WHO FOOD ADDITIVES SERIES: 63

FAO JECFA MONOGRAPHS 8

Safety evaluation of certain contaminants in food

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

ACRYLAMIDE
(addendum)
(pages 1 – 151)

World Health Organization, Geneva, 2011

Food and Agriculture Organization of the United Nations, Rome, 2011

ACRYLAMIDE (addendum)

First draft prepared by

U. Mueller,[1] A. Agudo,[2] C. Carrington,[3] D. Doerge,[4] K.-E. Hellenäs,[5] J.-C. Leblanc,[6] M. Rao,[7] A. Renwick,[8] W. Slob[9] and Y. Wu[10]

[1] Risk Assessment – Chemical Safety, Food Standards Australia New Zealand, Canberra, Australia
[2] Cancer Epidemiology Research Program, Catalan Institute of Oncology, L'Hospitalet de Llobregat, Spain
[3] Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, United States of America (USA)
[4] National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR, USA
[5] National Food Administration, Uppsala, Sweden
[6] Chemical Exposure and Quantitative Risk Assessment Unit, French Agency for Food, Environment and Occupational Health Safety (ANSES), Maisons-Alfort, France
[7] Quality Control Department, Department of the President's Affairs, Al Ain, United Arab Emirates
[8] School of Medicine, University of Southampton, Ulverston, England
[9] National Institute of Public Health and the Environment (RIVM), Bilthoven, the Netherlands
[10] National Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, Beijing, China

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

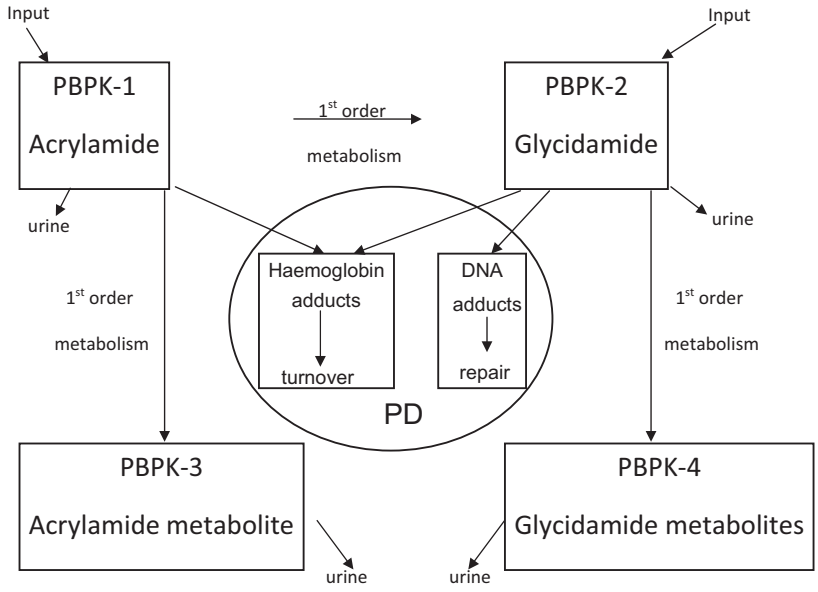
Several publications have reported various approaches to PBPK modelling of acrylamide absorption, metabolism and disposition, with the goal of predicting human internal exposures to acrylamide and glycidamide.

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

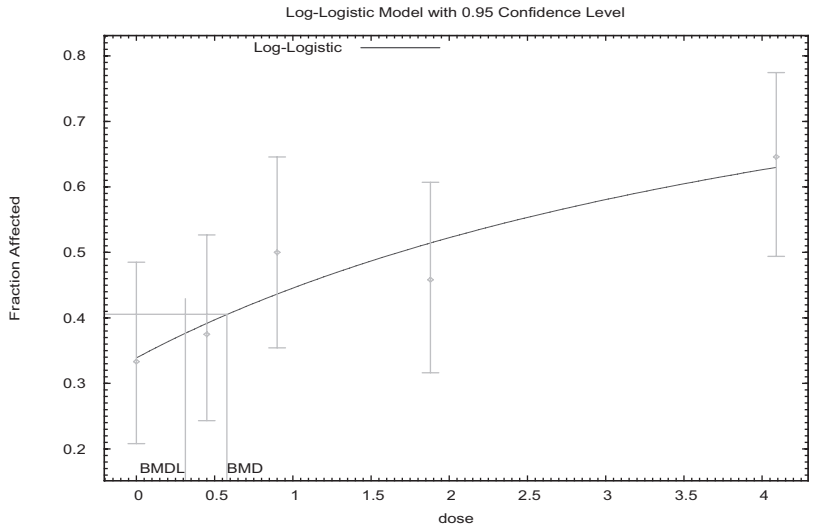
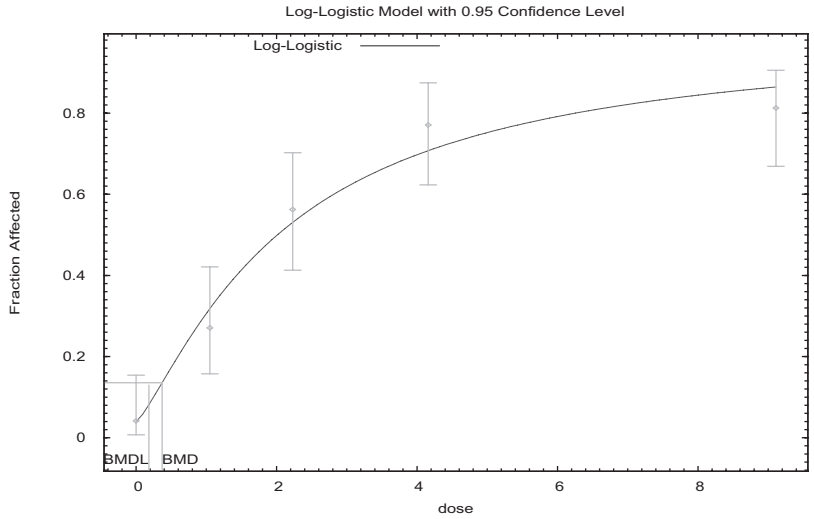


Figure 3. Log-logistic model for female rat mammary gland fibroadenoma



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.

