fertilizers, irrigation systems and water used in food processing and food preparation. However, washing can reduce surface contamination in vegetables and fruits.

10.7 Levels and patterns of contamination in food commodities

At the present meeting, the Committee reviewed data from six countries on the occurrence of perchlorate in water and different foods analysed between 2004 and 2009 (see Table 1 in section 7.1). Analytical data on 35 073 samples of groundwater and drinking-water indicate that the perchlorate level in 98% of samples was below the LOR. In drinking-water, perchlorate levels exceeded 20 µg/l in 1% of the samples only. The USEPA reported that only 160 out of 3870 water supplies (4%) had perchlorate levels above the LOR (4 µg/l), in the range of 4–420 µg/l, with a mean concentration of 9.9 µg/l; this mean concentration is the average of the detected concentrations. Analytical data in 1866 samples (vegetables, fruits, rice, milk, infant formula, fish and fish products, and beverages, such as juices, beer and wine) were reviewed, and perchlorate levels in 33% of samples were found to be below the LOR. Weighted mean (mean of reported mean levels weighted by number of samples) perchlorate levels in raw vegetables were in the range of 4.8–110 µg/kg (potato, 4.8 µg/kg; carrot, 6.6 µg/kg; spinach, 110 µg/kg; lettuce, 11.6 µg/kg; tomato, 14 µg/kg; squash, 75 µg/kg; eggplant, 78 µg/kg; broccoli, 19 µg/kg; cauliflower, 7 µg/kg; cabbage, 10 µg/kg); in fruits, the weighted mean levels ranged from 0.5 to 28 µg/kg (oranges, 5 µg/kg; apples, 0.5 µg/kg; grapes, 28 µg/kg; melons, 19 µg/kg). Other weighted mean perchlorate levels were as follows: rice, 1 µg/kg; whole wheat flour, 3.5 µg/kg; milk, 6.8 µg/kg; beer, 1 µg/kg; and wine, 6 µg/kg. The mean perchlorate levels in human milk in China (n = 24 composite samples, with each composite sample representing milk from 50 mothers) and the USA (n = 652) were found to be 19.7 µg/l (range 2.1–136 µg/l) and 9.3 µg/l (range 0.01–411 µg/l), respectively. Limited data show that the weighted mean perchlorate level in infant formula was 10 µg/kg. The Committee

noted that sampling and analysis were carried out on targeted foods. However, general surveys involving a broader range of foods in different countries were lacking. In view of the widespread presence of perchlorate in the environment, it is probable that it will be found more widely in drinking-water and food.

10.8 Food consumption and dietary exposure assessment

The Committee evaluated occurrence and dietary exposure data for perchlorate from submissions from China, Japan, Canada and the USA and from the literature. International estimates of dietary exposure were prepared using food consumption information from the 13 GEMS/Food consumption cluster diets and perchlorate concentrations discussed above. The range of estimated dietary exposures to perchlorate is 0.03–0.22 µg/kg bw per day for the 13 GEMS/Food consumption cluster diets. Milk consumption accounts for a large portion of the dietary exposure to perchlorate for most clusters, ranging from 7% (clusters C and G) to 42% (cluster J) of the total. These estimates do not include dietary exposure from drinking-water. Using the WHO default drinking-water consumption for adults of 2 litres per day and the mean concentration from the USEPA data of 9.9 µg/l in samples in which perchlorate was detected, the Committee estimated that additional perchlorate exposure from drinking-water could be 20 µg/day (0.33 µg/kg bw per day).

The Committee also reviewed national dietary exposure estimates from Canada and the USA. The Canadian estimates were made based on data taken from foods available in a public market using a deterministic approach. Perchlorate concentrations were combined with food consumption data available from the Nutrition Canada Food Consumption Survey (<18 years old) and the Nova Scotia Provincial Nutrition Survey (adults >18 years old, including women of childbearing age). Mean dietary exposures to perchlorate were higher for children than for adults. The mean dietary exposure was approximately 0.04 µg/kg bw per day for children 1–11 years of age compared with 0.03 µg/kg bw per day for adults, including women of childbearing age.

