ACRYLAMIDE

First draft prepared by

S.H. Henry,[1] A. Agudo,[2] S. Barlow,[3] L. Castle,[4] J. Garey,[5] K.-E. Hellenas,[6] K. Koehler,[1] J.C. Larsen,[7] J.-C. LeBlanc,[8] M. Paule,[5] J. Schlatter,[9] W. Slob[10] and D.R. Doerge[5]

[1] Food and Drug Administration, College Park, Maryland, USA
[2] Catalan Institute of Oncology, Hospitalet del Llobregat, Barcelona, Spain
[3] Harrington Road, Brighton, East Sussex, United Kingdom
[4] Central Science Laboratory, Sand Hutton, York, United Kingdom
[5] National Center for Toxicological Research, Little Rock, Arkansas, USA
[6] National Food Administration, Uppsala, Sweden
[7] Division of Toxicology and Risk Assessment, Danish Institute of Food and Veterinary Research, Søborg, Denmark
[8] Institut Nationale de la Recherche Agronomique/Institut Nationale Agronomique Paris-Grignon, Paris, France
[9] Food Toxicology Section, Swiss Federal Office of Public Health, Zurich, Switzerland
[10] National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands

1. EXPLANATION

Acrylamide (CH_2=CHCONH$_2$, CAS Registry Number 79-06-1) is an important industrial chemical that has been used since the mid-1950s as a chemical intermediate in the production of polyacrylamides, which are used as flocculants for clarifying drinking-water and in other industrial applications. It is well established that acrylamide is neurotoxic in humans, as revealed by the consequences of occupational and accidental exposures. In addition, experimental studies in animals have shown that acrylamide has reproductive toxicity and is genotoxic and carcinogenic.

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

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

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

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

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

Acrylamide has not been evaluated previously by the Committee.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Absorption

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

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

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

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

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

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

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

(b) Distribution

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(c) Excretion

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

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

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

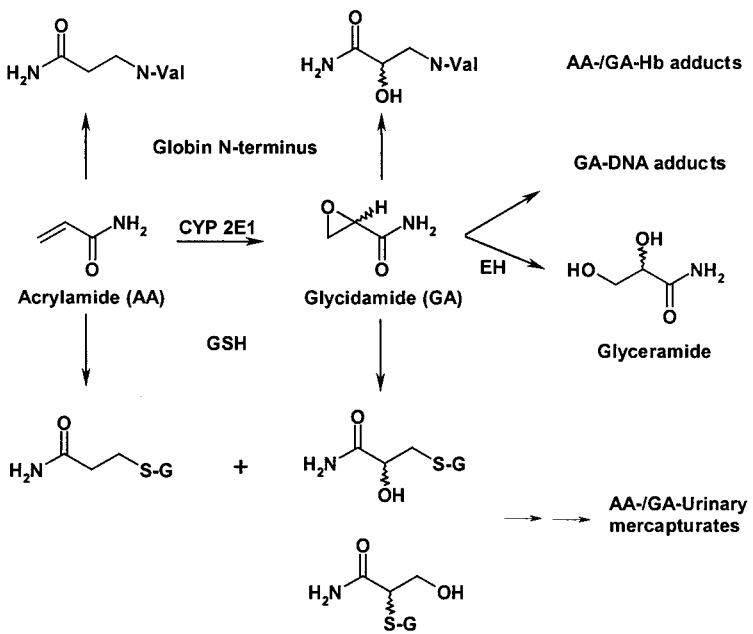
2.1.2 Biotransformation

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

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

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

Figure 1. Metabolism of acrylamide



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

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

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

2.1.3 *Effects on enzymes and other biochemical parameters*

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

2.1.4 *Physiologically based pharmacokinetic (PBPK) modelling*

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

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

2.2 Toxicological studies

2.2.1 Acute toxicity

Acute toxic effects from oral dosing (Table 2) were seen only above acrylamide doses of 100 mg/kg bw, and reported median lethal doses (LD_{50}s) are generally above 150 mg/kg bw (Dearfield et al., 1995).

2.2.2 Short-term studies of toxicity

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

(a) Mice

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

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

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

Table 2. Acute oral toxicity of acrylamide

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

Table 2. (contd)

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

From European Commission (2002a)

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

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

(b) Rats

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

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

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

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

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

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

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

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

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

(c) Cats

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

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

(d) Dogs

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

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

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

(e) Non-human primates

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

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

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

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

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

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

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

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

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

2.2.3 *Long-term studies of toxicity and carcinogenicity*

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

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

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

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

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

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

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

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

F, female; M, male

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

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

[c] Only clitoral glands with gross lesions were examined histologically.