11. REFERENCES

Academy of Health and Food Science of Democratic People's Republic of Korea (2009). Acrylamide occurrence in food. Data submitted to FAO/WHO.

AFSSA (2009). Estimates of acrylamide occurrence in food and dietary exposure to acrylamide from the second Total Diet Study. Preliminary unpublished data submitted to FAO/WHO from the French Food Safety Agency.

Alpmann A, Morlock G (2008). Rapid and sensitive determination of acrylamide in drinking water by planar chromatography and fluorescence detection after derivatization with dansulfinic acid. *Journal of Separation Science*, 31:71–77.

Amrein TM et al. (2007). Occurrence of acrylamide in selected foods and mitigation options. *Food Additives and Contaminants*, 24(Suppl. 1):13–25.

Anese M et al. (2009). Effect of low-temperature long-time pre-treatment of wheat on acrylamide concentration in short dough biscuits. *Molecular Nutrition and Food Research*, 53(12):1526–1531.

Arisseto AP et al. (2007). Determination of acrylamide levels in selected foods in Brazil. *Food Additives and Contaminants*, 24(3):236–241.

Arisseto AP et al. (2009). Contribution of selected foods to acrylamide dietary exposure by a population of Brazilian adolescents. *Food Science and Technology*, 42:207–211.

Arribas-Lorenzo G, Morales FJ (2009). Dietary exposure to acrylamide from potato crisps to the Spanish population. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 26(3):289–297.

Aureli F et al. (2007). An absorption study of dietary administered acrylamide in swine. *Food and Chemical Toxicology*, 45:1202–1209.

Baka S, Erim FB (2007). NACE for the analysis of acrylamide in food. *Electrophoresis,* 28:4108–4113.

Baum M et al. (2008). Fate of ^{14}C-acrylamide in roasted and ground coffee during storage. *Molecular Nutrition and Food Research*, 52(5):600–608.

Becalski A et al. (2003). Acrylamide in foods: occurrence, sources, and modeling. *Journal of Agricultural and Food Chemistry*, 51(3):802–808.

Beland FA (2008). *Tumorigenicity and mutagenicity of acrylamide and its metabolite glycidamide in the neonatal mouse bioassay.* Washington, DC, The Toxicology Forum. Unpublished study submitted to FAO/WHO by the United States National Center for Toxicological Research, Jefferson, AK.

Beland FA (2010). *Technical report for experiment No. 2150.05 and 2150.07. Genotoxicity and carcinogenicity of acrylamide and its metabolite, glycidamide, in rodents: two year chronic study of acrylamide in B6C3F1 mice and F334 rats.* Unpublished study. Submitted to FAO/WHO by the United States National Center for Toxicological Research, Jefferson, AK.

Bermudo E et al. (2004). Microemulsion electrokinetic chromatography for the analysis of acrylamide in food. *Electrophoresis*, 25:3257–3262.

Bermudo E et al. (2006a). Analysis of acrylamide in food samples by capillary zone electrophoresis. *Journal of Chromatography A*, 1120:199–204.

Bermudo E et al. (2006b). Analysis of acrylamide in food products by in-line preconcentration capillary zone electrophoresis. *Journal of Chromatography A*, 1129:129–134.

Bermudo E et al. (2007). Field amplified sample injection–capillary electrophoresis–tandem mass spectrometry for the analysis of acrylamide in foodstuffs. *Journal of Chromatography A*, 1159:225–232.

Bermudo E et al. (2008). Liquid chromatography coupled to tandem mass spectrometry for the analysis of acrylamide in typical Spanish products. *Talanta*, 76:389–394.

Biedermann M, Grob K (2008). In GC-MS, acrylamide from heated foods may be coeluted with 3-hydroxy propionitrile. *European Food Research and Technology*, 227:945–948.

Bjellaas T et al. (2005). Determination and quantification of urinary metabolites after exposure to acrylamide. *Xenobiotica*, 35:1003–1018.

Bjellaas T et al. (2007a). Urinary acrylamide metabolites as biomarkers for short-term dietary exposure to acrylamide. *Food and Chemical Toxicology*, 45:1020–1026.

Bjellaas T et al. (2007b). Comparison of estimated dietary intake of acrylamide with hemoglobin adducts of acrylamide and glycidamide. *Toxicological Sciences*, 98(1): 110–117.

Boettcher MI et al. (2005). Mercapturic acids of acrylamide and glycidamide as biomarkers of the internal exposure to acrylamide in the general population. *Mutation Research*, 580:167–176.

Boettcher MI et al. (2006). Excretion of mercapturic acids of acrylamide and glycidamide in human urine after single oral administration of deuterium-labelled acrylamide. *Archives of Toxicology*, 80:55–61.

Bowyer JF et al. (2008a). The effects of subchronic acrylamide exposure on gene expression, neurochemistry, hormones, and histopathology in the hypothalamus–pituitary–thyroid axis of male Fischer 344 rats. *Toxicology and Applied Pharmacology*, 230:208–215.

Bowyer JF et al. (2008b). Corrigendum to "The effects of subchronic acrylamide exposure on gene expression, neurochemistry, hormones, and histopathology in the hypothalamus–pituitary–thyroid axis of male Fischer 344 rats" (Erratum to: *Toxicology and Applied Pharmacology*, 230: 208–215). *Toxicology and Applied Pharmacology*, 232:498.

Bowyer JF et al. (2009). The mRNA expression and histological integrity in rat forebrain motor and sensory regions are minimally affected by acrylamide exposure through drinking water. *Toxicology and Applied Pharmacology*, 240:401–411.

Brantsaeter AL et al. (2008). Exploration of different methods to assess dietary acrylamide exposure in pregnant women participating in the Norwegian Mother and Child Cohort Study (MoBa). *Food and Chemical Toxicology*, 46:2808–2814.

Burek JD et al. (1980). Subchronic toxicity of acrylamide administered to rats in the drinking water followed by up to 144 days of recovery. *Journal of Environmental Pathology and Toxicology*, 4:157–182.

Calleman CJ (1996). The metabolism and pharmacokinetics of acrylamide: implications for mechanisms of toxicity and human risk estimation. *Drug Metabolism and Disposition*, 28:527–590.