For the USA, an estimate of dietary exposure to perchlorate was made using urinary biomarker concentrations of perchlorate, available from the 2001–2002 NHANES. The dietary exposure (which would include exposure from water) was estimated using the ratio of the perchlorate to creatinine concentrations in urine, assuming that 100% of perchlorate in the diet is absorbed and excreted unmetabolized. The 50th-percentile estimate for the total adult population was 0.06 µg/kg bw per day, whereas the 95th-percentile estimate was 0.23 µg/kg bw per day.

An analysis of perchlorate dietary exposures in the USA from the USFDA's TDS from 2003–2008 was submitted to the Committee. The mean estimates for all age subgroups 14 and over were approximately 0.1 µg/kg bw per day, with 90th-percentile estimates less than 0.23 µg/kg bw per day. Dietary exposure was greatest for 2-year-olds; the mean exposure for this group was 0.44 µg/kg bw per day, with a 90th-percentile exposure of 0.73 µg/kg bw per day.

The Committee concluded that the analyses from the USFDA's TDS provide the best estimates of dietary exposure to perchlorate, as they do not depend on

analyses of raw commodities and include water and beverages made from water that might be contaminated with perchlorate. Although all of the national estimates are in general agreement, the Committee noted that the highest estimates were from the TDS, suggesting that the other estimates (and the international estimates prepared by the Committee) may not include all dietary sources of perchlorate.

The highest estimate of dietary exposure from the USFDA's TDS of 0.7 µg/kg bw per day for 2-year-old children and the mean estimate of 0.1 µg/kg bw per day for those 14 years of age and older were chosen for comparison with the health-based guidance value.

10.9 Dose–response analysis

The pivotal study for dose–response analysis was the human clinical study on 16 male and 21 female healthy adult volunteers published by Greer and co-workers in 2002 (Greer et al., 2002), in which perchlorate was given in drinking-water at doses of 0.007, 0.02, 0.1 or 0.5 mg/kg bw per day for 14 days. The uptake of iodide into the thyroid was measured at baseline before administration of perchlorate and on days 2 and 14 of administration at both 8 and 24 h after administration of radiolabelled iodine. These data were used for modelling.

The Committee selected a critical effect size of 50% inhibition of iodide uptake as the benchmark response. This choice was made because human clinical data from healthy adults following both short-term and chronic exposure to perchlorate have shown that such a level of inhibition is not associated with any changes in TSH or thyroid hormone levels. The Committee noted that a benchmark response of 50% inhibition was within the observed range of the study.

PROAST software (version 23.2) for analysis of continuous data was used. In the clinical study, each subject served as his or her own control, with baseline values measured 1 day before the start of the 14-day perchlorate exposure period. In the BMD analysis, the baseline value for iodide uptake in each subject was set at 100%. Analysis of the post-treatment values for iodide uptake at 8 and 24 h and the values from exposure days 2 and 14 as covariates showed that these factors did not have any significant impact. Therefore, the two models used for continuous data—the exponential and Hill models—were fitted to all the data combined. Similar values for the BMD_{50} and $BMDL_{50}$ were obtained from the two models, as shown in Table 5 in section 9. The lower of the two $BMDL_{50}$ values (rounded to two significant figures) of 0.11 mg/kg bw per day was used as the point of departure.

11. EVALUATION

As perchlorate has a very short half-life and is rapidly cleared from the body, it is considered appropriate to derive a provisional maximum tolerable daily intake (PMTDI). The $BMDL_{50}$ of 0.11 mg/kg bw per day for inhibition of uptake of radiolabelled iodide by the thyroid was chosen as the point of departure for derivation of a PMTDI. As it is based on human data, there is no need to apply any interspecies uncertainty factor.