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

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

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

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

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

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

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

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

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

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

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

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

2.2.4 *Genotoxicity*

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

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

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

Table 5. Results of acrylamide genotoxicity testing

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

Table 5. (contd)

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

Table 5. (contd)

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

Table 5. (contd)

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

Table 5. (contd)

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

Table 5. (contd)

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

Table 5. (contd)

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

Table 5. (contd)

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

Table 5. (contd)

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

Table 5. (contd)

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

Table 5. (contd)

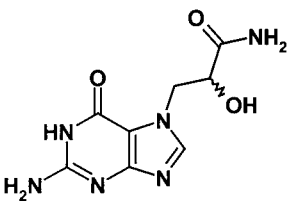
Assay	Test system	Dose/concentration	HID or LED	Result	Reference
DNA adducts (contd)	Rat (Sprague-Dawley), liver, brain, testis	18, 54 mg/kg bw, gavage	18	Positive	Manière et al. (2005)
	Rat (Sprague-Dawley) liver, brain, testis, adrenal, bone marrow, leukocytes	18, 36, 54 mg/kg bw, gavage	54	Positive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

Table 5. (contd)

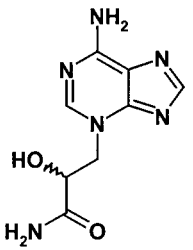
Assay	Test system	Dose/concentration	HID or LED	Result	Reference
	Mouse NIH/3T3 cells	2–200 µg/ml	0.0125	Positive	Banerjee & Segal (1986)
	Mouse C3H/10T1/2 cells	0.01–0.3 mg/ml	0.3	Negative	Abernethy & Boreiko (1987)
	Mouse BALB/c 3T3 cells	0.5–2 mmol/l	1	Positive	Tsuda et al. (1993)
	Syrian hamster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

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

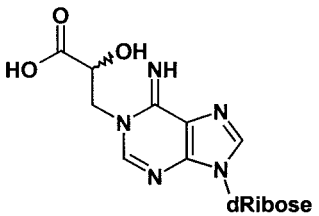
Figure 2. DNA adducts from glycidamide



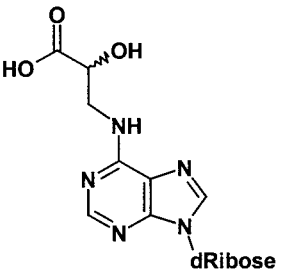
N7-(2-carbamoyl-2-hydroxyethyl)-guanine (N7-GA-Gua)



N3-(2-carbamoyl-2-hydroxyethyl)-adenine (N3-GA-Ade)



N1-(2-carboxy-2-hydroxyethyl)-2'-deoxyadenosine (N1-GA-dA)



N^6-(2-carboxy-2-hydroxyethyl)-2'-deoxyadenosine (N^6-GA-dA)

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

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

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

2.2.5 Reproductive toxicity

(a) Fertility and multigenerational toxicity

(i) Mice

Intraperitoneal route

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

Oral route

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

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

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

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

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

(ii) Rats

Oral route

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

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

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

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

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

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

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

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

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

(b) Developmental toxicity

(i) Mice

Intraperitoneal route

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

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

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

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

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

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

Oral route

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

(ii) Rats

Oral route

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

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

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

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

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

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

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

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

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

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

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

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

2.2.6 Special studies

(a) Covalent binding to nucleic acids and proteins

(ii) DNA adducts

In vitro

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

In vivo

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

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

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

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

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

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

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

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

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

(ii) Haemoglobin adduct formation

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

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

(b) Neurotoxicity

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(c) Hormonal activity/effects

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

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

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

(d) Thyroid function

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

(e) Related contaminants

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

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

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

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

Table 6. (contd)

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

Table 6. (contd)

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

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

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

2.3 Observations in domestic animals

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

2.4 Observations in humans

2.4.1 Biomarkers of exposure

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

2.4.2 Biomarkers of effects

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

2.4.3 Clinical observations

No clinical observations were located in the literature.

2.4.4 Epidemiological studies

(a) Cancer

(i) Occupational exposure

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

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

found for pancreatic cancer (SMR = 2.26, 95% confidence interval [CI] 1.03–4.29) for subjects with cumulative exposure greater than 0.3 mg/m^3-years; however, no consistent exposure–response relationship was observed. This estimate was based on nine pancreatic cancer cases (Marsh et al., 1999).

A reanalysis of the data proposed to combine the two intermediate categories of exposure (0.001–0.029 and 0.03–0.29 mg/m^3-years) to avoid the small number of cases, obtaining a monotonically increasing risk with increasing exposure (Schulz et al., 2001).

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

(ii) Dietary exposure

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

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

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

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

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

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

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

(a) Effects other than cancer

(i) Neurotoxicity

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

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

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

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

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

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

(ii) Genotoxicity

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

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

3. ANALYTICAL METHODS

3.1 Chemistry

Acrylamide (CH_2=CH-CO-NH_2; 2-propenamide) is a white crystalline solid. It has a relative molecular mass of 71, a melting point of 84.5 ± 0.3 °C and a high boiling point (136 °C at 3.3 kPa) (Cyanamid, 1969; Habermann, 1991). Acrylamide is extremely soluble in water (2155 g/l at 30 °C), in lower alcohols (1550 g/l in methanol and 860 g/l in ethanol) and in other polar organic solvents (396 g/l in acetonitrile, 126 g/l in ethyl acetate and 631 g/l in acetone). It is virtually insoluble in non-polar organic solvents such as heptane and carbon tetrachloride (<1 g/l) (Cyanamid, 1969; Habermann, 1991). The limited conjugation involving π-electrons means that acrylamide lacks a strong chromophore for ultraviolet (UV) detection and does not fluoresce. The solubility behaviour dictates the extraction strategy used for analysis, and the low molecular mass and low volatility have consequences with respect to the measurement techniques used.

