ACRYLAMIDE

This substance was considered by a previous Working Group, in June 1985 (IARC, 1986). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

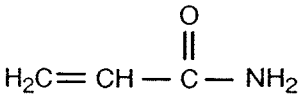
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 79-06-1
Chem. Abstr. Name: 2-Propenamide
IUPAC Systematic Name: Acrylamide
Synonyms: Acrylic acid amide; acrylic amide; ethylenecarboxamide; propenamide; propenoic acid amide; vinyl amide

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_3H_5NO Relative molecular mass: 71.08

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: White crystalline solid (monoclinic or triclinic crystal structure) (Habermann, 1991)
(*b*) *Boiling-point*: 136 °C at 3.3 kPa (Habermann, 1991)
(*c*) *Melting-point*: 84–85 °C (Lide, 1991)
(*d*) *Density*: 1.122 g/ml at 30 °C (Habermann, 1991)
(*e*) *Spectroscopy data*: Infrared [prism, 2998; grating, 8284], nuclear magnetic resonance, ultraviolet [3515] and mass spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991).
(*f*) *Solubility*: Soluble in water (215.5 g/100 ml at 30 °C), acetone (63.1 g/100 ml at 30 °C), chloroform (2.7 g/100 ml at 30 °C), diethyl ether, ethanol (86.2 g/100 ml at 30 °C), ethyl acetate (12.6 g/100 ml at 30 °C) and methanol (155 g/100 ml at 30 °C); very slightly soluble in heptane (6.8 mg/100 ml at 30 °C) (Budavari, 1989; Lide, 1991)

(*g*) *Volatility*: Vapour pressure, 0.9 Pa at 25 °C (Habermann, 1991)
(*h*) *Stability*: The solid is stable at room temperature but polymerizes on melting or exposure to ultraviolet light (Budavari, 1989)
(*i*) *Octanol–water partition coefficient (P)*: log P, –0.78 (Sangster, 1989)
(*j*) *Conversion factor*: $mg/m^3 = 2.91 \times ppm$[a]

1.1.4 *Technical products and impurities*

Acrylamide is available as a solid (either crystals or briquettes) and in aqueous solution. Most commercial preparations of acrylamide consist of a 50% aqueous solution stabilized with 25–30 ppm cupric ion or ethylenediaminetetraacetic acid, ferric ion, nitrite (Mannsville Chemical Products Corp., 1985; Habermann, 1991), hydroquinone, *tert*-butylpyrocatechol, *N*-phenyl-2-naphthylamine and other antioxidants (Budavari, 1989). Typical specifications for a 50% aqueous solution are: assay, 48–54 wt%; pH, 5.0–6.5; polymer (based on monomer), 100 ppm max.; acrylonitrile (based on solution), 100 ppm; and Cu^{++} inhibitor, 15–25 ppm max. (Habermann, 1991; Dow Chemical Co., 1992). Typical specifications for the crystalline monomer are: white, free-flowing crystal; assay, 98% min.; water, 0.8% max.; iron (as FeO), 15 ppm max.; water-insoluble material, 0.2% max.; and butanol-insoluble material, 1.5% max. (Habermann, 1991).

1.1.5 *Analysis*

EPA Method 8015 can be used to determine the concentrations of various non-halogenated volatile organic compounds, including acrylamide, in water, soil or sediment by gas chromatography with flame ionization detection. Aqueous process waste samples can be analysed by direct injection or the purge-and-trap method (EPA Method 5030); ground water and slightly contaminated soil and sediment samples must be analysed by the purge-and-trap method (US Environmental Protection Agency, 1986).

Biological monitoring of exposure to acrylamide involves determination of adducts of acrylamide with haemoglobin by gas chromatography–mass spectrometry, as tested experimentally (Farmer *et al.*, 1986; Bailey *et al.*, 1987) and in humans (Bergmark *et al.*, 1993).

Methods have been reported for the determination of acrylamide in water, wipe samples and polyacrylamide (Going & Thomas, 1979; Skelly & Husser, 1978). Water samples were reduced in volume by evaporation and analysed by gas chromatography with a nitrogen-selective thermionic detector. The detection limit was approximately 1 μg/L. Polyacrylamide samples were extracted with 80% methanol and 20% water (pH 3.75) for 3 h, and the extracts were analysed by high-performance liquid chromatography with an ultraviolet detector at 200 nm. The limit of detection of the monomer was approximately 0.5 μg/g. An analytical method for acrylamide in air and surface wipe samples, adapted from Skelly and Husser (1978), was described by Cummins *et al.* (1992). Air samples collected on a glass-fibre filter and XAD-7 solid sorbent tubes were desorbed with 5% methanol in water and analysed by

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[a]Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming normal temperature (25 °C) and pressure (101.3 kPa)

high-performance liquid chromatography with ultraviolet detection. Wipe samples obtained on moistened glass-fibre filters were extracted with water prior to analysis.

1.2 Production and use

1.2.1 *Production*

Acrylamide has been available commercially since the mid-1950s. The principal process for making acrylamide used to be reaction of acrylonitrile with hydrated sulfuric acid and separation of the product from its sulfate salt. This process yielded satisfactory amounts of monomer, either as crystals or in solution, but it also produced unwanted sulfates and other by-products. As far as can be determined, acrylamide is not produced by this process today (Mannsville Chemical Products Corp., 1985; Habermann, 1991; Smith & Oehme, 1991).

Catalytic hydration of acrylonitrile with copper metal or Raney copper catalyst was introduced in 1971. In this process, a solution of acrylonitrile in water is passed over a fixed bed of catalyst at 85 °C, and the acrylonitrile is converted directly to acrylamide. Levels of by-products are minimal, depending on the purity of the acrylonitrile. This method is now used throughout Europe, Japan and the USA (Mannsville Chemical Products Corp., 1985; Habermann, 1991; Smith & Oehme, 1991).

In 1985, microorganisms began to be used for the production of acrylamide from acrylonitrile by an enzymatic hydration process. This is one of the first uses of biocatalysts in the manufacture of chemicals in the petrochemical industry (Mannsville Chemical Products Corp., 1985; Habermann, 1991).

The largest volume of acrylamide is produced in Japan; there are also large production facilities in Europe and the USA. The estimated annual production capacity of the Japanese producers is 77 000 tonnes, that of the US producers, about 70 000 tonnes and that of the European producers, about 50 000 tonnes (Habermann, 1991). In Japan, 54 000 tonnes are estimated to have been produced in 1992 (Japan Petrochemical Industry Association, 1993).

Information available in 1991 indicated that acrylamide was produced by five companies in Japan, three each in Mexico and the USA, two in the United Kingdom and one each in Brazil, China, the Netherlands and Switzerland (Chemical Information Services Ltd, 1991).

1.2.2 *Use*

Acrylamide is a vinyl monomer which improves the aqueous solubility, adhesion and cross-linking of polymers. Water-soluble polyacrylamides are the largest application of acrylamide and are used in enhanced oil recovery as mobility control agents in water flooding, additives for oil well drilling fluids, and aids in fracturing, acidifying and other operations. Other uses of polyacrylamides are as flocculants for wastewater treatment, in the mining industry and various other process industries, soil stabilization, papermaking aids and thickeners, polymers for promoting adhesion, dye acceptors, and additives for textiles, paints, and cement. Acrylamide monomer is also used directly as a component of photopolymerization systems, in adhesives and grouts, and in cross-linking agents in vinyl polymers (WHO, 1985; Mannsville Chemical Products Corp., 1985; US Environmental Protection Agency, 1990). The patterns of use for acrylamide in the USA for several years are presented in Table 1.

Table 1. End-use patterns (%) for acrylamide in the USA

Use	Year		
	1985	1988	1991
Water treatment	45	45	65
Oil drilling	20	20	5
Pulp and paper	20	20	20
Mineral processing	10	10	5
Miscellaneous[a]	5	5	5

From Anon. (1985, 1988, 1991)
[a]Includes use as monomer (e.g. cross-linking agent, grout)

1.3 Occurrence

1.3.1 *Natural occurrence*

Acrylamide is not known to occur as a natural product.

1.3.2 *Occupational exposure*

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health between 1981 and 1983 indicated that 10 700 employees were potentially exposed to acrylamide at work in the USA (US National Institute for Occupational Safety and Health, 1993). Of this number, 30% were estimated to be exposed to acrylamide and 70% to materials containing acrylamide. The estimate is based on a survey of US companies and did not involve measurements of actual exposures. The US Environmental Protection Agency (1990) estimated that 700–1000 workers were exposed to acrylamide in the manufacture and processing of acrylamide monomer, 1800 in soil grouting and 100 000–200 000 in laboratories using polyacrylamide gels, such as in gel electrophoresis (Ducatman & Coumbis, 1991) and chromatography (Dearfield *et al.*, 1988).

Box 1 is a list of industries in which exposure to acrylamide monomer and polymer may occur. Occupations in which there is potential exposure to acrylamide include: chemical production; construction and maintenance involving pipe grouting and sealing; soil, tunnel and dam stabilization; water and wastewater treatment; and preparation of polyacrylamide gels in the laboratory. The potential for exposure to acrylamide has been decreased by the use of commercially prepared polyacrylamide gel plates (Ducatman & Coumbis, 1991). Exposure to acrylamide polymer may lead to exposure to acrylamide, since the polymer may contain small amounts of monomer (Mallevialle *et al.*, 1984).

Box 1. Industries in which there is potential exposure to acrylamide monomer and polymer

Acrylamide monomer

Acrylamide manufacture from acrylonitrile
Acrylamide polymer production
Adhesive and grout manufacture
Biotechnology laboratories

Acrylamide polymer

Water and wastewater treatment
Organic chemical manufacture
Inorganic chemical manufacture
Adhesive and grout manufacture
Coating applications
Moulded part manufacture
Textile products, weaving and fabric mills
Steel industry (blast furnace, metal part fabrication)
Water flocculent manufacture
Paper and pulp production
Lumber and wood production
Construction (soil and sand stabilization)
Crude oil production
Petroleum refining
Mineral processing
Concrete production
Sugar production
Hospitals
Biotechnology laboratories

Adapted from US National Institute for Occupational Safety and Health (1976, 1993)

Table 2 lists published data on exposure to acrylamide. Exposures may occur in monomer and polymer production from inhalation of dry powder or crystalline monomer, dermal contact with the monomer or dermal contact with solutions of acrylamide. Inhalation from solutions containing acrylamide is probably minimal because of their low volatility. The use of closed reactor vessels and automated addition of raw materials would be expected to reduce exposure. When acrylamide monomer is mixed or added by hand, the potential exposure is greater. Dermal exposure is known to account for a large number of cases of neurotoxic poisoning, but the dose was not given in any of the reports (Tilson, 1981; Dearfield *et al.*, 1988; He *et al.*, 1989; US Environmental Protection Agency, 1990; Myers & Macun, 1991).