The Committee noted that the $BMDL_{50}$ was derived from a study of relatively short duration but that there are efficient homeostatic mechanisms to cope with short-term and long-term inhibition of iodide uptake, up to (at least) 50%, in healthy children and adults. The Committee also noted that there is at least a 4-fold margin between the value of the $BMDL_{50}$ and the estimate of more than 0.4 mg/kg bw per day that would probably be necessary as a sustained exposure in order to trigger hypothyroidism in normal adults. The Committee therefore concluded that it was not necessary to apply an uncertainty factor to account for the short duration of the pivotal study.

In considering the size of any necessary uncertainty factor for interindividual human differences, the Committee took account of the fact that the effect of perchlorate on inhibition of iodide uptake by the thyroid and on the subsequent synthesis of thyroid hormones in potentially vulnerable groups—such as pregnant women, fetuses, neonates and young infants, those with iodine-deficient diets and those with clinical or subclinical hypothyroidism—may differ from that in healthy adults. The Committee concluded that an uncertainty factor of 10 would be appropriate to cover any differences in the general population, including those in potentially vulnerable subgroups. Applying this 10-fold factor to the $BMDL_{50}$ and rounding to one significant figure, a PMTDI of 0.01 mg/kg bw was established for perchlorate.

The estimated dietary exposures of 0.7 µg/kg bw per day (highest) and 0.1 µg/kg bw per day (mean), including both food and drinking-water, are well below the PMTDI. The Committee considered that these estimated dietary exposures were not of health concern.

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ANNEXES

ANNEX 1

REPORTS AND OTHER DOCUMENTS RESULTING FROM PREVIOUS MEETINGS OF THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

1. *General principles governing the use of food additives* (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. *Procedures for the testing of intentional food additives to establish their safety for use* (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. *Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants)* (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, Vol. I. *Antimicrobial preservatives and antioxidants*, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. *Specifications for identity and purity of food additives (food colours)* (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, Vol. II. *Food colours*, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. *Evaluation of the carcinogenic hazards of food additives* (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. *Evaluation of the toxicity of a number of antimicrobials and antioxidants* (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
7. *Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents* (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).
8. *Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants* (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).
9. *Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants*. FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).

10. *Specifications for identity and purity and toxicological evaluation of food colours*. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.
11. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases* (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).
12. *Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases.* FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.
13. *Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances* (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
14. *Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non nutritive sweetening agents* (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
15. *Toxicological evaluation of some flavouring substances and non nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.
16. *Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.
17. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics* (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.
18. *Specifications for the identity and purity of some antibiotics.* FAO Nutrition Meetings Series, No. 45A, 1969; WHO/Food Add/69.34.
19. *Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances* (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.
20. *Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances.* FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36.
21. *Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives.* FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.
22. *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, diethylpyrocarbamate, and octyl gallate.* FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.
32. *Toxicological evaluation of certain food additives with a review of general principles and of specifications* (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).
33. *Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents.* FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974.
34. *Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers.* FAO Food and Nutrition Paper, No. 4, 1978.
35. *Evaluation of certain food additives* (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.

36. *Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives.* FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.
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ANNEX 2