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

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

3.2 Description of analytical methods

3.2.1 Introduction

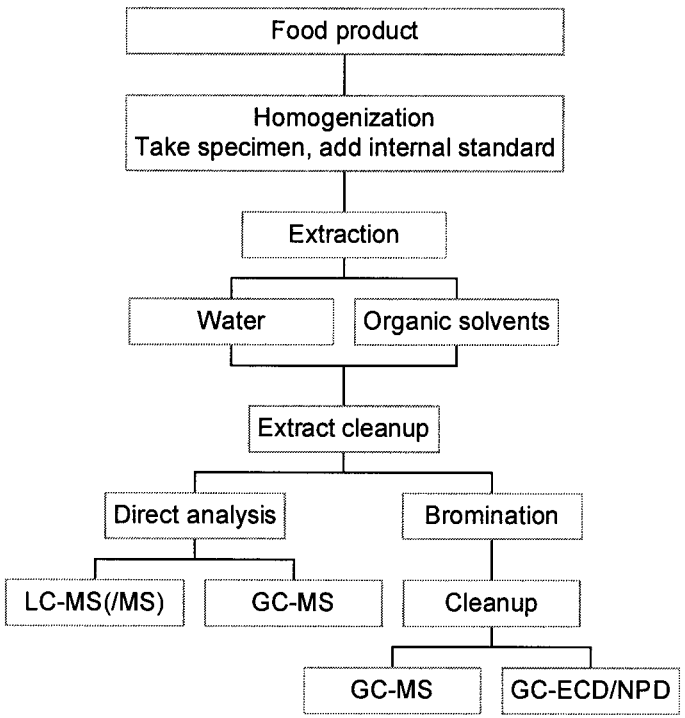
The formation of acrylamide is discussed in section 5.

3.2.2 Screening tests

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

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

Figure 3. General schematic for acrylamide analysis



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

3.2.3 Quantitative methods

(a) Addition of internal standard

Nearly all published methods use internal standards. Both deuterium-labelled ($^{2}H_3$-AA) and carbon-labelled ($^{13}C_3$-AA) substances are available commercially and are used widely. The triple label in each takes the internal standard well away from the mass ions used to measure acrylamide itself in methods that employ MS.

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

(b) Extraction using water

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

(c) Extraction using organic solvents

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

(d) Efficiency of extraction

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

(e) Determination by GC-MS after bromination

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

In some methods, the first-formed 2,3-dibromopropionamide is dehydrobrominated to form the more stable 2-bromopropenamide by treatment with triethylamine before GC-MS analysis. In this case, one of the three deuterium labels is lost if $^2H_3$-AA is used as the internal standard, but quantification via the monobromo derivative is still successful (Andrawes et al., 1987; Nemoto et al., 2002; Pittet et al., 2004).

(i) Confidence in the identification and quantification aspects

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

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

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

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

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

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

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

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

(iv) Br-GC-MS: Conclusions

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

(f) Determination by GC-MS with no derivatization

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

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

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

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

(g) Determination by LC

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

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

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

(h) Determination by LC-MS

(i) Extract cleanup requirements for LC-MS

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

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

(ii) Chromatographic performance in LC-MS

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

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

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

(iii) Detection by LC-MS

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

(iv) Confidence in LC-MS identification of acrylamide

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

(v) Quantitative aspects of LC-MS analysis

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

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

(vi) LC-MS methods: conclusions

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

(i) Summary of the performance of analytical methods used

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

4. SAMPLING

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

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

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

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

5. EFFECTS OF PROCESSING

5.1 Heat-induced formation of acrylamide in foods

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

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

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

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

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

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

5.2 Chemical mechanisms

5.2.1 Formation from amino acid and sugar by Maillard reactions

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

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

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

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

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

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

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

5.2.2 Formation via acrylic acid

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

5.2.3 Formation from 3-aminopropionamide

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

5.2.4 Elimination of acrylamide

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

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

5.2.5 Analogues: formation of other compounds

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

5.3 Formation factors

5.3.1 Heating temperature and time

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

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

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

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

5.3.2 Precursors

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

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

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

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

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

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

5.3.3 *Acidity*

The acrylamide accumulation in asparagine–glucose solutions at 150 °C increased exponentially with increasing pH in the range 4–7 (Jung et al., 2003). The acrylamide content of heated potato tissue adjusted to pH values from 6 to10 exhibited a maximum around pH 8 (Rydberg et al., 2003).

5.3.4 *The food matrix*

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

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

5.3.5 *Water activity*

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

6. LEVELS AND PATTERNS OF CONTAMINATION OF FOOD COMMODITIES

6.1 Surveillance data

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

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

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

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

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

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

[a] Number of analytical results for individual samples and for composite samples.
[b] LOR means limit of reporting (detection and quantification limit).