Exposure may occur to either the dust or aqueous solutions of the monomer in water and wastewater treatment, preparation of chromatography and permeation gels in laboratories, underground pipe grouting operations and underground injection for soil stabilization. In these cases, the solid is mixed with water and a cross-linking or catalytic agent just prior to use. Exposures would be expected to depend strongly on the task (mixing and handling solutions) and to be intermittent.

Table 2. Occupational exposures to acrylamide

Industry, operation and process	Type of sample	No. of samples	Air concentration (mg/m^3)		Year of measurement	Country	Reference
			Mean	Range			
Acrylamide and polyacrylamide production							
Monomer and polymer production					1991	China	Bergmark *et al.* (1993);
Polymerization room	Area	12	3.27	0.19–8.8			Deng *et al.* (1993);
Monomer synthesis	Area	18	1.07	0.11–3.01			Calleman *et al.* (1994)
Monomer and polymer production					1985	China	He *et al.* (1989)
Polymerization	Area	NR	NR	5.56–9.02			
Polymerization after renovation	Area	NR	0.0324	NR			
Monomer and polymer production					1984–85	USA	Hills & Greife (1986)
Monomer operators	Personal	19	0.065 GM	0.001–0.227			
Polymer operators	Personal	27	0.031 GM	0.001–0.181			
Monomer material handlers	Personal	4	0.085 GM	0.017–0.260			
Polymer material handlers	Personal	4	0.023 GM	0.018–0.035			
Maintenance	Personal	14	0.013 GM	0.001–0.132			
Utility operators	Personal	4	0.116 GM	0.004–0.392			
Plantwide	Area	19	0.269 GM	0.014–8.291			
Plantwide	Area	18	0.029 GM	0.003–0.157			
Plantwide	Area	18	0.006 GM	0.004–0.009			
Plantwide	Area	28	0.010 GM	0.001–0.015			
Monomer and polymer production	Personal TWA	NR	NR	< 0.1	> 1970	USA	Sobel *et al.* (1986)
Continuous monomer production	Personal TWA	NR	NR	0.1–1	< 1957		
	Personal TWA	NR	NR	0.1–0.6	1957–70		
Monomer production					1976	USA	US National Institute
Reactor operator	Personal 4-h	1	0.48	–			for Occupational Safety
Dryer operator	Personal 4-h	1	0.52	–			and Health (1976)
Packing	Personal 4-h	2	0.64	0.52–0.76			
					1971–75		
Control room	Personal 8-h	NR		0.1–0.4			
Bagging room	Personal 8-h	NR		0.1–0.9			
Processing	Personal 8-h	NR		0.1–0.4			

Table 2 (contd)

Industry, operation and process	Type of sample	No. of samples	Air concentration (mg/m^3)		Year of measurement	Country	Reference
			Mean	Range			
Other uses							
Sewer line repair					1990	USA	Cummins *et al.* (1992)
Grouting operation (2 sites)	Personal	12	0.010	0.003–0.02			
Grouting operation (3 sites)	Area	18	0.022	0.002–0.05			
Sewer line repair					1985	USA	Hills & Greife (1986)
Grouting operation	Personal	2	0.005	0.002–0.007			
Grouting operation	Area	2	0.005	0.001–0.009			
Sewer line repair					1988	USA	US Environmental Protection Agency (1990)
Grouting operation	Personal	6	0.10	0.008–0.36			
Grouting operation	Area	3	0.04	< 0.004–0.08			
Coal preparation plant					1992	USA	Hoekstra & Weber (1993)
Static thickening of coal waste	Personal	2	NR	< 0.001			
Slurry	Area	12	NR	< 0.001			
Polyacrylamide flocculent manufacture					1986	South Africa	Myers & Macun (1991)
Workshop, warehouse, laboratory, powder plant	Personal	3	[0.02]				
Cleaner, closed reactor operator	Personal	1	[0.06]				
Acrylamide warehouse	Personal	2	[0.12]				
Exposed laboratory worker	Personal	1	[0.21]				
Closed reactor operator handling acrylamide	Personal	3	[0.27]				
Foreman, laboratory worker, manager	Personal	1	[0.36]				
Skip hoist operator, shooter-polymerizer, hyster driver	Personal	4	[0.48]				
Drier operator, hyster driver, making, shooting	Personal	7	[0.51]				
Making, dissolving	Personal	3	[0.75]				
Research laboratories					1991	USA	Rohwein (1991)
Preparation of polyacrylamide gels	Personal	1	1.5				
	Personal	1	0.9				
	Personal	1	0.5				
	Personal	9	N/Q				

NR, not reported; TWA, time-weighted average; GM, geometric mean; N/Q, detectable but not quantifiable

1.3.3 *Environmental occurrence*

Environmental releases of acrylamide from industrial facilities in the USA in 1991 were reported as (tonnes per year): 29.1 into air, 2.1 into water, 2100 during underground injection and 0.7 onto land (US National Library of Medicine, 1993).

Because polyacrylamide is used in water treatment, residues of acrylamide may be found in potable water. In most countries, such residues are limited to 0.25 μg/L by maintaining the concentration of acrylamide monomer in the polyacrylamide used for water treatment at < 0.05%. Concentrations of acrylamide in effluents from factories where polyacrylamide is used generally range from < 1 to 50 μg/L. In the vicinity of local grouting operations (e.g. for repair of sewer lines and soil stabilization), high levels of acrylamide may be found in wells and groundwater; a concentration of 400 mg/L was reported in one such well (WHO, 1985).

Levels of acrylamide have seldom been measured in ambient air, except as described under *Occupational exposure* above. Monitoring of concentrations in air close to six acrylamide-producing plants in the USA failed to detect any acrylamide (detection limit, 0.1 μg/m^3) (WHO, 1985).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for acrylamide in a number of countries and regions are presented in Table 3.

Guidelines and regulations have been set for acceptable levels of acrylamide in polyacrylamide, depending on the end use (WHO, 1985). For example, the US Food and Drug Administration (1993) established regulations for the use of polymers and copolymers of acrylamide in products in contact with food (21 CFR 175.105; 175.300; 177.1010), limiting the residual acrylamide in the polymer to 0.05% for acrylate–acrylamide resins for use in food treatment or as boiler-water additives (21 CFR 173.5; 173.310), and to 0.2% in polyacrylamide used as a film former in the imprinting of soft-shell gelatin capsules and in acrylamide copolymer resins used in paper or paperboard in contact with food (21 CFR 172.255; 173.315; 176.110; 176.170; 176.180; 178.3520).

In Germany, the level of polyacrylamide used in foodstuff packaging is limited to 0.3% and the level of residual acrylamide monomer in polyacrylamide to 0.2% (WHO, 1985). In the United Kingdom, it is recommended that commercial polyelectrolytes used as coagulants during the preparation of potable waters contain no more than 0.05% acrylamide. Unregulated polyelectrolytes may be used for effluent treatment and such polyacrylamides may contain up to 5% monomer (Brown *et al.*, 1980). In Germany, the level of residual monomer in polyacrylamide used in hair sprays is limited to 0.01% (WHO, 1985).

Table 3. Occupational exposure limits and guidelines for acrylamide

Country or region	Year	Concentration (mg/m^3)	Interpretation
Argentina	1991	0.03	TWA; carcinogen; skin
Australia	> 1983	0.03	TWA; probable human carcinogen; skin
Austria	1982	0.3	TWA
Belgium	> 1984	0.03	TWA; probable human carcinogen; skin
Canada	1986	0.3	TWA; intended change
		0.6	STEL; intended change
Denmark	1988	0.3	TWA; suspected carcinogen; skin
Finland	1993	0.3	TWA
		0.9	STEL
France	1993	0.03	TWA
Germany	1993	None	Animal carcinogen and germ-cell mutagen; skin
Hungary	1978	0.3	Ceiling; suspected of having carcinogenic potential; skin; irritant
Indonesia	1978	0.3	TWA; skin
Italy	1978	0.3	TWA; skin
Japan	1983	0.3	TWA; skin
Mexico	1989	0.3	TWA; skin
Netherlands	1986	0.3	TWA; skin
Norway	1990	0.03	TWA; carcinogen; skin
Republic of Korea	1983	0.3	TWA
		0.6	STEL
Romania	1983	0.3	TWA
		0.5	Ceiling
Sweden	1991	0.3	TWA; skin
		0.9	STEL (15 min)
Switzerland	1987	0.3 (provisional)	TWA; carcinogen; skin
Taiwan	1982	0.3	TWA
United Kingdom	1992	0.3	TWA; skin
		0.6	STEL (10 min)
USA			
ACGIH (TLV)	1994	0.03	TWA; suspected human carcinogen[a]; skin
OSHA (PEL)	1993	0.3	TWA; skin
NIOSH (REL)	1992	0.03	TWA; suspected human carcinogen; skin
Venezuela	1978	0.3	TWA; skin
		0.6	Ceiling

From Cook (1987); Arbejdstilsynet (1988); Direktoratet for Arbeidstilsynet (1990); ILO (1991); US National Institute for Occupational Safety and Health (NIOSH) (1992); American Conference of Governmental Industrial Hygienists (ACGIH) (1993); Deutsche Forschungsgemeinschaft (1993); Institut National de Recherche et de Sécurité (1993); Työministeriö (1993); UNEP (1993); US Occupational Safety and Health Administration (OSHA) (1993)

TWA, time-weighted average; STEL, short-term exposure limit; TLV, threshold limit value; PEL, permissible exposure level; REL, recommended exposure level

[a]Substance identified by other sources as a suspected or confirmed human carcinogen

2. Studies of Cancer in Humans

2.1 Cohort studies

In 1986, Sobel *et al.* reported the findings of a study on mortality among 371 employees who had had potential exposure to acrylamide during monomer and polymerization operations at a facility in Michigan, USA. The acrylamide monomer process began in 1955, and polymer production was initiated in 1965. Environmental concentrations of acrylamide in the monomer production areas had been measured using personal samplers and showed a decrease over time: before 1957, the 8-h time-weighted average concentration ranged from 0.1 to 1.0 mg/m^3, and in 1957–70 it was 0.1–0.6 mg/m^3; after 1970, all measured values were below 0.1 mg/m^3. Potential for exposure to acrylonitrile also existed in the monomer production area. Exposure to acrylamide occurred during polymer production due to inhalation of polyacrylamide dust containing residual acrylamide. Dust concentrations were above 2 mg/m^3 (time-weighted average) in packaging and drying operations and lower in other jobs; the residual amount of acrylamide in polymer dust was estimated to be about 1%. Other potential sources of acrylamide were dermal absorption and ingestion of polyacrylamide dust; however, their extent could not be assessed. Workers were identified from personnel census lists for 1955–79 as having worked in the production and pilot plants of interest. All study subjects were white; six of the 371 were women. A detailed work history was obtained for each: 76% of the workers had been employed for fewer than four years, and only 19% had started work before 1960. Fourteen subjects had also been exposed to organic dyes for five or more years. Mortality was examined from date of first potential exposure to 31 December 1982. Death certificates were obtained for all those who had died. Standardized mortality ratios (SMRs) were estimated, and expected deaths were calculated from mortality rates for US white males. Twenty-nine deaths were observed and 38 were expected (SMR, 76). The number of deaths from all cancers was slightly greater than expected (11 observed; SMR, 139; 95% confidence interval [CI], 70–249). The increase was related specifically to cancers of the digestive tract (four observed; SMR, 202; 95% CI, 57–539) and of the respiratory system (four observed; SMR, 138; 95% CI, 38–353). When workers with previous exposure to organic dyes were excluded, no increase in the rate for respiratory tract cancer was seen and two cases of digestive tract cancer were observed with 1.6 expected.