ABBREVIATIONS USED IN THE MONOGRAPHS

β-NF	β-naphthoflavone
3-Ac-DON	3-acetyl-deoxynivalenol
3-APA	3-aminopropionamide
8-OHdG	8-hydroxy-2′-deoxyguanosine
15-Ac-DON	15-acetyl-deoxynivalenol
AA	acrylamide
AA-GS	acrylamide–glutathione conjugate
AAMA	*N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine
AAS	atomic absorption spectrometry
AB	arsenobetaine
ABT	aminobenzotriazole
AC	acetylcholine
ACE	angiotensin-converting enzyme
ADP	adenosine diphosphate
AFS	atomic fluorescence spectrometry
AH	aniline hydroxylase
AIC	Akaike's information criterion
ALT	alanine aminotransferase
AM	arbuscular mycorrhizae
AOAC	Association of Official Analytical Chemists
AP	alkaline phosphatase
API	atmospheric pressure ionization
ARfD	acute reference dose
ASE	accelerated solvent extraction
AST	aspartate aminotransferase
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
AUC	area under the curve
BALF	bronchoalveolar lavage fluid
BCC	basal cell carcinoma
BDA	*cis*-2-butene-1,4-dial
BHT	butylated hydroxytoluene
BMC	benchmark concentration
BMD	benchmark dose
BMD_{10}	benchmark dose for a 10% response
BMDL	95% lower confidence limit on the benchmark dose
$BMDL_{10}$	95% lower confidence limit on the benchmark dose for a 10% response
BMDS	Benchmark Dose Software
$BMDU_{10}$	95% upper confidence limit on the benchmark dose for a 10% response
BMI	body mass index
BMR	benchmark response

BrdU	5-bromo-2′-deoxyuridine
bw	body weight
CALIPSO	Fish and Seafood Consumption Study and Biomarkers of Exposure to Trace Elements, Pollutants and Omega-3
CAPS	Cancer of the Prostate in Sweden
CAS	Chemical Abstracts Service
CCD	charge coupled device
CD	conductivity detector
cDNA	complementary DNA
CE	capillary electrophoresis
CHO	Chinese hamster ovary
CI	confidence interval
CIAA	Confederation of the Food and Drink Industries of the European Union
CIS	cytokine-inducible SH2 domain protein
C_{max}	maximum plasma concentration
CNAS	Chinese National Standards Agency
COX-2	cyclooxygenase-2
CSFII	Continuing Survey of Food Intakes by Individuals
CSM	Cohort of Swedish Men
CV	coefficient of variation
CV-AAS	cold vapour atomic absorption spectrometry
CV-AFS	cold vapour atomic fluorescence spectrometry
CYP	cytochrome P450
dAdo	2′-deoxyadenosine
dCyd	2′-deoxycytidine
DDCH	Danish Diet, Cancer and Health
DES	diethylstilbestrol
dGuo	2′-deoxyguanosine
DMA	dimethylarsenic species
DMA^{III}	dimethylarsinous acid
DMA^{V}	dimethylarsinic acid
DMAE	dimethylarsionylethanol
$DMDTA^{V}$	dimethyldithioarsinic acid
$DMMTA^{V}$	dimethylmonothioarsinic acid
DNA	deoxyribonucleic acid
DON	deoxynivalenol
DQ	1,12-bis(trimethylammonium) dodecane
dw	dry weight
DW	drinking-water
DWEL	drinking-water equivalent level
EC	European Commission; electron capture
ECD	electron capture detection
ECOD	7-ethoxycoumarin-*O*-deethylase
EFSA	European Food Safety Authority

Eh	redox potential
ELISA	enzyme-linked immunosorbent assay
EMS	ethyl methanesulfonate
EPA	Environmental Protection Agency (USA)
EPHX1	microsomal epoxide hydrolase
EPIC	European Prospective Investigation into Cancer and Nutrition
Eq	equivalent to
ER+	estrogen receptor positive
EROD	ethoxyresorufin-*O*-deethylase
ESI	electrospray ionization
EU	European Union
f	female
F	filial generation (e.g. F_1)
FAO	Food and Agriculture Organization of the United Nations
FAPAS	Food Analysis Performance Assessment Scheme
FASI	field amplified sample injection
FFQ	food frequency questionnaire
FHB	*Fusarium* head blight
FID	flame ionization detection
FSANZ	Food Standards Australia New Zealand
FSH	follicle stimulating hormone
GA	glycidamide
GABAergic	γ-aminobutyric acid-releasing
GA-GS	glycidamide–glutathione conjugate
GAMA	*N*-acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)-L-cysteine
GC	gas chromatography
GD	gestation day
GEMS/Food	Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
GGT	gamma glutamyl transferase
GH	growth hormone
GIS	geographic information system
GPT	glutamate–pyruvate transaminase
GS	glutathione
GSH	glutathione
GST	glutathione *S*-transferase
GST-P	glutathione *S*-transferase-pi
Gua	guanine
HBD-1	human β-defensin-1
H&E	haematoxylin and eosin
HG	hydride generation
HgT	total mercury
HPLC	high-performance liquid chromatography
HPRT	hypoxanthine guanine phosphoribosyltransferase