Casado FJ, Montano A (2008). Influence of processing conditions on acrylamide content in black ripe olives. *Journal of Agricultural and Food Chemistry*, 56(6):2021–2027.

Casella IG, Pierri M, Contursi M (2006). Determination of acrylamide and acrylic acid by isocratic liquid chromatography with pulsed electrochemical detection. *Journal of Chromatography A*, 1107:198–203.

Castle L, Eriksson S (2005). Analytical methods used to measure acrylamide concentrations in foods. *Journal of AOAC International*, 88:274–284.

Chen F et al. (2008). Survey of acrylamide levels in Chinese foods. *Food Additives & Contaminants. Part B, Surveillance*, 1(2):85–92.

Chevolleau S et al. (2007). Analysis of hemoglobin adducts of acrylamide and glycidamide by liquid chromatography–electrospray ionization tandem mass spectrometry, as exposure biomarkers in French population. *Journal of Chromatography A*, 1167:125–134.

Chinese CDC (2009). Chinese total diet individual food consumption 2000 and 2007, and 2007 Chinese total diet concentration. Data submitted to FAO/WHO by the Chinese Center for Disease Control and Prevention, Institute of Nutrition and Food Safety.

Chu FL, Yaylayan VA (2009). FTIR monitoring of oxazolidin-5-one formation and decomposition in a glycolaldehyde–phenylalanine model system by isotope labeling techniques. *Carbohydrate Research*, 344(2):229–236.

Chu SG, Metcalfe CD (2007). Analysis of acrylamide in water using a coevaporation preparative step and isotope dilution liquid chromatography tandem mass spectrometry. *Analytical Chemistry*, 79:5093–5096.

Churchwell MI et al. (2005). Improving LC-MS sensitivity through increases in chromatographic performance: comparisons of UPLC-ES/MS/MS to HPLC-ES/MS/MS. *Journal of Chromatography B*, 825:134–143.

CIAA (2009a). *The CIAA acrylamide "toolbox"*. Brussels, Confederation of the Food and Drink Industries of the EU (http://www.ciaa.be/documents/brochures/ac_toolbox_20090216.pdf).

CIAA (2009b). Presentation made by the Confederation of the Food and Drink Industries of the EU at a meeting with the European Commission's "Expert Group on Environmental and Industrial Contaminants", 26 October 2009, Brussels, Belgium.

Ciesarova Z, Suhaj M, Horvathova J (2008). Correlation between acrylamide contents and antioxidant capacities of spice extracts in a model potato matrix. *Journal of Food and Nutrition Research*, 47(1):1–5.

Claus A, Carle R, Schieber A (2008). Acrylamide in cereal products: a review. *Journal of Cereal Science*, 47:118–133.

Claus A et al. (2006). Pyrolytic acrylamide formation from purified wheat gluten and gluten-supplemented wheat bread rolls. *Molecular Nutrition and Food Research*, 50(1):87–93.

Collins JJ et al. (1989). Mortality patterns among workers exposed to acrylamide. *Journal of Occupational Medicine*, 31(7):614–617.

Dabrio M et al. (2008). Production of a certified reference material for the acrylamide content in toasted bread. *Food Chemistry*, 110: 504–511.

Dearfield KL et al. (1995). Acrylamide: a review of its genotoxicity and an assessment of heritable genetic risk. *Mutation Research*, 330:71–99.

Doerge DR et al. (2005a). Toxicokinetics of acrylamide and glycidamide in B6C3F1 mice. *Toxicology and Applied Pharmacology*, 202:258–267.

Doerge DR et al. (2005b). Toxicokinetics of acrylamide and glycidamide in Fischer 344 rats. *Toxicology and Applied Pharmacology*, 208:199–209.

Doerge DR et al. (2007). Urinary excretion of acrylamide and metabolites in Fischer 344 rats and B6C3F1 mice administered a single dose of acrylamide. *Toxicology Letters*, 169:34–42.

Doerge DR et al. (2008). Using dietary exposure and physiologically based pharmacokinetic/pharmacodynamic modeling in human risk extrapolations for acrylamide toxicity. *Journal of Agricultural and Food Chemistry*, 56:6031–6038.

Doroshyenko O et al. (2009). In vivo role of cytochrome P450 2E1 and glutathione-*S*-transferase activity. *Cancer Epidemiology, Biomarkers & Prevention*, 18(2):433–443.

Duale N et al. (2009). Biomarkers of human exposure to acrylamide and relation to polymorphisms in metabolizing genes. *Toxicological Sciences*, 108:90–99.

Dunovská L et al. (2006). Direct determination of acrylamide in food by gas chromatography–high-resolution time-of-flight mass spectrometry. *Analytica Chimica Acta*, 578:234–240.

Dutch Food and Consumer Product Safety Authority (2009). Acrylamide levels in food. Data submitted to FAO/WHO.

Dybing E, Sanner T (2003). Risk assessment of acrylamide in foods. *Toxicological Sciences*, 75:7–15.

EC (2006). *European Union acrylamide monitoring database*. Geel, European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (http://www.irmm.jrc.be/html/activities/acrylamide/database.htm).

EC (2007). Commission recommendation of 3 May 2007 on the monitoring of acrylamide levels in food. *Official Journal of the European Union*, L123:33–40 (2007/331/EC; http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/l_123/l_12320070512en00330040.pdf).

EFSA (2009). Scientific report of EFSA prepared by Data Collection and Exposure Unit (DATEX) on "Monitoring of acrylamide levels in food". *The EFSA Scientific Report*, 285:1–26 (http://www.efsa.europa.eu/en/scdocs/doc/285r.pdf).

Ehling S, Hengel M, Shibamoto T (2005). Formation of acrylamide from lipids. *Advances in Experimental Medicine and Biology*, 561:223–233.

El-Ghorab AH, Fujioka K, Shibamoto T (2006). Determination of acrylamide formed in asparagine/D-glucose Maillard model systems by using gas chromatography with headspace solid-phase microextraction. *Journal of AOAC International*, 89:149–153.

Eriksson S, Karlsson P (2006). Alternative extraction techniques for analysis of acrylamide in food: influence of pH and digestive enzymes. *LWT – Food Science and Technology*, 39:393–399.