Mortality in four plants, three in the USA and one in the Netherlands, was studied in order to examine the possible cancer risk entailed by occupational exposure to acrylamide (Collins *et al.*, 1989). A total of 8854 men with potential exposure to acrylamide were employed in the plants between 1925 and 1976; 96% (8508; 7242 white and 1266 non-white) had been employed in the US facilities. Follow-up from 1925 to 1983 was complete for 94% of the cohort. Death certificates were obtained for 95% of the decedents in the USA and for 82% in the Netherlands. Exposure estimates were derived for each job in the four plants from ambient monitoring data available from 1977 onwards and from plant personnel knowledgeable about past processes and working conditions. An individual cumulative exposure index was calculated by combining the estimated average daily exposure and the number of days spent in each job held. Exposure to acrylamide was defined as cumulative exposure greater than 0.001 mg/m^3-year; 2293 men were exposed. Smoking histories were available

from medical records for 35% of the cohort. SMRs were estimated on the basis of expected deaths calculated from national death rates adjusted for age, calendar time and ethnicity. Analysis by plant showed no notable excess of any of the major categories of cause of death. Among exposed workers, there was a significant deficit for mortality from all causes (SMR, 81), but weak indications of an increased risk were noted for cancer of the pancreas (eight observed; SMR, 203 [95% CI, 87–400]) and Hodgkin's disease (five observed; SMR, 129 [95% CI, 42–300]). In addition, directly standardized relative risks were estimated from internal comparisons after adjustment for smoking status and latency, in addition to age and calendar period, for four categories of cumulative exposure (< 0.001, 0.001–0.03, 0.03–3.0 and > 3.0 mg/m^3-year). For none of the cancer sites examined did the relative risk depart significantly from unity. No trend in cancer mortality was seen with increasing cumulative exposure. In particular, for pancreatic cancer, relative risks of 0.90, 1.4, 1.1 and 1.3 were calculated by the Working Group for the four categories.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

In a screening assay based on increased multiplicity and incidence of lung tumours in a strain of mice highly susceptible to development of this neoplasm, groups of 40 female and 40 male A/J mice, eight weeks of age, received doses of 0 (control), 6.25, 12.5 or 25.0 mg/kg bw acrylamide (purity, > 99%) in 0.2 ml distilled water by oral gavage three times per week for eight weeks. Animals were killed seven months after the beginning of treatment. The authors reported a significant, dose-related increase in the number of mice with lung adenomas and in the number of lung adenomas per mouse ($p < 0.01$, logistic regression model) (Bull *et al.*, 1984a).

3.1.2 *Rat*

Groups of 90 male and 90 female Fischer 344 rats, five to six weeks of age, were administered 0, 0.01, 0.1, 0.5 or 2 mg/kg bw acrylamide per day in drinking-water for two years. The solutions were prepared twice a week from recrystallized acrylamide (purity, 96–99%; water content, 0.4–3.8%). Groups of 10 males and 10 females were killed at 6, 12 and 18 months, such that 60 animals of each sex were available for study. By the end of the study, survival had been reduced in groups of each sex receiving the highest dose. After adjustment for survival, there were significant increases in the incidences of thyroid gland tumours and peritoneal mesotheliomas in the region of the testis in males and tumours of the mammary gland, central nervous system, thyroid, oral cavity, uterus and clitoral gland in females (Table 4) (Johnson *et al.*, 1986).

Table 4. Numbers of Fischer 344 rats with tumours after receiving acrylamide in the drinking-water for two years

Type of tumour	Sex	Dose (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*
Testis, mesotheliomas of the tunica albuginea	M	3/60	0/60	7/60	11/60*	10/60*
Adrenal gland[a], phaeochromocytomas	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[b]	F	0/2	1/3	3/4	2/4	5/5*
Pituitary adenomas[a]	F	25/59	30/60	32/60	27/60	32/60*

From Johnson *et al.* (1986)

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

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

*$p = 0.05$; pair-wise Mantel–Haenszel comparison with the control group adjusted for mortality

3.2 Intraperitoneal administration

Mouse: In a screening assay similar to that described in section 3.1.1, groups of 16 female and 16 male A/J mice, eight weeks of age, received intraperitoneal injections of 0 (control), 1, 3, 10, 30 or 60 mg/kg bw acrylamide (purity, > 99%) dissolved in distilled water three times per week for eight weeks. A further control group was left untreated. Treatment with 60 mg/kg bw acrylamide was discontinued owing to the appearance of peripheral neuropathy and poor survival; animals in the other groups survived until six months after the beginning of treatment, at which time they were killed. The incidences of lung adenomas were: males—untreated controls, 5/16; vehicle controls, 2/16; 1 mg, 8/16; 3 mg, 6/16; 10 mg, 10/17; and 20 mg, 14/15 ($p < 0.01$, logistic regression model); females—untreated controls, 7/14; vehicle controls, 1/15; 1 mg, 6/17; 3 mg, 9/17; 10 mg, 11/14; and 30 mg, 14/15 ($p < 0.01$, logistic regression model). The average numbers of lung adenomas per mouse were 0.31 in untreated male controls, 0.06 in male vehicle controls, 0.75 in males given 1 mg, 0.69 in males given 3 mg, 0.88 in males given 10 mg and 1.87 in males given 30 mg; the numbers were 0.5 in untreated female controls, 0.13 in female vehicle controls, 0.35 in females given 1 mg, 0.88 in those given 3 mg, 1.57 in those given 10 mg and 2.53 in those given 30 mg (Bull *et al.*, 1984a).

3.3 Initiation–promotion studies

Mouse: Groups of 40 female Sencar mice, six to eight weeks of age, received 12.5, 25.0 or 50.0 mg/kg bw acrylamide (purity, > 99%) six times, either by gastric intubation in 0.2 ml

distilled water, by skin application in 0.2 ml ethanol or by intraperitoneal injection in 0.2 ml distilled water over a period of two weeks (total doses, 75, 150 or 300 mg/kg bw). Two weeks later, topical applications of 1 μg 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in 0.2 ml acetone were given on the back skin three times per week for 20 weeks. Vehicle control groups received distilled water by the same schedule. Groups of 20 mice received 300 mg/kg bw acrylamide followed by acetone instead of TPA. Nearly all animals survived until 52 weeks (34–38 per group), at which time they were killed; only gross lesions of the skin were examined histologically. Dose-related increases in the incidence of skin tumours were seen when acrylamide was given in combination with TPA by all routes. The numbers of skin tumour-bearing animals (papillomas and carcinomas combined) are shown in Table 5 (Bull *et al.*, 1984a).

Table 5. Skin tumours initiated in Sencar mice by administration of acrylamide by various routes

Route	Total dose (mg/kg bw)	No. of skin tumour-bearing animals/total no. of animals
Oral	0 + TPA	2/40
	75 + TPA	12/40
	150 + TPA	23/40
	300 + TPA	30/40
	300	0/20
Intraperitoneal	0 + TPA	0/40
	75 + TPA	10/40
	150 + TPA	13/40
	300 + TPA	21/40
	300	0/20
Topical	0 + TPA	7/40
	75 + TPA	4/40
	150 + TPA	11/40
	300 + TPA	18/40
	300	0/20

From Bull *et al.* (1984a); TPA, 12-*O*-tetradecanoylphorbol 13-acetate

Groups of 40 female Swiss-ICR mice [age unspecified] were administered total doses of 0, 75, 150 or 300 mg/kg bw acrylamide (purity, > 99%), divided into six equal portions over a two-week period, in water by oral gavage. Two weeks after the last dose, animals were given skin applications of 2.5 μg/mouse TPA dissolved in 0.2 ml acetone three times per week for 20 weeks. A further group of animals received a total dose of 300 mg/kg bw acrylamide administered as described above and received skin applications of 0.2 ml acetone alone. After 52 weeks, the surviving animals were killed and skin and lung tissues were evaluated. In the different groups, 32–36 animals survived to termination of the experiment. In animals receiving 0, 75, 150 or 300 mg/kg bw acrylamide plus TPA, the numbers with skin tumours were 0/40, 4/40, 4/40 and 13/40 [$p < 0.001$, Cochran-Armitage trend test], and those with

lung tumours were 4/36, 8/34, 6/36 and 11/34 [$p > 0.05$, Cochran-Armitage trend test], respectively. The numbers of skin tumours per mouse were 0, 0.10, 0.13 and 0.43 in the control and treated groups, respectively. Increased incidences of skin and lung tumours were also observed in the group treated with 300 mg/kg bw acrylamide plus acetone; the incidence of skin tumours was 10/40 [controls, 0/40; $p < 0.001$, Fisher exact test] and that of lung tumours was 14/36 [controls, 4/36; $p = 0.06$, Fisher exact test] (Bull *et al.*, 1984b).

4. Other Data Relevant for an Evaluation of Carcinogenicity and Its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

In a study in China, haemoglobin (Hb) adducts of acrylamide and glycidamide were determined in 41 workers who were exposed to acrylamide for periods ranging between one month and 11.5 years and who had concurrent medical and neurological examinations. The levels of N-terminal valine adducts of acrylamide ranged from 0.3 to 34 nmol/g Hb, with directly proportional formation of glycidamide adducts to the same residue in Hb. Background levels of valine adducts in Hb were not found in a group of 10 controls, with one possible exception, indicating that endogenous production of or environmental contamination with acrylamide is low or nonexistent (Bergmark *et al.*, 1993). The average ratio between the doses of glycidamide and acrylamide received (the concentration of free electrophilic agents integrated over time) in this group of workers was found to be 0.3. On the basis of the Hb adduct levels and the air concentrations in two of the workshops in this study (1.1 and 3.3 mg/m^3) and assuming 100% uptake of acrylamide through the air and an alveolar ventilation rate of 0.2 L/min per kg, it was concluded that there had been only minor exposure by inhalation and that dermal uptake was the dominant route of exposure.

Free acrylamide in plasma, mercapturic acids in urine (confounded by co-exposure to acrylonitrile) and several diagnostic indicators of neurotoxicity were determined in the same groups of workers described above. For workers in the acrylamide synthesis room, the average level of free acrylamide in plasma was 1.8 μmol/L and that of valine adducts was 13.4 nmol/g Hb; 64 μmol *S*-(2-carboxyethyl)cysteine were found in 24-h urine, but some was the result of exposure to acrylonitrile. The levels of free acrylamide in plasma were in good agreement with those expected on the basis of Hb adduct levels (Calleman *et al.*, 1994).