HPT	hypothalamic–pituitary–thyroid
HR	hazard ratio
HS	headspace
HSD	Honestly Significant Difference
HS-SPME	headspace solid-phase microextraction
IARC	International Agency for Research on Cancer
IC	ion chromatography
IC_{50}	median inhibitory concentration
ICP-AES	inductively coupled plasma atomic emission spectrometry
ICP-MS	inductively coupled plasma mass spectrometry
IFN	interferon
IgA	immunoglobulin A
IGF	insulin-like growth factor
IGFAL	insulin-like growth factor acid-labile subunit
IGFBP3	insulin-like growth factor binding protein 3
IgG	immunoglobulin G
IgM	immunoglobulin M
IL	interleukin
INRAN	National Research Institute for Food and Nutrition (Italy)
ip	intraperitoneal
IP	intraperitoneal
IPCS	International Programme on Chemical Safety (WHO)
IRMM	Institute for Reference Materials and Measurements (European Commission)
IRR	incidence rate ratio
IU	international unit
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
K_m	Michaelis-Menten constant
LB	lower bound
LC	liquid chromatography
LD_{50}	median lethal dose
LD_{lo}	lowest reported lethal dose
LDH	lactate dehydrogenase
LH	luteinizing hormone
LI	labelling index
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOEL	lowest-observed-effect level
LOQ	limit of quantification
LOR	limit of reporting
LPS	lipopolysaccharides
m	male
MAE	microwave-assisted digestion

MCP-1	monocyte chemotactic protein-1
MDL	method detection limit
MeHg	methylmercury
MEL	maximum exposure level
MIP-AES	microwave-induced plasma atomic emission spectrometry
MMA	monomethylarsenic species
MMA^{III}	monomethylarsonous acid
MMA^{V}	monomethylarsonic acid
$MMMTA^{V}$	monomethylthioarsonic acid
MMS	methyl methanesulfonate
MOE	margin of exposure
MRL	minimum reporting limit; minimal risk level
mRNA	messenger ribonucleic acid
MROD	methoxyresorufin-*O*-deethylase
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NA	no association; not available
NAC	*N*-acetylcysteine
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NAG	*N*-acetyl-β-glucosaminidase
NAS	National Academy of Sciences (USA)
NCTR	National Center for Toxicological Research (USA)
ND	not determined; not detected
NDNS	National Diet and Nutrition Survey
N7-GA-Gua	N7-glycidamide–guanine
NHANES	National Health and Nutrition Examination Survey (USA)
NHS	Nurses' Health Study
NIS	sodium–iodide symporter
NIV	nivalenol
NK	natural killer
NLCS	Netherlands Cohort Study
NMR	nuclear magnetic resonance
NMSC	non-melanoma skin cancer
NNS	National Nutrition Survey
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NQ	not quantified
NS	non-smoker
NTP	National Toxicology Program (USA)
OECD	Organisation for Economic Co-operation and Development