Exon JH (2006). A review of the toxicology of acrylamide. *Journal of Toxicology and Environmental Health. Part B, Critical Reviews*, 9:397–412.

FAO/WHO (2009). *Report of the Thirty-second Session of the Codex Alimentarius Commission, Rome, Italy, 29 June – 4 July, 2009.* Rome, Food and Agriculture Organization of the United Nations and World Health Organization (ALINORM 09/32/41; http://www.codexalimentarius.net/download/report/722/al32_41e.pdf).

Fennell TR et al. (2005). Metabolism and hemoglobin adduct formation of acrylamide in humans. *Toxicological Sciences*, 85:447–459.

Fennell TR et al. (2006). Kinetics of elimination of urinary metabolites of acrylamide in humans. *Toxicological Sciences*, 93(2):256–267.

Fernandes JO, Soares C (2007). Application of matrix solid-phase dispersion in the determination of acrylamide in potato chips. *Journal of Chromatography A*, 1175:1–6.

Fohgelberg P et al. (2005). The acrylamide intake via some common baby food for children in Sweden during their first year of life—an improved method for analysis of acrylamide. *Food and Chemical Toxicology*, 43:951–959.

Foot RJ et al. (2007). Acrylamide in fried and roasted potato products: a review on progress in mitigation. *Food Additives and Contamination*, 24(Suppl. 1):37–46.

Friedman M, Levin CE (2008). Review of methods for the reduction of dietary content and toxicity of acrylamide. *Journal of Agricultural and Food Chemistry*, 56(15):6113–6140.

Friedman MA, Dulak LH, Stedman MA (1995). A lifetime oncogenicity study in rats with acrylamide. *Fundamental and Applied Toxicology*, 27:95–105.

Fuhr U et al. (2006). Toxicokinetics of acrylamide in humans after ingestion of a defined dose in a test meal to improve risk assessment for acrylamide carcinogenicity. *Cancer Epidemiology, Biomarkers & Prevention*, 15(2):266–271.

Garey J, Paule MG (2007). Effects of chronic low-dose acrylamide exposure on progressive ratio performance in adolescent rats. *NeuroToxicology*, 28:998–1002.

GEMS/Food-EURO (1995). *GEMS/Food-EURO second workshop on reliable evaluation of low-level contamination of food*. Report on a Workshop in the Frame of GEMS/Food-EURO, Kulmbach, Federal Republic of Germany, 26–27 May 1995 (http://www.who.int/foodsafety/publications/chem/en/lowlevel_may1995.pdf).

Geng ZM, Jiang R, Chen M (2008). Determination of acrylamide in starch-based foods by ion-exclusion liquid chromatography. *Journal of Food Composition and Analysis*, 21:178–182.

Gertz C, Klostermann S (2002). *Analysis of acrylamide and mechanisms of its formation in deep-fried products*. Weinheim, Wiley-VCH.

Gertz C, Klostermann S, Kochhar SP (2003). Deep frying: the role of water from food being fried and acrylamide formation. *Oléagineux, Corps Gras, Lipides*, 10:297–303.

Gökmen V et al. (2005). Determination of acrylamide in potato chips and crisps by high-performance liquid chromatography. *Journal of Chromatography A*, 1088:193–199.

Goldmann T et al. (2006). Impact of extraction conditions on the content of acrylamide in model systems and food. *Food Additives and Contaminants*, 23(5):437–445.

Granvogl M, Schieberle P (2006). Thermally generated 3-aminopropionamide as a transient intermediate in the formation of acrylamide. *Journal of Agricultural and Food Chemistry*, 54(16):5933–5938.

Granvogl M, Schieberle P (2007). Quantification of 3-aminopropionamide in cocoa, coffee and cereal products. *European Food Research and Technology*, 225(5):857–863.

Granvogl M et al. (2004). Quantitation of 3-aminopropionamide in potatoes—a minor but potent precursor in acrylamide formation. *Journal of Agricultural and Food Chemistry*, 52(15):4751–4757.

Granvogl M et al. (2007). Influence of sulphur fertilization on the amounts of free amino acids in wheat. Correlation with baking properties as well as with 3-aminopropionamide and acrylamide generation during baking. *Journal of Agricultural and Food Chemistry*, 55(10): 4271–4277.

Grob K (2007). Options for legal measures to reduce acrylamide contents in the most relevant foods. *Food Additives and Contaminants*, 24(Suppl. 1):71–81.

GSA (2006). *A survey of acrylamide in non-carbohydrate based foods*. Government of South Australia, Department of Health, Public Health, Food Policy and Programs Branch (project coordinated by P. Eckert) (http://www.health.sa.gov.au/PEHS/Food/survey-acrylamide-jan07.pdf).

Guo L et al. (2009). Acrylamide and glycidamide induce *cII* mutations in lung tissue of Big Blue mice. *Environmental and Molecular Mutagenesis*, 50:570 (abstract).

Hagmar L et al. (2005). Differences in haemoglobin adduct levels of acrylamide in the general population with respect to dietary intake, smoking habits and gender. *Mutation Research*, 580:157–165.

Hartmann EC et al. (2008). Hemoglobin adducts and mercapturic acid excretion of acrylamide and glycidamide in one study population. *Journal of Agricultural and Food Chemistry*, 56:6061–6068.

Hasegawa K et al. (2007). A rapid and inexpensive method to screen for common foods that reduce the action of acrylamide, a harmful substance in food. *Toxicology Letters*, 175:82–88.

Health Canada (2009). Acrylamide surveillance in foods. Data submitted to FAO/WHO by A. Becalski, Food Research Division, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario.

Hedegaard RV et al. (2008). Acrylamide in bread. Effect of prooxidants and antioxidants. *European Food Research and Technology*, 227(2):519–525.

Hendriksen HV et al. (2009). Evaluating the potential for enzymatic acrylamide mitigation in a range of food products using an asparaginase from *Aspergillus oryzae*. *Journal of Agricultural and Food Chemistry*, 57(1):4168–4176.

Heudorf U, Hartmann E, Angerer J (2009). Acrylamide in children—exposure assessment via urinary acrylamide metabolites as biomarkers. *International Journal of Hygiene and Environmental Health*, 212:135–141.