Crucial data are still lacking on the absorption and metabolism of acrylamide in humans: neither the degree of permeation through the skin nor its spectrum of urinary metabolites is known, and there are no reliable data on the rate of elimination of acrylamide.

4.1.2 *Experimental systems*

(*a*) *Acrylamide*

Oral absorption of acrylamide may be considered to be complete on the basis of the observation that urinary excretion of 10 mg/kg bw is similar when the compound is

administered by gavage or by intravenous injection into rats (Miller *et al.*, 1982). Frantz *et al.* (1985) reported that 26% of an aqueous solution was taken up through the skin of rats within 24 h, and a further 35% is available for further absorption. Uptake of acrylamide after inhalation has not been determined in any species.

In rats given 0.5–100 mg/kg bw of either [1-^{14}C]- or [2,3-^{14}C]acrylamide intravenously or orally, radioactivity was distributed rapidly throughout the body, with no selective accumulation in any tissue (Hashimoto & Aldridge, 1970; Miller *et al.*, 1982). Radioactivity was also distributed evenly among tissues of beagle dogs and miniature pigs (Ikeda *et al.*, 1987). After topical and oral administration of [2,3-^{14}]acrylamide to SENCAR and BALB/c mice, few strain differences were seen in either its tissue distribution or its association with DNA, RNA or proteins. Comparable concentrations were observed after administration by either route in all tissues except the skin, where the label concentration after topical administration was approximately 100 times higher than after oral dosing (Carlson & Weaver, 1985). Autoradiographic studies have shown, however, that, nine days after treatment, there is preferential retention of radioactivity in the reproductive tract of male mice. The same study also demonstrated that, 24 h after oral administration of 120 mg/kg bw acrylamide to pregnant mice on days 13.5 or 17.5 of gestation, the compound was distributed in the fetuses and, on the latter day, intense accumulation occurred in fetal skin (Marlowe *et al.*, 1986). This finding is consistent with those of previous studies that have shown that acrylamide crosses the placenta in rats, rabbits, dogs and pigs (Ikeda *et al.*, 1983, 1985).

After a single oral dose of [2,3-^{14}C]acrylamide (10 mg/kg bw) to male Fischer 344 rats, 53–67% was excreted within 24 h and 65–82% within seven days. More than 90% of the excreted radioactivity appeared in the urine. Urinary excretion was biphasic; the initial component, presumed to represent acrylamide and its metabolites, had a half-life of about 5 h, and the terminal component, presumed to result from the release of metabolites from tissue depots, had a half-life of eight days. Faecal excretion represented 4.8 and 6% after 24 h and seven days, respectively. Radiolabel was distributed rapidly to some tissues, but an initial absorption phase was noted for fat, liver, kidney, testis and plasma. Elimination from most tissues was biphasic, with a terminal half-life of about eight days. The tissues that contained the highest amounts of total radioactivity were muscle (48%), skin (15%), blood (12%) and liver (7%), in proportion to the relative mass of these tissues. Less than 1% of the dose was located in brain, spinal cord or sciatic nerve at any time; only erythrocytes concentrated the label. After intravenous injection of [2,3-^{14}C]acrylamide (10 mg/kg bw), the estimated first-order rate constants of elimination of the parent compound ranged from 0.23/h to 0.51/h in different tissues, with 0.4/h in the blood compartment. Only the testis exhibited an absorption phase, with a delay until peak concentration. Total recovery of acrylamide in tissues and excreta of animals was 96.8%, and no radioactivity was found in expired air (Miller *et al.*, 1982). In contrast, after administration of [1-^{14}C]acrylamide, 5 and 6% of the dose was excreted as carbon dioxide in beagle dogs (Ikeda *et al.*, 1987) and rats (Hashimoto & Aldridge, 1970), respectively, indicating the existence of an unidentified metabolic pathway resulting in the cleavage of the 1–2 carbon bond. It must be noted that studies of the tissue distribution of acrylamide based on measurements of total radioactivity suffer from uncertainty, owing to the inclusion of all metabolites and adducts.

After administration of high doses to rats, the mercapturic acid of acrylamide, resulting from direct or glutathione *S*-transferase-catalysed reaction with glutathione (Dixit *et al.*, 1981), is the main urinary metabolite (Edwards, 1975; Miller *et al.*, 1982). Mass spectrometric techniques have been used to show, however, that an epoxide metabolite, glycidamide, is formed *in vitro* and *in vivo* in rats (Calleman *et al.*, 1990) and that its formation is strongly dose dependent (Calleman *et al.*, 1992). The epoxide is excreted either intact as glyceramide following hydrolysis or as a mercapturic acid derivative following conjugation of either of its reactive carbons with glutathione (Sumner *et al.*, 1992) (Fig. 1 and Table 6). The fact that the proportion of urinary metabolites originating from glycidamide is higher in mice (59%) than in rats (33%) seems to indicate a higher conversion rate of acrylamide to glycidamide after an oral dose of 50 mg/kg bw in mice. Since the percentage of acrylamide metabolized *in vivo* to glycidamide increased from 13% in rats administered 100 mg/kg bw to about 50% after a dose of 5 mg/kg bw (Bergmark *et al.*, 1991), the percentage of urinary metabolites derived from glycidamide may be expected to increase and that from acrylamide, *N*-acetyl-*S*(2-carbamoylethyl)cysteine, to decrease at low doses. Specific urinary metabolites of acrylamide have not been studied in any species given doses lower than 50 mg/kg bw.

Table 6. Urinary metabolites collected from three mice and three rats given 50 mg/kg bw acrylamide orally, expressed as mean percentages of the total urinary metabolites excreted in 24 h

Metabolite	Mice	Rats
N-Acetyl-*S*-(2-carbamoylethyl)cysteine	41.2	67.4
N-Acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)cysteine	21.3	15.7
N-Acetyl-*S*-(1-carbamoyl-2-hydroxyethyl)cysteine	11.7	9.0
Glycidamide	16.8	5.5
Glyceramide	5.3	2.4

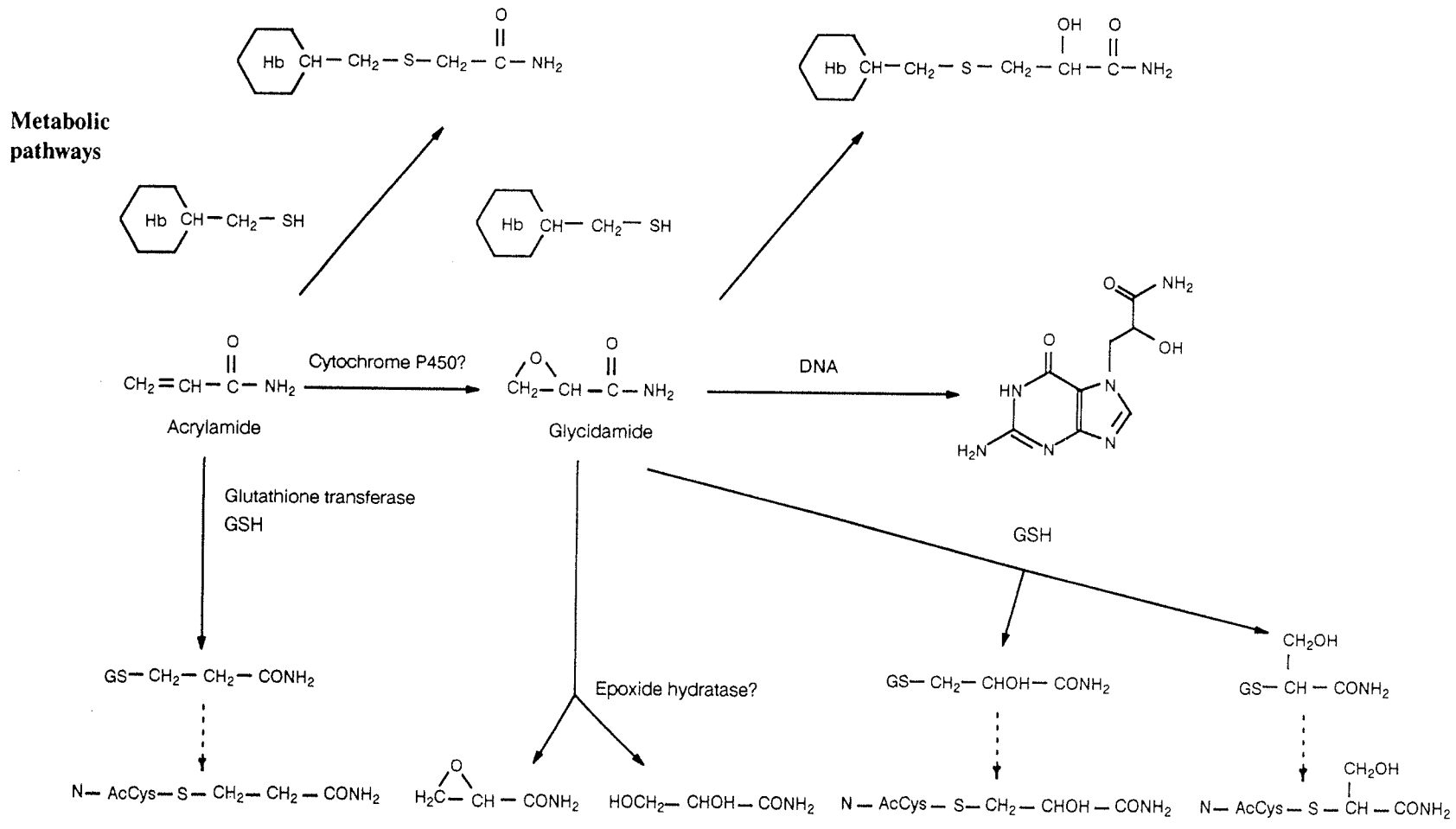
From Sumner *et al.* (1992)

Acrylamide reacts with tissue nucleophiles in Michael-type additions, and glycidamide reacts according to a nucleophilic substitution mechanism; both reactions result in covalent binding of the electrophile. Nonspecific covalent binding of radiolabelled acrylamide with proteins, RNA and DNA has been studied in different tissues of SENCAR and BALB/c mice after both topical and oral administration (Carlson & Weaver, 1985).

Covalent binding of acrylamide to central nervous system proteins may play a role in its neurotoxicity. In attempts to identify a putative target enzyme of acrylamide-induced peripheral neuropathy, inhibition of such enzymes as neuron-specific enolase (Howland *et al.*, 1980a,b), creatine kinase (Matsuoka *et al.*, 1990) and transglutaminase (Bergamini & Signorini, 1990) has been demonstrated *in vivo*, as has covalent binding to a range of proteins in the nervous system (Carrington *et al.*, 1991).