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

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

6.2 National occurrence

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

6.2.1 Australia

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

6.2.2 Austria

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

6.2.3 Belgium

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

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

6.2.4 Canada

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

6.2.5 China

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

6.2.6 China, Hong Kong Special Administrative Region

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

6.2.7 Denmark

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

6.2.8 France

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

6.2.9 Germany

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

6.2.10 Japan

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

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

6.2.11 The Netherlands

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

6.2.12 Norway

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

6.2.13 Sweden

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

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

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

6.2.14 Switzerland

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

6.2.15 Syria

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

6.2.16 United Arab Emirates

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

6.2.17 United Kingdom

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

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

6.2.18 United States

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

6.2.19 Summary of national occurrence data

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

6.3 International occurrence

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

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

Most of the samples reported were analysed by LC-MS, GC-MS or LC-MS-MS; the LOD or LOQ was <30 µg/kg. In order to take into account the censored data in the calculation of dietary exposure, international recommendations described in the GEMS/Food report have been applied for data below the detection limit (LOD) and below the quantification limit (LOQ) (WHO, 1995). As the percentage of non-quantified values was less than 60% for major contributing foods, the following treatment was used: data below LOD = ½ LOD and data below LOQ = ½ LOQ. This approach concerns 13% of the data from Europe, 31% from North America, 51% from the Pacific region and 32% from Asia.

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

7. DIETARY INTAKE ASSESSMENT

7.1 Exposure to acrylamide from non-food sources

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

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

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

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

Table 8. (contd)

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

Table 8. (contd)

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

[a] LOR means limit of reporting (detection and quantification limit).
[b] Results that were below the reporting limits (LOD or LOQ) have been assumed to be half of those limits.
[c] Coefficient of variation (standard deviation divided by the mean, %).
[d] According to correspondence with the GEMS/Food commodities group, only the mean concentrations of acrylamide given in bold type were used to estimate international intake.
[e] Concentrations for brewed coffee ([concentration of acrylamide in coffee-as-consumed] × 28, to convert concentration in beverage into concentration in coffee powder).

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

- **Drinking-water intake:** Exposure from contamination of drinking-water by industrial releases into domestic water supplies is not a general exposure source because acrylamide is rapidly biodegraded. There is no bioaccumulation in the aquatic food-chain because acrylamide is highly water-soluble and not lipophilic. Drinking-water is commonly treated with polyacrylamide resins to remove suspended particulates. This practice was estimated to produce acrylamide concentrations much lower than 0.5 µg/l. The estimated upper-bound exposure is 0.01 µg/kg bw per day from drinking 2 litres of water per day.
- **Occupational exposures:** Exposure to acrylamide is possible for workers in a wide range of industries that use polyacrylamide: paper and pulp, construction, foundry, oil drilling, textiles, cosmetics, food processing, plastics, mining and agricultural occupations. Workers could be exposed by inhaling dusts or vapours and through dermal contact with monomers and polymers. Researchers or technicians who prepare polyacrylamide gels may also experience variable and intermittent exposures to acrylamide. Based on geometric means of 0.01–0.13 mg/m^3 and an upper-bound exposure of 0.3 mg/m^3 (permissible exposure level), the expert panel estimated mean and upper-bound workplace (United States and Europe) acrylamide inhalation exposures at 1.4–18.6 µg/kg bw per day and 43 µg/kg bw per day, respectively. Skin exposure and uptake are unknown and difficult to measure.

7.2 National assessments of intake from diet

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

7.2.1 Australia

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

7.2.2 Belgium

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

7.2.3 Canada

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

7.2.4 China

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

and mean food consumption data at the food group commodities level. Concentrations reported below the reporting limits (<LOR = 29%) have been assigned to a concentration equal to ½ LOR. The mean intake for adult Chinese has been estimated to be 1.1 µg/kg bw per day. The main food contributors to total exposure are cereal-based foods: rice (40%), roots and tubers (42%) and wheat and wheat products (18%).

7.2.5 China, Hong Kong Special Administrative Region

Hong Kong SAR submitted the results of intake from recent publications (FEHD, 2003; Leung et al., 2003). Food consumption data were collected in 1995 from the Hong Kong adult survey and in 2000 from a secondary school student survey. Dietary exposures of acrylamide were generated in a deterministic fashion using mean concentration levels and food consumption data. Concentrations reported below the reporting limits (<LOR = 38%) have been assigned a concentration equal to ½ LOR. The intake estimates for adults and students are, respectively, 0.3 µg/kg bw per day and 0.4 µg/kg bw per day for the average consumer. No information was available on the primary food contributors to total exposure.

7.2.6 Czech Republic

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

7.2.7 Denmark

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

7.2.8 France

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

reported below the reporting limits (<LOR = 20%) have been assigned a concentration equal to ½ LOR. The intake estimates for the whole population older than 15 years is 0.5 µg/kg bw per day for the average consumer and 1.3 µg/kg bw per day for the 95th-percentile consumer. Children aged from 3 to 14 years have exposures ranging from 1 to 2.5 µg/kg bw per day. The main food contributors to total exposure are potato chips (27–28%), bread (12–29%), pastry and sweet biscuits (20%) and rolls (9–11%).