Hoenicke K et al. (2004). Analysis of acrylamide in different foodstuffs using liquid chromatography–tandem mass spectrometry and gas chromatography–tandem mass spectrometry. *Analytica Chimica Acta*, 520:207–215.

Hogervorst JG et al. (2007). A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiology, Biomarkers & Prevention*, 16:2304–2313.

Hogervorst JG et al. (2008a). Dietary acrylamide intake and the risk of renal cell, bladder, and prostate cancer. *American Journal of Clinical Nutrition*, 87:1428–1438.

Hogervorst JG et al. (2008b). Dietary acrylamide intake is not associated with gastrointestinal cancer risk. *Journal of Nutrition*, 138:2229–2236.

Hogervorst JG et al. (2009a). Lung cancer risk in relation to dietary acrylamide intake. *Journal of the National Cancer Institute*, 101:651–662.

Hogervorst JG et al. (2009b). Dietary acrylamide intake and brain cancer risk. *Cancer Epidemiology, Biomarkers & Prevention*, 18:1663–1666.

Japan Ministry of Health, Labour and Welfare (2009). Acrylamide levels in food. Data submitted to FAO/WHO.

Jin X et al. (2009). *Effects of dietary acrylamide on systematic oxidative stress and inflammatory markers in rats*. Unpublished. Toxicology Research Division, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario. Submitted to FAO/WHO by Health Canada.

Johansson F et al. (2005). Mutagenicity and DNA repair of glycidamide-induced adducts in mammalian cells. *Mutation Research*, 580(1–2):81–89.

Johnson KA et al. (1986). Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicology and Applied Pharmacology*, 85:154–168.

Jones K et al. (2006). Correlation of haemoglobin–acrylamide adducts with airborne exposure: an occupational survey. *Toxicology Letters*, 162:174–180.

Karasek L, Szilláguy S, Wenzl T (2008). *Proficiency test on the determination of acrylamide in potato crisps. Final report.* Luxembourg, Office for Official Publications of the European Communities (JRC Scientific and Technical Reports, EUR 23276 EN-2008; http://irmm.jrc.ec.europa.eu/html/activities/acrylamide/EUR23276EN.pdf).

Kellert M et al. (2006). Quantitation of mercapturic acids from acrylamide and glycidamide in human urine using a column switching tool with two trap columns and electrospray tandem mass spectrometry. *Journal of Chromatography A*, 1131:58–66.

Kirman CR et al. (2003). A physiologically based pharmacokinetic model for acrylamide and its metabolite, glycidamide, in the rat. *Journal of Toxicology and Environmental Health. Part A*, 66:253–274.

Knol JJ et al. (2005). Toward a kinetic model for acrylamide formation in a glucose–asparagine reaction system. *Journal of Agricultural and Food Chemistry*, 53:6133–6139.

Koch M et al. (2009). Development of two certified reference materials for acrylamide determination in foods. *Journal of Agricultural and Food Chemistry*, 57(18):8202–8207.

Konings EJ et al. (2007). Acrylamide in cereal and cereal products: a review on progress in level reduction. *Food Additives and Contaminants*, 24(Suppl. 1):47–59.

Kopp EK, Dekant W (2009). Toxicokinetics of acrylamide in rats and humans following single oral administration of low doses. *Toxicology and Applied Pharmacology*, 235:135–142.

Kopp EK et al. (2008). Rapid and sensitive HILIC-ESI-MS/MS quantitation of polar metabolites of acrylamide in human urine using column switching with an online trap column. *Journal of Agricultural and Food Chemistry*, 56:9828–9834.

Koyama N et al. (2006). Genotoxicity of acrylamide and glycidamide in human lymphoblastoid TK6 cells. *Mutation Research*, 603:151–158.

Kütting B, Uter WW, Drexler H (2008). The association between self-reported acrylamide intake and haemoglobin adducts as biomarkers of exposure. *Cancer Causes and Control*, 19:273–281.

Kütting B et al. (2009). Acrylamide as environmental noxious agent: a health risk assessment for the general population based on the internal acrylamide burden. *International Journal of Hygiene and Environmental Health*, 212:470–480.

Larsson SC, Akesson A, Wolk A (2009a). Long-term dietary acrylamide intake and breast cancer risk in a prospective cohort of Swedish women. *American Journal of Epidemiology*, 169:376–381.

Larsson SC, Akesson A, Wolk A (2009b). Long-term dietary acrylamide intake and risk of epithelial ovarian cancer in a prospective cohort of Swedish women. *Cancer Epidemiology, Biomarkers & Prevention*, 18:994–997.

Larsson SC, Akesson A, Wolk A (2009c). Long-term dietary acrylamide intake and risk of endometrial cancer in a prospective cohort of Swedish women. *International Journal of Cancer*, 124:1196–1199.

Larsson SC, Akesson A, Wolk A (2009d). Dietary acrylamide intake and prostate cancer risk in a prospective cohort of Swedish men. *Cancer Epidemiology, Biomarkers & Prevention*, 18:1939–1941.

Levine RA, Smith RE (2005). Sources of variability of acrylamide levels in a cracker model. *Journal of Agricultural and Food Chemistry*, 53(11):4410–4416.

Love J, Grounds P (2006). *Chemical food safety: acrylamide in New Zealand food*. Prepared by Institute of Environmental Science & Research Ltd, Christchurch, for New Zealand Food

Safety Authority (http://www.nzfsa.govt.nz/science/research-projects/acrylamide/FW0545_Acrylamide_2006.pdf).

MAPA (2005). *La alimentacion en Espana*. Madrid, Ministerio de Agricultura, Pesca y Alimentacion.

Marín JM et al. (2006). Study of different atmospheric-pressure interfaces for LC-MS/MS determination of acrylamide in water at sub-ppb levels. *Journal of Mass Spectrometry*, 41:1041–1048.

Marsh GM et al. (1999). Mortality patterns among workers exposed to acrylamide: 1994 follow up. *Occupational and Environmental Medicine*, 56:181–190.

Marsh GM et al. (2007). Mortality patterns among workers exposed to acrylamide: updated follow up. *Journal of Occupational and Environmental Medicine*, 49:82–95.

Martins C et al. (2007). Cytogenetic damage induced by acrylamide and glycidamide in mammalian cells: correlation with specific glycidamide–DNA adducts. *Toxicological Sciences*, 95:383–390.