It has also been shown that a single intraperitoneal administration of acrylamide to rats of 25–100 mg/kg bw results in increased lipid peroxidation and decreased glutathione content and glutathione *S*-transferase activity in liver, while only the glutathione content was decreased in the brain (Srivastava *et al.*, 1983).

Fig. 1. Metabolism of acrylamide in mice and rats *in vivo*



Adapted from Sumner *et al.* (1992); Hb, haemoglobin; AcCys, acetylcysteine

(*b*) *Formation of haemoglobin adducts*

Hashimoto and Aldridge (1970) noted that a large percentage (12%) of an administered dose of acrylamide bound to Hb of red blood cells. On the basis of this observation, Bailey *et al.* (1986) developed a gas chromatographic–mass spectrometric technique to measure the formation of *S*-(2-carboxyethyl)cysteine in rats given 0.1–5 mg/kg bw acrylamide intravenously, after total hydrolysis of the protein. Using a modification of this technique, Calleman *et al.* (1990) identified the cysteine adduct, *S*-(2-hydroxy-2-carboxyethyl)cysteine, formed by reaction of glycidamide with Hb. Bergmark *et al.* (1991) developed a method to determine Hb adducts formed by both acrylamide and glycidamide with cysteine residues in rats. The Hb binding index of acrylamide with cysteine was 6400 pmol acrylamide residue/g Hb per μmol acrylamide/kg bw, which is higher than the indices for all other compounds so far studied in rats and higher than that of glycidamide, which was 1820 pmol glycidamide residue/g Hb per μmol glycidamide/kg bw after peritoneal injection at 50 mg/kg bw. Background levels of both cysteine adducts were determined in control rats. While Hb adduct formation was a linear function of dose when the metabolite glycidamide was administered to animals, Hb adduct formation by either acrylamide or glycidamide was strongly dose-dependent in rats treated with the parent compound. Thus, a cumulative dose of 100 mg/kg bw acrylamide given intraperitoneally to Sprague-Dawley rats produced Hb adduct levels ranging from 8.6 μmol/g Hb of *S*-(2-carboxyethyl)cysteine and 0.36 μmol/g Hb of *S*-(2-carboxy-2-hydroxy-2-ethyl)cysteine when given as a single injection to 3.6 and 0.75 μmol/g Hb, respectively, when administered as daily doses of 3.3 mg/kg bw for 30 days. After correction for the life-span of the erythrocytes and the increase in body weight of the animals, this dose-rate effect resulted in ratios between the estimated doses received (the concentration in the blood compartment of free electrophilic agents integrated over time) of glycidamide and acrylamide of [0.08, 0.32 and 0.47], depending on whether the dose was fractionated equally over 1, 10 or 30 days, respectively.

The observed dose-rate effect was presumed to be a result of the Michaelis-Menten kinetics of the metabolic conversion of acrylamide to glycidamide, and a mathematical model has been developed to describe the formation of Hb adducts in animals treated with acrylamide (Calleman *et al.*, 1992, 1993). Using the experimental data of Bailey *et al.* (1986) and Bergmark *et al.* (1991), Calleman *et al.* (1992) estimated the maximal metabolic rate (V_{max}), the Michaelis-Menten constant (K_m) and the first-order rates of elimination of acrylamide and glycidamide from all other processes by means of the model (Table 7). At a low concentration, the ratio of the dose received (the concentration in the blood compartment of free electrophilic agents integrated over time) of glycidamide to that of acrylamide was estimated to be 0.58.

(*c*) *Formation of adducts with cytoskeletal proteins*

Binding of ^{14}C-acrylamide to rat neurofilament and microtubular proteins has been studied *in vitro* (Lapadula *et al.*, 1989). Binding to microtubule-associated proteins 1 and 2 was at least an order of magnitude greater than that to tubulin. Other proteins that bound acrylamide significantly were heavy- and medium-weight neurofilaments and an unidentified 53 kDa protein (Table 8).

Table 7. Pharmacokinetic parameters of acrylamide and glycidamide determined in the blood of rats *in vivo*

Parameter	Compound	
	Acrylamide	Glycidamide
Linear model		
k_{el}	0.36/h[a] 0.40/h[b] 0.37/h[c] 0.50/h[d]	0.48/h[c]
$t_{½}$	1.4–1.9/h[c,d]	1.5/h[c]
Non-linear model		
V_{max}	19 μmol/h[e]	
Km	66 μmol/h[e]	
k'_{el}	0.21/h[f]	

k_{el}, first-order rate of elimination; $t_{½}$, half-life; V_{max}, maximal metabolic rate; K_m, Michaelis-Menten constant; k'_{el}, first-order rate of elimination from all processes other than metabolic conversion to glycidamide

[a]Calculated by Calleman *et al.* (1992) from Edwards (1975); 100 mg/kg intravenously

[b]Calculated by Calleman *et al.* (1992) from Miller *et al.* (1982); 10 mg/kg orally

[c]From Bergmark *et al.* (1991); average for 0.5–100 mg/kg intraperitoneally

[d]From Calleman *et al.* (1992); low-dose estimate

[e]From Calleman *et al.* (1992); metabolic conversion of acrylamide to glycidamide

[f]From Calleman *et al.* (1992)

Table 8. Specific activities of binding of ^{14}C-acrylamide to cytoskeletal proteins

Protein	^{14}C-Acrylamide bound (mmol/mol protein)
Microtubule-associated protein-1	2.21
Microtubule-associated protein-2	0.89
Tubulin	0.04
Heavy-weight neurofilament	0.53
Medium-weight neurofilament	0.31
Light-weight neurofilament	0.06
53-kDa protein	0.43
Glial fibrillary acidic protein	0.02

From Lapadula *et al.* (1989)

(*d*) *Formation of adducts with protamines*

Sperm from the vasa deferentia of mice that had been injected intraperitoneally with 125 mg/kg ^{14}C-acrylamide was analysed for radioactivity in the sperm head, sperm DNA and sperm protamines at approximately daily intervals for three weeks. The radioactivity associated with purified protamine closely paralleled the total radioactivity associated with the sperm. Very little radioactivity (< 0.5%) was associated with sperm DNA. The period of maximal protamine alkylation (about day 8 after dosing; Fig. 2) corresponded to the period of maximal sensitivity for induction of dominant lethal mutations. Analysis of acid-hydrolysed protamine showed that 31% of the protamine adducts co-eluted with *S*-carboxyethylcysteine (Sega *et al.*, 1989).

4.1.3 *Comparison of humans and experimental animals*

The formation of glycidamide and acrylamide adducts on the N-terminal valine of Hb is directly proportional in man and rat. Comparison of free acrylamide in plasma, valine adducts on Hb and urinary *S*-(2-carboxyethyl)cysteine indicate that the rate of elimination of acrylamide is at least five times lower in man than in rats. Therefore, since the integrated concentration–time ratio for glycidamide to acrylamide adducts in man (0.3, see p. 402) is about one-half of that for rats (0.58, see p. 402) at low doses, the tissue dose of glycidamide may be higher in man than in rats, on the basis of an equal uptake of acrylamide.

4.2 Toxic effects

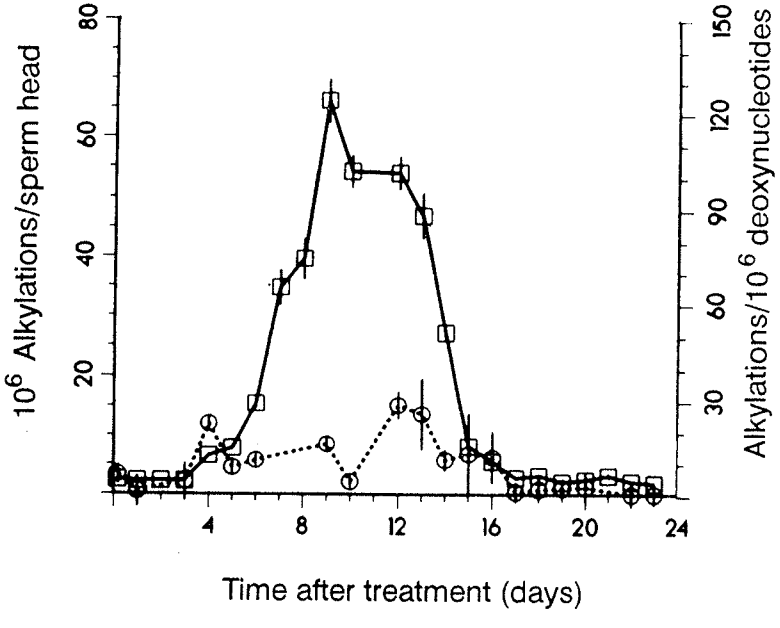
The toxicity of acrylamide has been reviewed (Dearfield *et al.*, 1988; King & Noss, 1989; US Environmental Protection Agency, 1990; Molak, 1991).

4.2.1 *Humans*

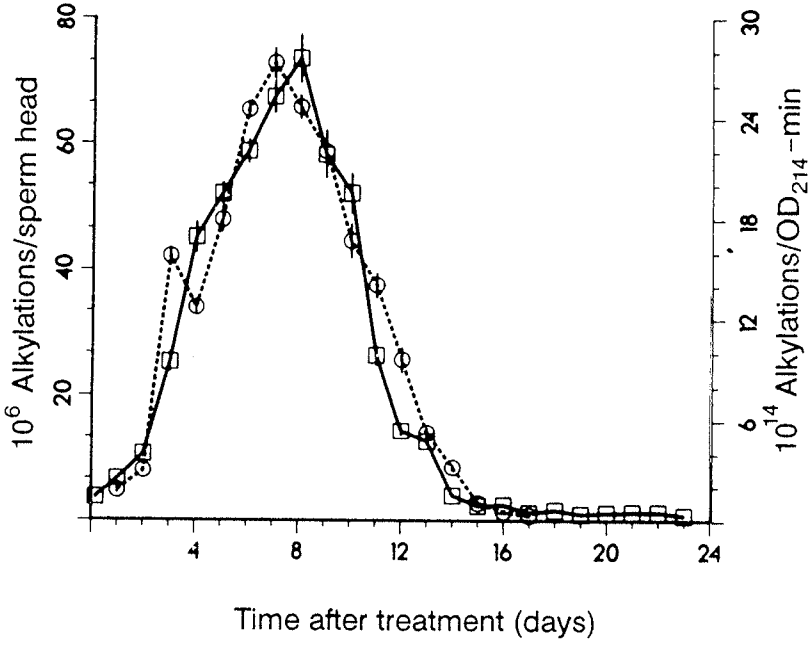
The clinical neurological signs and symptoms resulting from human exposure have been summarized (US Environmental Protection Agency, 1990). They include numbness of hands and feet, peeling of skin and impairment of sensation of vibration, touch and pain; loss of ankle reflexes, muscular atrophy, loss of body weight and ataxia are seen in severe cases. Recovery from mild forms of neuropathy is usually complete within a few months, whereas in severe cases there may never be full recovery. Recent studies of humans poisoned with acrylamide have directed attention to exposures to this substance in developing countries (He *et al.*, 1989; Myers & Macun, 1991) and prompted the development of techniques for human biomonitoring.