7.2.9 Germany

Germany submitted the results of intakes from two recent publications (Mosbach-Schulz et al., 2003; European Union, 2004). Using a deterministic approach, intakes reported in the Mosbach-Schulz et al. (2003) report are based on the mean results from the acrylamide measurement programme performed by the German food surveillance programme and from the national individual consumption study covering 25 000 participants from 1985 to 1989. Concentrations reported below the reporting limits (<LOR = 7%) have been assigned to a concentration equal to ½ LOR. The intake estimates for the population aged from 4 to 79 years ranged from 0.6 to 1.2 µg/kg bw per day for the average consumer to 3.2 µg/kg bw per day and 5.1 µg/kg bw per day, respectively, for the 95th- and 99th-percentile child consumer. No information was available on the main food contributors to total exposure.

7.2.10 The Netherlands

The Netherlands submitted the results of the survey of acrylamide from a recent publication (Konings et al., 2003). The individual food consumption survey, called the National Food Consumption Survey, as carried out in 1998 on 6257 non-institutionalized persons aged 1–97 years, was used. Dietary exposure distributions for acrylamide were generated in a probabilistic fashion using the Monte Carlo Risk Analysis Program (Monte Carlo Risk Analysis Program version 1.2, RIKILT, Wageningen) combining the distribution of food consumption and available distribution of acrylamide levels. Concentrations reported below the reporting limits (<LOR = 39%) have been assigned to a concentration equal to ½ LOR, while samples likely to contain no acrylamide at all were assumed to have acrylamide concentrations equal to 0. The intake estimates for the whole population aged from 1 to 97 years ranged from 0.5 to 1 µg/kg bw per day for the average consumer and from 0.6 to 1.1 µg/kg bw per day for the 95th-percentile consumer. Main food contributors to total exposure in the adult and children populations are potato crisps (31–46%), potato chips (18–23%), Dutch spiced cake (11–20%), coffee (13%), biscuits (10%) and bread (10%).

7.2.11 New Zealand

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

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

7.2.12 Norway

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

7.2.13 Sweden

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

7.2.14 Switzerland

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

7.2.15 United Arab Emirates

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

7.2.16 United Kingdom

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

7.2.17 United States

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

aged 2+ years. Both surveys were used. Dietary exposure distributions for acrylamide were generated in a probabilistic way using a Monte Carlo sampling combining the distribution of acrylamide levels and the distribution of food consumption obtained in each survey. Concentrations reported as being below the reporting limits (<LOR = 34%) have been assigned as being equal to ½ LOR. The intake estimates for the whole population aged 2+ years ranged from 0.4–0.5 µg/kg bw per day for the average consumer to 0.8–0.9 µg/kg bw per day for the 90th-percentile consumer. For children (2–5 years), intakes ranged from 1.0–1.3 µg/kg bw per day for the average consumer to 2.2–2.3 µg/kg bw per day for the 90th-percentile consumer. The main food group contributors to total exposure are french fries (23–30%), breakfast cereals (11–23%), toast (12–13%), cookies (10–13%), potato chips (11–15%), cereals (29%) and starchy vegetables (52%).

7.2.18 Summary of national intake estimates

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

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

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

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

The Committee concluded that based on national estimates, an intake of 1 µg/kg bw per day of acrylamide could be taken to represent the average for the general population and that an intake of 4 µg/kg bw per day could be taken to

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

Country	Population group	Average/50th percentile (µg/kg bw per day)	90th, 95th, 97.5th, 99th percentile (µg/kg bw per day)	Reference	Comments
Australia	Whole population (>2 years)	0.4–0.5	1.4–1.5 (P95)	Croft et al. (2004)	National individual food consumption
	Children (2–6 years)	1.0–1.3	3.2–3.5 (P95)		Deterministic modelling (mean occurrence data, <LOR = 0 and LOR)
					Major contributing foods: hot potato chips (25–27%), toast (9–13%), breakfast cereals (7–14%), potato crisps (6–12%)
Belgium	Adolescents M (13–18 years)	0.6 (P50)	1.3 (P95)	Matthys et al. (2005)	National individual food consumption
	Adolescents F (13–18 years)	0.5 (P50)	0.9 (P95)		Probabilistic modelling (MCRA, <LOR = ½ LOR)
					Major contributing foods: french fries (30%), breads (11%) and biscuits (11%)
Canada	Adults (20–39 years)	0.4	–	Vavasour (2005)	National individual food consumption
	Pre-teens and teens (10–19 years)	1.0	–		Deterministic modelling (mean occurrence data, <LOR = 0 and LOR)
					No information on major contributing foods
China	Adults (15–>70 years)	1.1	–	Chen (2005)	National individual food consumption
					Deterministic modelling (mean occurrence data, <LOR = ½ LOR)
					Major contributing foods are roots and tubers (42%), cereal-based rice (40%) and wheat and wheat products (18%)