OR	odds ratio
P	parental generation (e.g. P_1)
P95	95th percentile
P97.5	97.5th percentile
PB	phenobarbital
PBPK	physiologically based pharmacokinetic
PBTK	physiologically based toxicokinetic
PCB	pentachlorobiphenyl
PCF	perchlorate in food
PCR	polymerase chain reaction
PCW	perchlorate in water
PD	pharmacodynamic
PET	polyethylene terephthalate
PK	pharmacokinetic
PMTDI	provisional maximum tolerable daily intake
PND	postnatal day
POR	prevalence odds ratio
ppm	parts per million
PQL	practical quantification limit
PRG	preliminary remediation goal
PROD	pentoxyresorufin-*O*-deethylase
PTWI	provisional tolerable weekly intake
PY	pyrazole
QT_c	corrected QT interval
RDS	replicative DNA synthesis
RfD	reference dose
RNA	ribonucleic acid
RR	relative risk
RSD	relative standard deviation
RT-PCR	reverse transcriptase polymerase chain reaction
S	smoker
S9	9000 × *g* supernatant fraction from rodent liver
SAHC	*S*-adenosylhomocysteine
SAM	*S*-adenosylmethionine
SAR	Special Administrative Region
SCC	squamous cell carcinoma
SCE	sister chromatid exchange
SCOOP	Scientific Co-operation on Questions relating to Food
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SID-MS	speciated isotope dilution mass spectrometry
SIR	standardized incidence ratio
SMC	Swedish Mammography Cohort
SMR	standardized mortality ratio

SO	sulfoxide
SOCS	suppressors of cytokine signalling
SPDE	solid-phase dynamic extraction
SPE	solid-phase extraction
StAR	steroidogenic acute regulatory
SWLHC	Swedish Women's Lifestyle and Health Cohort
T_3	triiodothyronine
T_4	thyroxine
TBG	thyroxine binding globulin
TCC	transitional cell carcinoma
TDI	tolerable daily intake
TDS	total diet study
TG	transgenic
TGFα	transforming growth factor-alpha
TK	thymidine kinase
TLC	thin-layer chromatography
TMA^+	tetramethylarsonium ion
TMAO	trimethylarsine oxide
TMAP	trimethylarsoniopropionate
TNF-α	tumour necrosis factor-alpha
TPA	12-*O*-tetradecanoyl phorbol-13-acetate
TRH	thyrotropin releasing hormone
TSH	thyroid stimulating hormone
UB	upper bound
UDS	unscheduled DNA synthesis
UPLC	ultraperformance liquid chromatography
USA	United States of America
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
UV	ultraviolet
Val	valine
V_{max}	maximum rate
WHO	World Health Organization
WT	wild type
ww	wet weight
ZEA	zearalenone

ANNEX 3

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Rome, 16–25 February 2010

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ANNEX 4

SUMMARY OF TOXICOLOGICAL EVALUATIONS

Acrylamide

Dietary exposure estimates:

Mean 0.001 mg/kg body weight (bw) per day

High 0.004 mg/kg bw per day

Effect	NOAEL/ $BMDL_{10}$ (mg/kg bw per day)	MOE at		Conclusion/comments
		Mean dietary exposure	High dietary exposure	
Morphological changes in nerves in rats	0.2 (NOAEL)	200	50	The Committee noted that while adverse neurological effects are unlikely at the estimated average exposure, morphological changes in nerves cannot be excluded for individuals with a high dietary exposure to acrylamide.
Mammary tumours in rats	0.31 ($BMDL_{10}$)	310	78	The Committee considered that for a compound that is both genotoxic and carcinogenic, these MOEs indicate a health concern.
Harderian gland tumours in mice	0.18 ($BMDL_{10}$)	180	45	

$BMDL_{10}$, lower limit on the benchmark dose for a 10% response; bw, body weight; MOE, margin of exposure; NOAEL, no-observed-adverse-effect level

Arsenic

The inorganic arsenic lower limit on the benchmark dose for a 0.5% increased incidence of lung cancer ($BMDL_{0.5}$) was determined from epidemiological studies to be 3.0 µg/kg bw per day (2–7 µg/kg bw per day based on the range of estimated total dietary exposure) using a range of assumptions to estimate total dietary exposure to inorganic arsenic from drinking-water and food. The Committee noted that the provisional tolerable weekly intake (PTWI) of 15 µg/kg bw (equivalent to 2.1 µg/kg bw per day) is in the region of the $BMDL_{0.5}$ and therefore was no longer appropriate. The Committee withdrew the previous PTWI.