In the study carried out in China (described in detail in section 4.1.1; Calleman *et al.*, 1994), significant differences were found in the frequencies of signs and symptoms of neurotoxicity, and differences were seen in vibration sensitivity and electroneuromyographic measurements between the exposed group of workers and controls. These diagnostic indicators of acrylamide neurotoxicity formed the basis for a neurotoxicity index, reflecting a clinical diagnosis of peripheral neuropathy in humans. This index correlated better with biomarkers that reflect long exposure than with momentary measures of air concentrations, and the average levels of Hb adducts paralleled the average neurotoxicity indices in different workshops in the factory.

Fig. 2. Alkylation of sperm heads, sperm DNA and sperm protamine recovered during three weeks after intraperitoneal administration of ^{14}C-acrylamide at 125 mg/kg bw



Alkylation of sperm heads (squares) and DNA (circles) taken from the vasa deferentia. Error bars represent ± 1 SD.



Alkylation of sperm heads (squares) and sperm protamine (circles) recovered from the caudal epididymides. OD_{214}, optical density at 214 nm. Error bars represent ± 1 SD.

From Sega *et al.* (1989)

4.2.2 *Experimental systems*

(*a*) *Acrylamide*

Acrylamide is a cumulative neurotoxicant in several species of experimental animals; notably, it causes peripheral neuropathy (Tilson, 1981). The disorder has been termed central–peripheral distal axonopathy (Spencer & Schaumburg, 1977), since it is characterized by distal retrograde degeneration of long, large-diameter axons in both the central and peripheral nervous systems. Treatment of experimental animals with acrylamide results in reduced water and food consumption, decreased weight gain, weakness and ataxia in hindlimbs, decrease in axonal transport, accumulation of neurofilaments and demyelination of nerves (US Environmental Protection Agency, 1987). An effect of dose rate has been suggested from studies in rats (O'Shaughnessy & Losos, 1986). The central nervous system was affected more strongly than the peripheral nervous system in rats administered acrylamide by intraperitoneal injection at doses of up to 50 mg/kg bw per day for four or 10 days than when the rats received doses of up to 12 mg/kg bw per day for 90 days. The mechanism of the neurotoxic action of acrylamide is not known, but at least three hypotheses have been proposed: inhibition of glycolytic enzymes (Spencer *et al.*, 1979), although non-neurotoxic chemicals can also inhibit these enzymes (Tanii & Hashimoto, 1985); alterations in axonal transport (Miller & Spencer, 1985; Harry, 1992); and filament degradation (Sager & Matheson, 1988; Tanii *et al.*, 1988; Carrington *et al.*, 1991). These hypotheses are not mutually exclusive. The last, in particular, may affect our understanding of the genotoxicity of acrylamide and its neoplastic properties in experimental systems. In PtK1 kidney epithelial cells, acrylamide caused networks of both vimentin and keratin filaments to collapse into a juxtanuclear aggregate (Eckert, 1986). Such effects occur at concentrations that do not affect microtubular polymerization (Sager & Matheson, 1988), and they are reversible (Denèfle & Zhu, 1989). The lack of effect of acrylamide on polymerization of microtubular protein was confirmed *in vitro*. Furthermore, the effects in cells of acrylamide and colchicine (a potent anti-microtubular agent) are additive (Shiver *et al.*, 1992).

(*b*) *Glycidamide*

Glycidamide depresses body weight in rats (Hashimoto *et al.*, 1988) and has a potent effect on rats undergoing the rotarod test for neurotoxicity; in contrast to acrylamide, it does not affect hindlimb splay (Costa *et al.*, 1992). Although glycidamide was as cytotoxic as acrylamide to mouse neuroblastoma cells *in vitro*, it did not cause a decrease in the number of neurites (Walum *et al.*, 1992). These findings indicate that glycidamide is not the agent that causes peripheral neuropathy, although potentially conflicting results have been reported (Abou-Donia *et al.*, 1993).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

The reproductive, developmental and genotoxic effects of acrylamide have been reviewed (Dearfield *et al.*, 1988).

Male ddY mice receiving oral doses of 35 mg/kg bw acrylamide twice weekly for eight weeks developed testicular atrophy and degeneration of the germinal epithelium, but Sertoli and interstitial cells did not appear to be affected. Neurotoxic signs were observed, but when mice were treated concurrently with intraperitoneal injections of phenobarbital, the neurotoxic signs were significantly reduced and testicular toxicity was prevented, probably because of accelerated detoxification (Hashimoto *et al.*, 1981). Sakamoto *et al.* (1988) treated male ddY mice with a single oral dose of 100 or 150 mg/kg bw acrylamide and studied the histopathological changes in the testis over the following 10 days. In both immature and adult mice, round spermatids, especially in the Golgi phase, were sensitive to the toxic effects of acrylamide, and all other cell types (spermatogonia, spermatocytes, Sertoli cells and Leydig cells) were unchanged.

Marlowe *et al.* (1986) using autoradiography, showed that 120 mg/kg bw acrylamide administered orally to Swiss-Webster mice crossed the placenta and was distributed in the fetus on days 13.5 and 17.5 of gestation; on day 17.5, intense accumulation occurred in fetal skin. When acrylamide or its metabolites were administered to male Swiss-Webster mice, they appeared to bind to mature spermatids, since activity was observed in the seminiferous tubules and the head of the epididymis by 9 h after administration; by nine days, activity was present only in the tail of the epididymis and in the glans penis.

A reduced content of striatal dopamine receptors was seen in male but not female neonates at 14 days of age but not at 21 days after oral administration of 20 mg/kg bw acrylamide per day to pregnant Fischer 344 rats on days 7–16 of gestation (Agrawal & Squibb, 1981). Increased numbers of dopamine receptors were seen in the striatum of adult acrylamide-treated rats one day but not eight days after treatment for 10 days (Agrawal *et al.* 1981).

Reproductive toxicity was investigated in male and female Long-Evans rats by Zenick *et al.* (1986). For the studies in males, groups of 15 rats were treated with 0, 50, 100 or 200 ppm (mg/L) acrylamide in the drinking-water from 70 days of age onwards. Sexual behaviour was assessed by mating them with hormonally primed, ovariectomized females before and at two-week intervals during treatment. Sperm count, motility and morphology were assessed by recovering the ejaculate from the female reproductive tract before treatment began and after nine weeks of treatment. After 10 weeks of treatment, the control and 100-ppm groups were mated with untreated females and the females were killed on day 17 of gestation to record the number of implants. Males were killed after 11 weeks of treatment; one testis and epididymis were examined histologically and the others for spermatid and sperm counts. Severe toxicity and death were observed by week 5 in the 200-ppm group, and treatment of this group was terminated at week 6. No mortality or weight loss was observed in the other groups, but some hind limb splaying was observed in the 100-ppm group at week 8. Treatment with 50 or 100 ppm induced a significant increase in sexual activity, but reduced ejaculatory ability and reduced sperm counts were seen at 100 ppm: the sperm of only 1/15 animals reached the uterus after mating at week 9. No adverse effect on sperm count, motility

or morphology was observed at 50 ppm. Fertility at week 10 was also decreased at 100 ppm, with only 5/15 mated and 11/14 control females becoming pregnant; postimplantation loss was also increased (32% *versus* 8%; $p < 0.01$). On examination *post mortem* at week 11, no adverse effect was observed at 50 or 100 ppm on body or organ weights, on spermatid counts in the testis, on epididymal sperm counts or on testicular histology. The authors suggested that the reduced sperm count observed at 100 ppm in the mating trials resulted from disrupted copulatory behaviour which caused vaginal leakage of the sperm.

For the studies in females, Zenick *et al.* (1986) treated groups of 15 rats that had regular oestrous cycles with acrylamide at 0, 25, 50 or 100 ppm (equivalent to about 10–20 mg/kg bw at the highest dose) in the drinking-water; a pilot study had shown that a dose of 200 ppm was too toxic. After two weeks of treatment, the females were mated with untreated males, and exposure was continued throughout gestation and lactation; the pups were killed on postnatal day 42. Hindlimb splaying was observed during the first and second week of gestation in the 100-ppm group only, with depressed body weight from week 2 of treatment onwards. Dams in the 50-ppm group had depressed body weight only during the lactation phase. There was no adverse effect on mating performance or pregnancy rate and no significant difference in litter size or pup survival. There was a small but significant decrease in pup weight at birth in the 100-ppm group, which persisted until termination of the study on day 42. The pups from the 50-ppm group also had reduced body weight from seven days of age onwards. At 25 ppm, female pup weight was depressed on days 7 and 14 only. Vaginal opening was delayed in the pups of the 100-ppm group (day 36.0 ± 1.6 *versus* day 33.2 ± 1.9; $p < 0.01$). The cumulative intake of males at the onset of hindlimb ataxia was 544 mg/kg bw at 8–10 weeks, but copulatory behaviour was affected before ataxia was observed. The increase in postimplantation loss (up to 50%) in the mated females in the only five successful matings may indicate a dominant lethal effect (reported in other studies). It is unclear from the study whether the decrease in pup weight was secondary to toxicity in the dams or was a direct effect of acrylamide acting on the pups via the milk.

Groups of 50 male Fischer 344 rats were dosed orally by gavage with 30 mg/kg bw acrylamide daily for five days and then mated with one female each per week for 10 weeks. Pre- and post-implantation losses were significantly raised in the first four and three weeks, respectively, after exposure, both returning to control values for the remaining six weeks. This indicates that acrylamide induces dominant lethal mutations in mature spermatozoa and late-stage spermatids (Working *et al.*, 1987).

Sublet *et al.* (1989) investigated the possibility that the reduction in fertility and the increase in pre-implantation loss observed after acrylamide treatment might be due to factors other than chromosomal damage. Groups of 15 male Long-Evans hooded rats were treated orally with doses ranging from 5 to 60 mg/kg bw acrylamide for five consecutive days and mated for 4–10 weeks after exposure. Reduced fertility and pre- and post-implantation loss were observed mainly in the first three weeks after treatment. In a subsequent experiment to study factors associated with reduced implantation frequencies, decreased entry of sperm from the vagina to the uterus was observed in the first week, and there were decreased percentages of motile sperm and decreased sperm velocity in weeks 2 and 3. Ova recovered 10–14 h after copulation showed a decreased fertilization rate.