Mastovska K, Lehotay SJ (2006). Rapid sample preparation method for LC-MS/MS or GC-MS analysis of acrylamide in various food matrices. *Journal of Agricultural and Food Chemistry*, 54:7001–7008.

Matissek R, Raters M (2005). Analysis of acrylamide in food: dedicated to Professor Dr. Werner Baltes. *Advances in Experimental Medicine and Biology*, 561:293–302.

Mei N et al. (2008). Genotoxic effects of acrylamide and glycidamide in mouse lymphoma cells. *Food and Chemical Toxicology*, 46:628–636.

Mei N et al. (2010). The genotoxicity of acrylamide and glycidamide in Big Blue rats. *Toxicological Sciences*, 115:412–421.

Mestdagh F et al. (2008). Importance of oil degradation components in the formation of acrylamide in fried foodstuffs. *Journal of Agricultural and Food Chemistry*, 56(15): 6141–6144.

Mestdagh FJ et al. (2005). Influence of oil type on the amounts of acrylamide generated in a model system and in french fries. *Journal of Agricultural and Food Chemistry*, 53(15): 6170–6174.

MHPRC (2004). *The nutrition and health status of the Chinese people*. Beijing, Ministry of Health of the People's Republic of China.

Miller MJ, Carter DE, Sipes IG (1982). Pharmacokinetics of acrylamide in Fischer-344 rats. *Toxicology and Applied Pharmacology*, 63:36–44.

Mills C et al. (2008). Dietary acrylamide exposure estimates for the United Kingdom and Ireland: comparison between semiprobabilistic and probabilistic exposure models. *Journal of Agricultural and Food Chemistry*, 56:6039–6045.

Ministry of Health, People's Republic of China (2005). *GB/T 5009.204-2005: GC-MS method for determination of acrylamide in food.* Issued on 9 September 2005. Beijing, Standard Press of China.

Ministry of Health, People's Republic of China (2010). *GB/T 5009.204-2010: Determination of acrylamide in foods*. Updated in 2010. Beijing, Standard Press of China.

Mizukami Y et al. (2006). Analysis of acrylamide in green tea by gas chromatography–mass spectrometry. *Journal of Agricultural and Food Chemistry*, 54:7370–7377.

Mucci LA, Adami HO, Wolk A (2006). Prospective study of dietary acrylamide and risk of colorectal cancer among women. *International Journal of Cancer*, 118:169–173.

Mucci LA, Sandin S, Magnusson C (2005). Acrylamide intake and breast cancer risk in Swedish women. *JAMA: the Journal of the American Medical Association*, 293:1326–1327.

Mucci LA et al. (2003). Dietary acrylamide and cancer of the large bowel, kidney, and bladder: absence of an association in a population-based study in Sweden. *British Journal of Cancer*, 88:84–89.

Mucci LA et al. (2004). Dietary acrylamide and risk of renal cell cancer. *International Journal of Cancer*, 109:774–776.

Muttucumaru N et al. (2008). Reducing acrylamide precursors in raw materials derived from wheat and potato. *Journal of Agricultural and Food Chemistry*, 56(15):6167–6172.

Neafsey P et al. (2009). Genetic polymorphism in CYP2E1: population distribution of CYP2E1 activity. *Journal of Toxicology and Environmental Health. Part B*, 12:362–388.

New Zealand Food Safety Authority (2009). Acrylamide levels in coffee. Data submitted to FAO/WHO.

Nielsen NJ et al. (2006). A liquid chromatography–tandem mass spectrometry method for simultaneous analysis of acrylamide and the precursors, asparagine and reducing sugars in bread. *Analytica Chimica Acta*, 557:211–220.

Norwegian Food Safety Authority (2006). [Analysis of acrylamide in selected products: potatoes, cereal and coffee.] In: *Report on the levels of acrylamide in Norwegian foods.* Norwegian Food Safety Authority (in Norwegian).

Olesen PT et al. (2008). Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health study. *International Journal of Cancer*, 122:2094–2100.

Ölmez H et al. (2008). A survey of acrylamide levels in foods from the Turkish market. *Journal of Food Composition and Analysis*, 21:564–568.

Paleologos EK, Kontominas MG (2005). Determination of acrylamide and methacrylamide by normal phase liquid chromatography and UV detection. *Journal of Chromatography A*, 1077:128–135.

Pelucchi C et al. (2004). Re: Fried potatoes and human cancer. *International Journal of Cancer*, 108:636–637.

Pelucchi C et al. (2006). Dietary acrylamide and human cancer. *International Journal of Cancer*, 118:467–471

Pelucchi C et al. (2007). Dietary acrylamide and renal cell cancer. *International Journal of Cancer*, 120:1376–1377.

Perez Locas C, Yaylayan V (2008). A. Further insight into thermally and pH-induced generation of acrylamide from glucose/asparagine model systems. *Journal of Agricultural and Food Chemistry*, 56(15):6069–6074.

Petersson EV et al. (2006). Critical factors and pitfalls affecting the extraction of acrylamide from foods: an optimisation study. *Analytica Chimica Acta*, 557:287–295.

Poland National Food and Nutrition Institute (2009). Acrylamide levels in food. Data submitted to FAO/WHO.

Preston A, Fodey T, Elliott C (2008). Development of a high-throughput enzyme-linked immunosorbent assay for the routine detection of the carcinogen acrylamide in food, via rapid derivatisation pre-analysis. *Analytica Chimica Acta*, 608:178–185.

Puppel N et al. (2005). DNA strand breaking capacity of acrylamide and glycidamide in mammalian cells. *Mutation Research*, 580(1–2):71–80.

Raju J, Mehta R (2009). *Short-term rat bio-assay using colon aberrant crypt foci as surrogate marker to assess colon cancer modulating effects of dietary acrylamide*. Unpublished report. Toxicology Research Division, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario. Submitted to FAO/WHO by Health Canada.

Rommens CM et al. (2008). Low-acrylamide french fries and potato chips. *Plant Biotechnology Journal*, 6(8):843–853.

Rosén J, Hellenäs KE (2002). Analysis of acrylamide in cooked foods by liquid chromatography–tandem mass spectrometry. *Analyst*, 127:880–882.