Table 9. (contd)

Country	Population group	Average/50th percentile (µg/kg bw per day)	90th, 95th, 97.5th, 99th percentile (µg/kg bw per day)	Reference	Comments
China, Hong Kong Special Administrative Region	Adults (>18 years)	0.3	–	FEHD (2003)	National individual food consumption
	School students	0.4	–		Deterministic modelling (mean occurrence data, <LOR = ½ LOR)
					No information on major contributing foods
Czech Republic	Whole population (>1 year)	0.3	–	National Institute of Public Health (2003)	The basis of the intake study is unknown because the publication on the web site was not available in English
					No information on major contributing foods
Denmark	Adults (15–80 years)	6.5 µg/day (0.09[a])	18 µg/day (P95)	Grandby & Fagt (2004)	National individual food consumption
					Probabilistic modelling (@risk software)
					Study implemented to estimate the dietary intake from coffee; contribution was estimated to be 20% of the total exposure found in Scandinavian countries
France	Adults (>15 years)	0.5	1.3 (P95)	AFSSA (2004)	National individual food consumption
	Children (3–14 years)	1.0	2.5 (P95)		Deterministic modelling (mean occurrence data, <LOR = ½ LOR)
					Major contributing foods: potato chips (27–28%), bread (12–29%), pastry and sweet biscuits (20%), rolls (9–11%)

Table 9. (contd)

Country	Population group	Average/50th percentile (µg/kg bw per day)	90th, 95th, 97.5th, 99th percentile (µg/kg bw per day)	Reference	Comments
Germany	Adults (18–79 years)	0.6	3.2–5.1 (P99)	Mosbach-Schulz et al. (2003)	National individual food consumption
	Children (15–18 years)	0.9			Deterministic modelling (mean occurrence data)
	Children (4–6 years)	1.2			No information on major contributing foods
	Adolescents (19–24 years)	0.7			
The Netherlands	Whole population (1–97 years)	0.5	0.6 (P95)	Konings et al. (2003)	National individual food consumption
	Children (1–6 years)	1.0	1.1 (P95)		Probabilistic method (MCRA-RIKILT, <LOR = ½ LOR)
	Children (7–18 years)	0.7	0.9 (P95)		Major contributing foods: potato crisps (31–46%), potato chips (18–23%), Dutch spiced cake (11–20%), coffee (13%), biscuits (10%) and bread (10%)
New Zealand	Whole population (>15 years)	0.3–0.5	1.2–1.4 (P95)	Food Standards Australia New Zealand (2004)	National individual food consumption
	Whole population, consumers only (>15 years)	0.4–0.5			Deterministic modelling (Australian mean occurrence data, <LOR = 0 and LOR)
					Major contributing foods: hot chips (25%), sweet biscuits (15%), baked potatoes (14%) and toasted bread (10%)

Table 9. (contd)

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

Table 9. (contd)

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

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

[a] Assuming a mean body weight of 70 kg.

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

7.3 Regional estimates of intake from GEMS/Food diet

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

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

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

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

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

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

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

8. PREVENTION AND CONTROL

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

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

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

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

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

From WHO (2003)

[a] Mean body weight = 60 kg.

[b] According to Stegen et al. (1997), a multiplicative conversion factor of 28 (1 g for 30 ml) has been applied for brewed coffee to convert contamination data into ground and roasted coffee.

8.1 Mitigation achievements

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

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

8.2 Mitigation experiments in food models

8.2.1 Lowering the amount of precursors

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

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

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

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

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

8.2.2 *Chemical interference with formation or elimination*

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

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

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

8.2.3 *Optimized time/temperature regimen*

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

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

8.2.4 *Drying*

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

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

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

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

9.1 Contribution of above data to assessment of risk

9.1.1 Pivotal data from biochemical and toxicological studies

(a) Metabolism and activation

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

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

(b) Neurotoxicity

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

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

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

(c) Reproductive and developmental toxicity

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

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

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

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

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

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

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

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

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

(d) Mutagenicity and clastogenicity

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

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

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

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

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

(e) Carcinogenicity

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

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

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

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

9.1.2 Pivotal data from human clinical/epidemiological studies

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

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

9.2 General modelling considerations

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

Table 11. Dose–response models used

Model	Model equation[a]	Constraints
One-stage	$R = a + (1-a)\,(1-\exp(-x/b))$	$0 \le a \le 1$,
Two-stage	$R = a + (1-a)\,(1-\exp(-(x/b)-c(x/b)^2))$	$0 \le a \le 1$
Log-logistic	$R = a+(1-a)/(1+\exp(c\,\log10(b/x)))$	$0 \le a \le 1$, $c \ge \ln(10)$
Log-probit	$R = a+(1-a)\,\Phi(c\,\log10(x/b))$	$0 \le a \le 1$
Weibull	$R = a + (1-a)\,(1-\exp(-(x/b)^c))$	$0 \le a \le 1$, $c > 1$
Proast M2	$y = \exp(bx)$, *th1*	
Proast M3	$y = \exp(b\,x^d)$, *th1*	$d \ge 1$
Proast M4	$y = c - (c-1)\exp(-bx)$, *th1*	