In a study of the effects of acrylamide on protein-deficient rats (Khanna *et al.*, 1988), 32 pregnant Wistar rats were fed either a normal protein diet (20% casein) or a low-protein diet (8% casein) from day 1 of gestation (day of vaginal plug). Half of each group was treated orally [presumably by gavage] from day 6 of gestation until weaning with 0.3 mg/kg bw acrylamide; controls received saline. At parturition, litters were culled to eight pups, so that each study group consisted of four litters of eight pups each. Postnatal development was studied up to weaning, on day 21 post-partum. Acrylamide had no effect on the weight of the pups of dams fed the normal diet but produced a significant reduction in the body weight of pups of dams fed the protein-deficient diet in comparison with that of their respective controls. Protein deficiency alone had no effect on the time of development of landmarks such as eye opening, pinna detachment, incisor eruption or reflex development, and acrylamide had no effect on the time of development of these landmarks in pups of the group fed the normal diet; however, acrylamide significantly retarded the appearance of the landmarks, including surface and air righting reflexes and locomotion, in pups of dams on the low-protein diet. It also reduced the numbers of dopamine and benzodiazepine binding sites in the striatum and cortex, respectively, in pups of the protein-deficient group. In a similar study (Khanna *et al.*, 1992), in which pregnant Wistar rats on high- and low-protein diets were dosed on days 6–17 of gestation, higher doses of acrylamide (3–10 mg/kg bw) given orally daily induced high mortality in dams fed the low-protein diet. In animals treated with 2 mg/kg bw acrylamide on days 6–17 and examined on day 18, there was decreased activity of brain monoamine oxidase and acetylcholine esterase; in pups of dams fed on the low-protein diet there was a decrease in striatal dopaminergic, cerebellar muscarinic and frontocortical diazepam binding sites.

In a combined study of mutagenicity and teratogenicity (Neuhäuser-Klaus & Schmahl, 1989), female T stock mice were mated with HT males and injected intraperitoneally with 75 mg/kg bw acrylamide once on day 12, or with 50 or 75 mg/kg bw on days 10, 11 and 12 of gestation; controls were injected with 10 ml/kg bw distilled water. The fetuses were examined macroscopically on day 18, and malformed fetuses and five controls were subjected to histological examination. Kinked tails and haemorrhages were observed in 4.2% of all treated fetuses and in 1.3% of controls ($p = 0.05$). The three daily doses of 75 mg/kg caused increased postimplantation loss and decreased fetal weight. Histological examination of eight treated fetuses showed hypoplasia of lymphatic organs and of centres of haematopoesis in liver and bone marrow. Positive results were observed in the spot test in pups that were allowed to be delivered; the doubling dose was calculated to be 30 mg/kg bw acrylamide. Kinked tails were also observed in these offspring.

A full study of the teratogenicity of acrylamide in Swiss CD-1 and Sprague-Dawley rats has been reported by Field *et al.* (1990). Groups of 30 mice (25–29 pregnant at term) were dosed by gavage with 0, 3, 15 or 45 mg/kg bw acrylamide per day from days 6 to 17 gestation (vaginal plug = day 0); fetuses were removed and examined on day 17. All fetuses were examined by dissection and by staining for skeletal malformations. Maternal toxicity was observed at the highest dose only, with half the animals showing hindlimb splaying by day 17 and reduced body weight gain. No effect was seen on the numbers of implantations, resorptions or live fetuses per litter or overall, or on specific types of malformations. Fetal body weight was reduced at the highest dose. The only other significant effect was a

dose-related trend in the percentage of fetuses per litter with extra ribs, from 3.4% ± 1.2 in controls to 13.9% ± 3.7 at the highest dose ($p < 0.05$). Groups of 29–30 rats (23–26 pregnant at term) were dosed by gavage with 0, 2.5, 7.5 or 15 mg/kg bw acrylamide per day from day 6 to 20 of gestation (vaginal plug = day 0) and the fetuses removed for examination as described above on day 20. Maternal weight gain (minus gravid uterine weight) was reduced at 7.5 and 15 mg/kg bw, but no other clinical sign of toxicity was observed. No adverse effect was observed on the numbers of implantations, resorptions or live fetuses per litter, fetal weight or malformations. The only effect observed was a dose-related trend in the percentage of fetuses per litter with skeletal variations, which were mainly rudimentary extra ribs (from 11.1% ± 3.6 in controls to 16.4% ± 4.0 at the highest dose). No malformations of the tail were observed in either mice or rats. The authors commented that the extra ribs may have been related more to maternal toxicity than to a direct effect of acrylamide on the fetuses. No maternal or fetal toxicity was seen at 15 mg/kg bw per day in mice; in rats, no effect on maternal toxicity was seen at 2.5 mg/kg bw per day and probably no effect on fetal toxicity 15 mg/kg bw per day.

The reproductive toxicity of acrylamide and its epoxide metabolite, glycidamide, was studied in male Sprague-Dawley rats by Costa *et al.* (1992). Groups of eight rats were injected intraperitoneally once a day with 50 mg/kg bw acrylamide for seven days or with 50 mg/kg bw glycidamide for 14 days. Twenty-four hours after the last dose, the animals were killed and the testes removed and weighed to measure epididymal and vas deferens sperm count and viability. Neither substance affected testicular weight. Vas deferens sperm count was reduced by both substances, and glycidamide but not acrylamide reduced testicular protein content, epididymal weight and vas deferens sperm viability.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see also Table 9 and Appendices 1 and 2)

(a) Formation of DNA adducts

Acrylamide reacts very slowly with DNA *in vitro*, with a rate constant of [9×10^{-12} L/mg DNA per h] at pH 7.0 and 37 °C, estimated on the basis of all types of DNA adducts combined. The principal site of reaction of acrylamide with calf thymus DNA was the N1 of adenine, and only 14% of the reaction products was with the N7 of guanine. In some DNA adducts formed *in vitro*, the amide group of the adducted acrylamide moiety underwent spontaneous hydrolysis to a carboxylic acid group (Solomon *et al.*, 1985).

Carlson and Weaver (1985) and Sega *et al.* (1990) measured the total amount of radioactivity associated with DNA in acrylamide-treated mice. Carlson *et al.* (1986) found more radioactivity associated with DNA after oral administration of [2,3-^{14}C]acrylamide than after topical administration in all tissues except the skin of SENCAR mice. This result appears to conflict with the data of Bull *et al.* (1984a), who found a higher frequency of skin tumours in the same strain of mice after oral than after topical administration. [The Working Group

noted that no attempt was made in any of these studies of tissue distribution to isolate specific DNA adducts, so that metabolic incorporation or contamination by non-covalently bound metabolites cannot be ruled out; it is not clear whether the skin samples analysed were from the application area.]

7-(2-Carbamoyl-2-hydroxyethyl)guanine formed by the metabolite glycidamide is the predominant adduct in DNA of rats and mice exposed to ^{14}C-acrylamide (Segerbäck *et al.*, 1994).

(*b*) *Mutation and allied effects*

Acrylamide did not increase the mutation frequency in bacteria, but it induced differential toxicity in one *rec* assay.

Somatic mutation and sex-linked recessive lethal mutations have been observed in *Drosophila melanogaster* after feeding of acrylamide to larvae. In contrast, no increase in the frequency of sex-linked recessive lethal mutations was observed in a single-injection experiment.

Unscheduled DNA synthesis in primary cultures of rat hepatocytes was not observed in two studies.

Mutations were induced at both the *tk* and *hprt* locus in mouse lymphoma L5178Y cells but not at the *hprt* locus in Chinese hamster V79H3 cells. In those studies that showed an effect, they were observed at single doses within each experiment and at relatively low (< 10%) survival.

There is consistent evidence for the induction of sister chromatid exchange and chromosomal aberrations in cultured mammalian cells, including cultures of human lymphocytes. Various mitotic disturbances have also been reported in cultured mammalian cells exposed to acrylamide. It induced significant transformation in BALB/c 3T3, C3H10T½ and NIH 3T3 cell lines.

[The Working Group noted that the activity of acrylamide in cultured mammalian cells was also seen in the absence of an exogenous metabolic activation system, implying that glycidamide might not be the responsible agent.]

Alkali-labile sites and DNA single-strand breakage have been observed in germ-line cells of mice. Conflicting results were reported for the induction of unscheduled DNA synthesis in rat liver *in vivo*.

In the Muta^{TM} mouse, a transgenic mouse, three-fold and six-fold increases in mutant frequency were reported. These are considered to be equivocal results from this developing assay, since there is considerable variation in control mutation frequencies.

An intraperitoneal dose of 50 mg/kg bw acrylamide induced a significant response in the mouse spot test, in which the spots may result not only from point mutations but also from chromosomal aberrations, chromosomal loss or somatic recombination. Additionally, in the morphological specific-locus test in mice, which allows detection of both small and large genetic lesions, a significant increase in mutation rate was seen in certain stages of spermatogenesis, particularly on days 5–12 after treatment (spermatozoa and spermatids). On the basis of cytogenetic evidence, four of the six verified specific locus mutations induced postmeiotically were considered to be multi-locus lesions. Testing of spermatogonial stages

yielded negative results with a fractionated treatment regimen of 5×50 mg/kg bw in one study but positive results after a single injection of 100 mg/kg bw in another.

Acrylamide induced sister chromatid exchange in splenocytes but not in bone marrow or spermatogonia of mice. In several studies, acrylamide induced micronuclei in mouse bone-marrow cells. [Whether the micronuclei indicate structural chromosomal aberration or aneuploidy induction cannot be concluded from these data.] Micronuclei have also been observed in mouse splenocytes and spermatids following exposure to acrylamide during meiosis. In two of three studies, acrylamide given at intraperitoneal doses of 50 or 100 mg/kg bw to mice *in vivo* induced chromosomal aberrations in bone-marrow cells; however, an intraperitoneal dose of 125 mg/kg bw did not induce chromosomal aberrations in mouse splenocytes.

Except in one study, chromosomal aberrations were not induced in differentiating spermatogonia by single or repeated doses of acrylamide; however, a clastogenic effect on spermatocytes was observed in three independent studies. The induction of dominant lethal mutation in rats and mice after various treatment regimens, including one dermal application, was demonstrated in several laboratories. The stages sensitive to dominant lethal effects (late spermatids to early spermatozoa) correspond to increased DNA breakage and parallel the pattern of sperm alkylation and protamine alkylation. Heritable translocations were also induced in male mice in two independent studies.

Studies of aneuploidy induction in bone marrow and spermatogonia *in vivo* gave either negative or inconclusive results, due to the combined reporting of polyploidy and aneuploidy in one study and to protocol uncertainties in another. Hyperploidy was observed in spermatids. Moreover, approximately one-third of the micronuclei found in spermatids after acrylamide treatment of prophase cells showed kinetochore-positive staining, which might suggest a mixed breakage–aneuploidy response (Collins *et al.*, 1992).

(*c*) *Mutagenicity of glycidamide*

Glycidamide is mutagenic to *Salmonella typhimurium* but not to *Klebsiella pneumoniae*. It is more mutagenic than acrylamide to mouse lymphoma cells. Glycidamide induced unscheduled DNA synthesis in a cell line, in primary cultures of rat hepatocytes and in mouse spermatids *in vivo*.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Acrylamide has been produced since the 1950s by hydration of acrylonitrile. It is used mainly to produce water-soluble polyacrylamides used as flocculents for clarifying drinking-water, for treating municipal and industrial waste waters and as flow control agents in oil-well operations. Other major uses of acrylamide are in soil stabilization, in grout for repairing sewers and in acrylamide gels used in biotechnology laboratories. The major routes of exposure at the workplace appear to be dermal absorption of acrylamide monomer from solution and inhalation of dry monomer or aerosols of acrylamide solution. Exposure occurs during acrylamide and polyacrylamide manufacture, during acrylamide grouting and during laboratory preparation of polyacrylamide gels.