Rosén J, Nyman A, Hellenäs KE (2007). Retention studies of acrylamide for the design of a robust liquid chromatography–tandem mass spectrometry method for food analysis. *Journal of Chromatography A*, 1172:19–24.

Rüdiger W (2004). Acrylamide in heated potato products—analytics and formation routes. *European Journal of Lipid Science and Technology*, 106(11):786–792.

Rufián-Henares JA, Morales FJ (2006). Determination of acrylamide in potato chips by a reverse-phase LC-MS method based on a stable isotope dilution assay. *Food Chemistry*, 97:555–562.

Rufián-Henares JA, Delgado-Andrade C, Morales FJ (2006). Relationship between acrylamide and thermal-processing indexes in commercial breakfast cereals: a survey of Spanish breakfast cereals. *Molecular Nutrition & Food Research*, 50:756–762.

Rufian-Henares JA, Arribas-Lorenzo G, Morales FJ (2007). Acrylamide content of selected Spanish foods: survey of biscuits and bread derivatives. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 24(4):343–350.

Rydberg P et al. (2003). Investigations of factors that influence the acrylamide content of heated foodstuffs. *Journal of Agricultural and Food Chemistry*, 51(24):7012–7018.

Sánchez J et al. (2008). Formation of hemoglobin adducts of acrylamide after its ingestion in rats is dependent on age and sex. *Journal of Agricultural and Food Chemistry*, 56:5096–5101.

Schouten LJ et al. (2009). Dietary acrylamide intake and the risk of head–neck and thyroid cancers: results from the Netherlands Cohort Study. *American Journal of Epidemiology*, 170:873–884.

Schulz MR et al. (2001). Dose–response relation between acrylamide and pancreatic cancer. *Occupational and Environmental Medicine*, 58:609.

Senyuva HZ, Gökmen V (2005). Survey on acrylamide in foods by an in-house validated LC-MS method. *Food Additives and Contaminants*, 22:204–209.

Settels E et al. (2008). Human CYP2E1 mediates the formation of glycidamide from acrylamide. *Archives of Toxicology*, 82:717–727.

Shipp GL et al. (2006). Acrylamide: review of toxicity data and dose–response analyses for cancer and noncancer effects. *Critical Reviews in Toxicology*, 36:481–608.

Sickles DW et al. (2007). Acrylamide effects on kinesin-related proteins of the mitotic/meiotic spindle. *Toxicology and Applied Pharmacology*, 222:111–121.

Sirot V et al. (2009). Core food of the French food supply: second Total Diet Study. *Food Additives and Contaminants*, 26(5):623–639.

Smith EA, Prues SL, Oehme FW (1996). Environmental degradation of polyacrylamides. 1. Effects of artificial environmental conditions: temperature, light, and pH. *Ecotoxicology and Environmental Safety*, 35:121–135.

Soares C, Cunha S, Fernandes J (2006). Determination of acrylamide in coffee and coffee products by GC-MS using an improved SPE clean-up. *Food Additives and Contaminants*, 23:1276–1282.

Sobel W et al. (1986). Acrylamide cohort mortality study. *British Journal of Industrial Medicine*, 43:785–788.

Sörgel F et al. (2002). Acrylamide: increased concentrations in homemade food and first evidence of its variable absorption from food, variable metabolism and placental and breast milk transfer. *Chemotherapy*, 48:267–274.

Sumner SC, MacNeela JP, Fennell TR (1992). Characterization and quantitation of urinary metabolites of [1,2,3-^{13}C]acrylamide in rats and mice using ^{13}C nuclear magnetic resonance spectroscopy. *Chemical Research in Toxicology*, 5:81–89.

Sumner SC et al. (1999). Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. *Chemical Research in Toxicology*, 12:1110–1116.

Sumner SC et al. (2003). Acrylamide: a comparison of metabolism and hemoglobin adducts in rodents following dermal, intraperitoneal, oral, or inhalation exposure. *Toxicological Sciences*, 75:260–270.

Swaen GMH et al. (2007). Mortality study update of acrylamide workers. *Occupational and Environmental Medicine*, 64:396–401.

Swedish National Food Administration (2009). *An on-going trend study on acrylamide levels in food products sold in Sweden. Interim summary report for the years 2005 to 2009*. Submitted to FAO/WHO by K-E Hellenäs, Swedish National Food Administration.

Sweeney LM et al. (2010). Development of a physiologically-based toxicokinetic model of acrylamide and glycidamide in rats and humans. *Food and Chemical Toxicology*, 48(2): 668–685.

Takahashi M et al. (2009). Limited lactational transfer of acrylamide to rat offspring on maternal oral administration during the gestation and lactation periods. *Archives of Toxicology*, 83:785–793.

Tardiff RG et al. (2010). Estimation of safe dietary intake levels of acrylamide for humans. *Food and Chemical Toxicology*, 48(2):658–667.

Tareke E et al. (2006). Relationships between biomarkers of exposure and toxicokinetics in Fischer 344 rats and B6C3F1 mice administered single doses of acrylamide and glycidamide and multiple doses of acrylamide. *Toxicology and Applied Pharmacology*, 217:63–75.

Tezcan F, Erim FB (2008). On-line stacking techniques for the nonaqueous capillary electrophoretic determination of acrylamide in processed food. *Analytica Chimica Acta*, 617:196–199.

Tran NL et al. (2010). Dietary acrylamide exposure and hemoglobin adducts—National Health and Nutrition Examination Survey (2003–04). *Food and Chemical Toxicology*, 48(11): 3098–3108.

Tyl RW et al. (2000). Rat two generation reproduction and dominant lethal study of acrylamide in drinking water. *Reproductive Toxicology*, 14:385–401.

Urban M et al. (2006). Urinary mercapturic acids and a hemoglobin adduct for the dosimetry of acrylamide exposure in smokers and nonsmokers. *Inhalation Toxicology*, 18:831–839.

USEPA (2010). *Toxicological review of acrylamide (CAS No. 79-06-1) in support of summary information on the Integrated Risk Information System (IRIS). March 2010*. Washington, DC, United States Environmental Protection Agency (EPA/635/R-07/009F; http://www.epa.gov/iris/toxreviews/0286tr.pdf).