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

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

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

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

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

9.2.1 Selection of models

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

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

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

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

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

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

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

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

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

9.2.2 *Selection of data*

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

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

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

9.2.3 Measure of intake

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

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

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

9.2.4 Measure of response

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

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

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

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

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

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

9.2.5 Selection of mathematical model

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

9.3 Estimates of BMDs and BMDLs

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

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

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

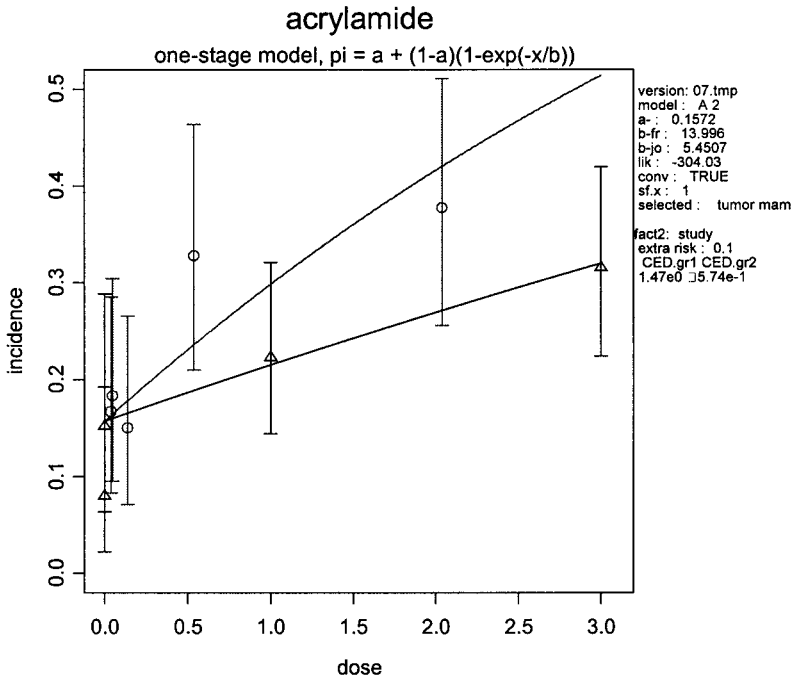
9.3.1 Mammary tumours

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

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

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

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



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

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

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

Table 13. Modelling results for total number of mammary tumours

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

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

[a] The BMD(L)s for the models that were considered most appropriate are printed in bold.
[b] Lower 5% confidence bound, based on 500 bootstrap runs.
[c] With constraint on shape parameter, to avoid infinite slope at dose zero.

9.3.2 *Testis tumours*

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

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

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

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

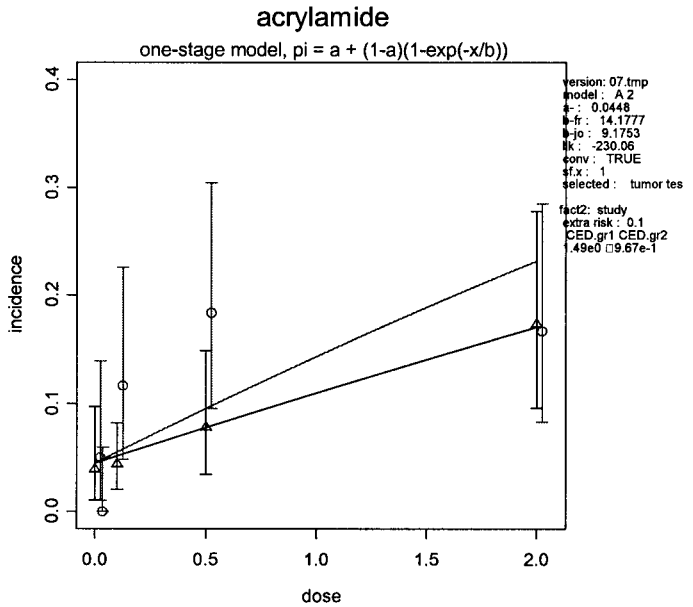


Table 14. Modelling results for total testis tumours[a]

	Study-dependent parameter	log-lik	No. of pars	mg/kg bw[b]	
				BMD_{10}	$BMDL_{10}$[c]
One-stage	Slope	−230.06	3	**0.97**	**0.63**
Two-stage					
Log-logistic[d]	Slope	−229.75	4		
Log-probit					
Weibull	Slope	−228.12	4	0.52	0.19
Weibull[d]	Slope	−230.06	4	**0.97**	**0.97**
Proast M4					
Saturated model		−222.41	10		

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

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

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

9.3.3 Central nervous system tumours

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

Table 15. Modelling results for total central nervous system tumours

	log-lik	No. of parameters	mg/kg bw[a]	
			BMD_{10}	$BMDL_{10}$[b]
One-stage	−116.97	2	**2.01**	**1.25**
Two-stage	−116.97	3		
Log-logistic	−115.82	3	**1.91**	**1.44**
Log-probit	−115.82	3	**1.88**	
Weibull	−115.82	3		
Proast M2	−116.82	2	**1.87**	**1.49**
Proast M3	−115.82	3	**1.94**	**1.59**
Saturated model	−110.97	10		