Deoxynivalenol (DON)

As 3-acetyl-deoxynivalenol (3-Ac-DON) is converted to deoxynivalenol (DON) in vivo and therefore contributes to the total DON-induced toxicity, the Committee decided to convert the provisional maximum tolerable daily intake (PMTDI) for DON to a group PTMDI of 1 µg/kg bw for DON and its acetylated derivatives (3-Ac-DON and 15-Ac-DON). In this regard, the Committee considered the toxicity of the acetylated derivatives to be equal to that of DON. The Committee concluded that, at this time, there was insufficient information to include DON-3-glucoside in the group PMTDI.

The Committee derived a group acute reference dose (ARfD) of 8 µg/kg bw for DON and its acetylated derivatives using the lowest lower limit on the benchmark dose for a 10% response ($BMDL_{10}$) of 0.21 mg/kg bw per day for emesis in pigs. Limited data from human case reports indicated that dietary exposures to DON up to 50 µg/kg bw per day are not likely to induce emesis.

The Committee concluded that all of the mean estimates of national exposure to DON were below the group PMTDI of 1 µg/kg bw. National reports showed dietary exposures that were above 1 µg/kg bw per day in only a few cases, only for children at upper percentiles. For acute dietary exposure, the estimate of 9 µg/kg bw per day, based on high consumption of bread and a regulatory limit for DON of 1 mg/kg food, was close to the group ARfD.

Group PTMDI: 1 µg/kg bw for DON and its acetylated derivatives

Group ARfD: 8 µg/kg bw for DON and its acetylated derivatives

Furan

Dietary exposure estimates:

Mean 0.001 mg/kg bw per day

High 0.002 mg/kg bw per day

Effect	$BMDL_{10}$ (mg/kg bw per day)	MOE at		Conclusion/comments
		Mean dietary exposure	High dietary exposure	
Hepatocellular adenomas and carcinomas in female mice	1.3	1300	650	The Committee considered that these MOEs indicate a human health concern for a carcinogenic compound that might act via a DNA-reactive genotoxic metabolite.

$BMDL_{10}$, lower limit on the benchmark dose for a 10% response; bw, body weight; DNA, deoxyribonucleic acid; MOE, margin of exposure

Mercury

The Committee established a PTWI for inorganic mercury of 4 µg/kg bw. The previous PTWI of 5 µg/kg bw for total mercury, established at the sixteenth meeting, was withdrawn.

The new PTWI for inorganic mercury was considered applicable to dietary exposure to total mercury from foods other than fish and shellfish. The upper limits of estimates of average dietary exposure to total mercury from foods other than fish and shellfish for adults (1 µg/kg bw per week) and for children (4 µg/kg bw per week) were at or below the PTWI.

PTWI: 4 µg/kg bw for inorganic mercury

Perchlorate

The Committee established a PMTDI of 0.01 mg/kg bw for perchlorate. The estimated dietary exposures of 0.7 µg/kg bw per day (highest) and 0.1 µg/kg bw per day (mean), including both food and drinking-water, are well below the PMTDI. The Committee considered that these estimated dietary exposures were not of health concern.

PMTDI: 0.01 mg/kg bw

This volume contains monographs prepared at the seventy-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met in Rome, Italy, from 16 to 25 February 2010.

The detailed monographs in this volume summarize the technical, analytical, dietary exposure and toxicological data on a number of contaminants in food: acrylamide, arsenic, deoxydivalenol, furan, mercury and perchlorate.

This volume and others in the WHO Food Additives series contain information that is useful to those who produce and use food additives and veterinary drugs and those involved with controlling contaminants in food, government and food regulatory officers, industrial testing laboratories, toxicological laboratories and universities.

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