USFDA (2006). *Survey data on acrylamide in foods: total diet study results. Tables 3 and 4: Acrylamide levels in food products sampled for the 2005 and 2006 total diet study*. Silver Spring, MD, United States Department of Health and Human Services, Food and Drug Administration (http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Acrylamide/ucm053566.htm).

Vesper HW et al. (2008). Cross-sectional study on acrylamide haemoglobin adducts in subpopulations from the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. *Journal of Agricultural and Food Chemistry*, 56:6046–6053.

Vikstrom AC et al. (2008). Internal doses of acrylamide and glycidamide in mice fed diets with low acrylamide contents. *Molecular Nutrition and Food Research*, 52(8):974–980.

Von Tungeln LS et al. (2009). DNA adduct formation and induction of micronuclei and mutations in B6C3F1/Tk mice treated neonatally with acrylamide or glycidamide. *International Journal of Cancer*, 124:2006–2015.

Walker K et al. (2007). Approaches to acrylamide physiologically based pharmacokinetic modeling for exploring child–adult dosimetry differences. *Journal of Toxicology and Environmental Health. Part A*, 70:2033–2055.

Wang HY et al. (2008). SPE/HPLC/UV studies on acrylamide in deep-fried flour-based indigenous Chinese foods. *Microchemical Journal*, 89:90–97.

Wenzl T, Anklam E (2007). European Union database of acrylamide levels in food: update and critical review of data collection. *Food Additives and Contaminants*, 24(Suppl. 1): 5–12.

Wenzl T, de la Calle MB, Anklam E (2003). Analytical methods for the determination of acrylamide in food products: a review. *Food Additives and Contaminants*, 20:885–902.

Wenzl T, Lachenmeier DW, Gökmen V (2007). Analysis of heat-induced contaminants (acrylamide, chloropropanols and furan) in carbohydrate-rich food. *Analytical and Bioanalytical Chemistry*, 389:119–137.

Wenzl T et al. (2006). Collaborative trial validation study of two methods, one based on high performance liquid chromatography–tandem mass spectrometry and on gas chromatography–mass spectrometry for the determination of acrylamide in bakery and potato products. *Journal of Chromatography A*, 1132:211–218.

Wenzl T et al. (2009). Validation by collaborative trial of an isotope dilution liquid chromatographic tandem mass spectrometric method to determine the content of acrylamide in roasted coffee. *Food Additives and Contaminants*, 26:1146–1152.

WHO (2006). *GEMS/Food consumption cluster diets (June revision)*. Geneva, World Health Organization, Department of Food Safety, Zoonoses and Foodborne Diseases.

Wilson KM et al. (2009a). Dietary acrylamide intake and risk of premenopausal breast cancer. *American Journal of Epidemiology*, 169:954–961.

Wilson KM et al. (2009b). Acrylamide exposure measured by food frequency questionnaire and haemoglobin adduct levels and prostate cancer risk in the Cancer of the Prostate in Sweden study. *International Journal of Cancer*, 124:2384–2390.

Wilson KM et al. (2009c). Validation of a food frequency questionnaire measurement of dietary acrylamide intake using haemoglobin adducts of acrylamide and glycidamide. *Cancer Causes and Control*, 20:269–278.

Wirfält E et al. (2008). Associations between estimated acrylamide intakes, and haemoglobin AA adducts in a sample from the Malmö Diet and Cancer cohort. *European Journal of Clinical Nutrition*, 62:314–323.

Woo G-H et al. (2007). Lack of preventive effects of dietary fibers or chlorophyllin against acrylamide toxicity in rats. *Food and Chemical Toxicology*, 45:1507–1515.

Yasuhara A et al. (2003). Gas chromatographic investigation of acrylamide formation in browning model systems. *Journal of Agricultural and Food Chemistry*, 51(14):3999–4003.

Yener Y, Dikmenli M (2009). Increased micronucleus frequency in rat bone marrow after acrylamide treatment. *Food and Chemical Toxicology*, 47:2120–2123.

Young JF, Luecke RH, Doerge DR (2007). Physiologically based pharmacokinetic/pharmacodynamic model for acrylamide and its metabolites in mice, rats and humans. *Chemical Research in Toxicology*, 20:388–399.

Zamora R, Delgado RM, Hidalgo FJ (2010). Model reactions of acrylamide with selected amino compounds. *Journal of Agricultural and Food Chemistry*, 58(3):1708–1713.

Zeiger E et al. (2009). Investigation of the low-dose response in the in vivo induction of micronuclei and adducts by acrylamide. *Toxicological Sciences*, 107(1):247–257.

Zhang Y, Ren YP, Zhang Y (2009). New research developments on acrylamide: analytical chemistry, formation mechanism, and mitigation recipes. *Chemical Reviews*, 109:4375–4397.

Zhang Y, Zhang GY, Zhang Y (2005). Occurrence and analytical methods of acrylamide in heat-treated foods: review and recent developments. *Journal of Chromatography A*, 1075:1–21.

Zhang Y et al. (2005). Determination of acrylamide in infant cereal-based foods by isotope dilution liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *Analytica Chimica Acta*, 551:150–158.

Zhang Y et al. (2006). Rapid determination of acrylamide contaminant in conventional fried foods by gas chromatography with electron capture detector. *Journal of Chromatography A*, 1116:209–216.

Zhang Y et al. (2007). An improved method validation for rapid determination of acrylamide in foods by ultra-performance liquid chromatography combined with tandem mass spectrometry. *Journal of Chromatography A*, 1142:194–198.

Zhao R et al. (2005). [Determination of acrylamide in heated starchy food by liquid chromatography electrospray ionization tandem mass spectrometry.] *Chinese Journal of Chromatography*, 23(3):289–291 (in Chinese).

Zhou S et al. (2008). Antigen synthetic strategy and immunoassay development for detection of acrylamide in foods. *Analyst*, 133:903–909.

Zhou X et al. (2007). Separation and determination of acrylamide in potato chips by micellar electrokinetic capillary chromatography. *Talanta*, 71:1541–1545.

Zhu YH et al. (2008). Application of the standard addition method for the determination of acrylamide in heat-processed starchy foods by gas chromatography with electron capture detector. *Food Chemistry*, 109:899–908.