Data from Johnson et al. (1986)

log-lik, log-likelihood value

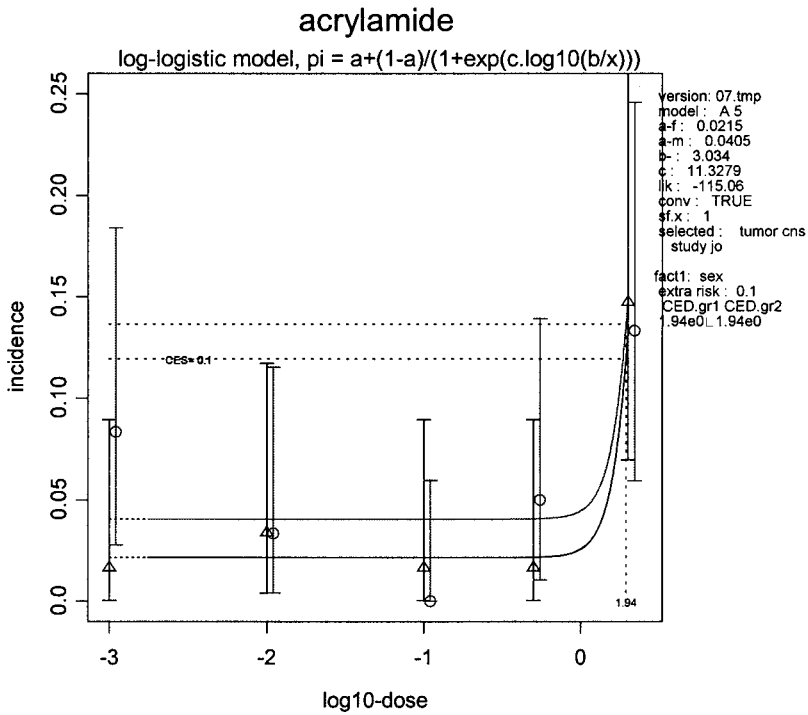
[a] The BMD(L)s for the models that were considered most appropriate are printed in bold.
[b] Lower 5% confidence bound, based on 500 bootstrap runs.

9.3.4 Thyroid tumours

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

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

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



9.4 Potency estimates in humans

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

10. COMMENTS

10.1 Absorption, distribution, metabolism and excretion

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

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

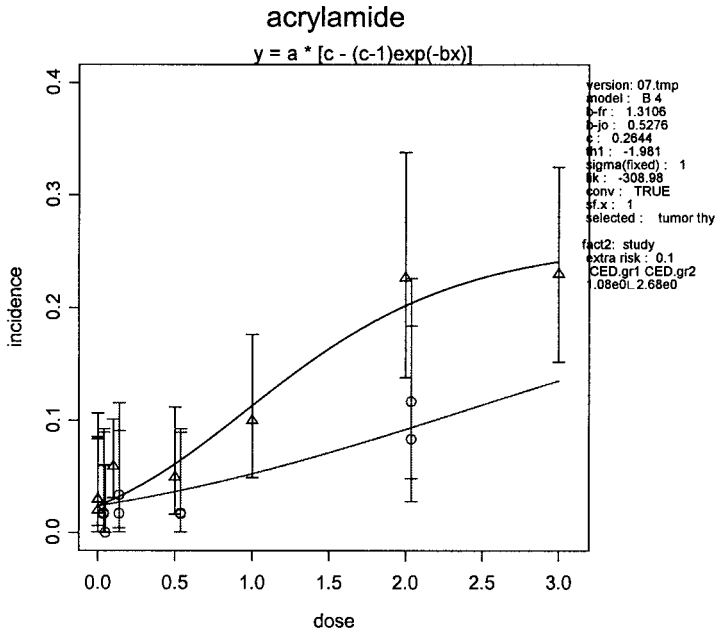


Table 16. Modelling results for thyroid tumours[a]

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

log-lik, log-likelihood value

[a] BMD(L)s relate to Friedman et al. (1995).
[b] Lower 5% confidence bound, based on 500 bootstrap runs.
[c] With constraint on shape parameter, to prevent infinite slope at dose zero.

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

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

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

10.2 Toxicological data

Single oral doses produced acute toxic effects only at doses of >100 mg/kg bw, and reported LD_{50}s are generally >150 mg/kg bw.

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

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

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

10.3 Genotoxicity

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

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

10.4 Carcinogenicity

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

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

10.5 Observations in humans

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

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

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

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

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

10.6 Analytical methods

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

10.7 Formation of acrylamide during cooking and heat processing

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

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

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

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

10.8 Prevention and control

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

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

10.9 Levels and pattern of food contamination

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

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

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

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

10.10 Dietary intake assessment

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

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

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

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

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

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

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

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

10.11 Dose–response analysis

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

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

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

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

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

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

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

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

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

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

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

11. EVALUATION

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

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

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

11.1 Recommendations

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

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

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