Table 9. Genetic and related effects of acrylamide

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BSD, *Bacillus subtilis* spore *rec* assay, differential toxicity	+	+	10000.0000	Tsuda *et al.* (1993)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500.0000	Lijinsky & Andrews (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	15000.0000	Bull *et al.* (1984a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500.0000	Hashimoto & Tanii (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	10000.0000	Knaap *et al.* (1988)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	10000.0000	Tsuda *et al.* (1993)
SA2, *Salmonella typhimurium* TA102, reverse mutation	–	–	10000.0000	Knaap *et al.* (1988)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500.0000	Lijinsky & Andrews (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	15000.0000	Bull *et al.* (1984a)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500.0000	Hashimoto & Tanii (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	10000.0000	Knaap *et al.* (1988)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	10000.0000	Tsuda *et al.* (1993)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500.0000	Lijinsky & Andrews (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	15000.0000	Bull *et al.* (1984a)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500.0000	Hashimoto & Tanii (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	10000.0000	Knaap *et al.* (1988)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	25000.0000	Tsuda *et al.* (1993)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	500.0000	Lijinsky & Andrews (1980)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2500.0000	Hashimoto & Tanii (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500.0000	Lijinsky & Andrews (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	15000.0000	Bull *et al.* (1984a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500.0000	Hashimoto & Tanii (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	10000.0000	Knaap *et al.* (1988)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	10000.0000	Tsuda *et al.* (1993)
ECW, *Escherichia coli* WP2 *uvr*A–, reverse mutation	–	–	25000.0000	Tsuda *et al.* (1993)
KPF, *Klebsiella pneumoniae*, forward mutation	–	–	10000.0000	Knaap *et al.* (1988)
DMM, *Drosophila melanogaster*, somatic mutation (and recombination)	+		70, larval feeding	Knaap *et al.* (1988)

Table 9 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DMM, *Drosophila melanogaster*, somatic mutation	+		100, larval feeding	Batiste-Alentorn *et al.* (1991)
DMM, *Drosophila melanogaster*, somatic mutation (and recombination)	+		70, larval feeding	Tripathy *et al.* (1991)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	-		2800, injection	Knaap *et al.* (1988)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+		70, larval feeding	Tripathy *et al.* (1991)
URP, Unscheduled DNA synthesis, rat hepatocytes *in vitro*	-	0	700.0000	Miller & McQueen (1986)
URP, Unscheduled DNA synthesis, rat hepatocytes *in vitro*	?	0	2100.0000	Barfnecht *et al.* (1987)
URP, Unscheduled DNA synthesis, rat hepatocytes *in vitro*	-	0	70.0000	Butterworth *et al.* (1992)
GCL, Gene mutation, Chinese hamster V79H3 *in vitro*, *hprt* locus	-	0	500.0000	Tsuda *et al.* (1993)
G5T, Gene mutation, mouse lymphoma L5178Y cells *in vitro*, *tk* locus	+	0	600.0000	Moore *et al.* (1987)
G5T, Gene mutation, mouse lymphoma L5178Y cells *in vitro*, *tk* locus	(+)	(+)	7500.0000	Knaap *et al.* (1988)
G51, Gene mutation, mouse lymphoma L5178Y cells *in vitro*, *hprt* locus	+	+	700.0000	Barfknecht *et al.* (1988) (abstract)
G51, Gene mutation, mouse lymphoma L5178Y cells *in vitro*, *hprt* locus	(+)	(+)	300.0000	Knaap *et al.* (1988)
SIC, Sister chromatid exchange, Chinese hamster cells *in vitro*	+	+	300.0000	Knaap *et al.* (1988)
SIC, Sister chromatid exchange, Chinese hamster V79H3 cells *in vitro*	+	0	70.0000	Tsuda *et al.* (1993)
CIC, Chromosomal aberrations, Chinese hamster CHL cells *in vitro*	+	+	150.0000	Sofuni *et al.* (1985)
CIC, Chromosomal aberrations, Chinese hamster cells, *in vitro*	+	+	100.0000	Knaap *et al.* (1988)
CIC, Chromosomal aberrations, Chinese hamster, V79H3 cells *in vitro*	+	0	140.0000	Tsuda *et al.* (1993)
*, DNA amplification, Chinese hamster -SV40 *in vitro*	(+)	0	150.0000	Vanhorick & Moens (1983)
CIM, Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	0	750.0000	Moore *et al.* (1987)
AIA, Aneuploidy, Chinese hamster DON cells *in vitro*	+	0	1000.0000	Warr *et al.* (1990)
AIA, Aneuploidy, Chinese hamster LUC 2 cells *in vitro*	(+)	0	500.0000	Warr *et al.* (1990)
AIA, Aneuploidy, Chinese hamster V79H3 cells *in vitro*	+	0	70.0000	Tsuda *et al.* (1993)
AIA, Aneuploidy, Chinese hamster V79 cells, *in vitro*	+	0	10.0000	Adler *et al.* (1993a)
TBM, Cell transformation, BALB/c3T3 mouse cells	+	0	70.0000	Tsuda *et al.* (1993)

Table 9 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
TCM, Cell transformation, C3H10T1/2 mouse cells	+	0	50.0000	Banerjee & Segal (1986)
TCL, Cell transformation, NIH 3T3 mouse cells	+	0	12.5000	Banerjee & Segal (1986)
UIH, Unscheduled DNA synthesis, human epithelial cells *in vitro*	(+)	0	710.0000	Butterworth *et al.* (1992)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	(+)	(+)	0.0000	Norppa & Tursi (1984)
DVA, DNA strand breaks, mouse spermiogenic stages *in vivo*	+		25×1 ip	Sega & Generoso (1990)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	-		100×1 po	Butterworth *et al.* (1992)
UVA, Unscheduled DNA synthesis, rat spermatocytes *in vivo*	+		30×5 po	Butterworth *et al.* (1992)
GVA, Gene mutation, Muta mouse, bone-marrow cells *in vivo*	?		50×5 ip	Hoorn *et al.* (1993)
MST, Mouse spot test	+		50×1 ip	Neuhäuser-Klaus & Schmahl (1989)
SLP, Specific locus mutation, mouse, postspermatogonia	+		50×5 ip	Russell *et al.* (1991)
SLP, Specific locus mutation, mouse, spermatozoa and spermatids	+		100×1 ip	Ehling & Neuhäuser-Klaus (1992)
SLO, Specific locus mutation, mouse, other stages	-		50×5 ip	Russell *et al.* (1991)
SLO, Specific locus mutation, mouse, spermatogonia	+		100×1 ip	Ehling & Neuhäuser-Klaus (1992)
SVA, Sister chromatid exchange, mouse bone-marrow cells *in vivo*	-		100×1 ip	Shiraishi (1978)
SVA, Sister chromatid exchange, mouse stem cells and spermatogonia treated *in vivo*, spermatogonia observed	-		100×1 ip	Shiraishi (1978)
SVA, Sister chromatid exchange, mouse splenocytes *in vivo*	+		50×1 ip	Backer *et al.* (1989)
MVM, Micronucleus formation, mouse bone-marrow cells *in vivo*	+		50×1 ip	Adler *et al.* (1988)
MVM, Micronucleus formation, mouse bone-marrow cells *in vivo*	+		100×1 ip	Čihák & Vontorková (1988)
MVM, Micronucleus formation, mouse bone-marrow cells *in vivo*	+		25×2 ip	Čihák & Vontorková (1988)
MVM, Micronucleus formation, mouse bone-marrow cells *in vivo*	+		42.5×1 ip	Čihák & Vontorková (1990)
MVM, Micronucleus formation, mouse bone-marrow cells *in vivo*	+		136×1 ip	Knaap *et al.* (1988)
MVM, Micronucleus formation, mouse splenocytes *in vivo*	+		50×1 ip	Backer *et al.* (1989)
MVM, Micronucleus formation, mouse spermatid *in vivo*	+		50×1 ip	Collins *et al.* (1992)

Table 9 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronucleus formation, mouse peripheral blood erythrocytes *in vivo*	+		50×1 ip	Cao *et al.* (1993)
CBA, Chromosomal aberrations, mouse bone-marrow cells *in vivo*	-		60×3 wk diet	Shiraishi (1978)
CBA, Chromosomal aberrations, mouse bone-marrow cells *in vivo*	-		100×1 ip	Shiraishi (1978)
CBA, Chromosomal aberrations, mouse bone-marrow cells *in vivo*	+		50×1 ip	Adler *et al.* (1988)
CBA, Chromosomal aberrations, mouse bone-marrow cells *in vivo*	+		100×1 ip	Čihák & Vontorková (1988)
CVA, Chromosomal aberrations, mouse splenocytes *in vivo*	?		125×1 ip	Backer *et al.* (1989)
CCC, Chromosomal aberrations, mouse spermatocytes treated *in vivo*, spermatocytes observed	(+)		60×3 wk diet	Shiraishi (1978)
CCC, Chromosomal aberrations, mouse spermatocytes treated *in vivo*, spermatocytes observed	+		100×1 ip	Kliesch *et al.* (1989) (abstract)
CCC, Chromosomal aberrations, mouse spermatocytes treated *in vivo*, spermatocytes observed	+		100×1 ip	Adler (1990)
CGC, Chromosomal aberrations, mouse spermatogonia treated *in vivo*, spermatocytes observed	(+)		100×1 ip	Shiraishi (1978)
CGC, Chromosomal aberrations, mouse spermatogonia treated *in vivo*, spermatocytes observed	-		100×1 ip	Kliesch *et al.* (1989) (abstract)
CGC, Chromosomal aberrations, mouse spermatogonia treated *in vivo*, spermatocytes observed	-		100×1 ip	Adler (1990)
CGC, Chromosomal aberrations (reciprocal translocations), rat spermatogonial stem cells treated *in vivo*, spermatocytes observed	?		5.8×80 drinking-water	Smith *et al.* (1986)
CGC, Chromosomal aberrations, mouse spermatogonial stem cells treated *in vivo*, spermatocytes observed	-		125×1 ip	Backer *et al.* (1989)
CGG, Chromosomal aberrations, mouse spermatogonial stem cells treated *in vivo*, spermatogonia observed	-		125×1 ip	Backer *et al.* (1989)
CGG, Chromosomal aberrations, mouse spermatogonial stem cells treated *in vivo*, spermatogonia observed	+		60×3 wk diet	Shiraishi (1978)

Table 9 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CGG, Chromosomal aberration, mouse spermatogonial stem cells and spermatogonia treated *in vivo*, spermatogonia observed	+		100×1 ip	Shiraishi (1978)
CGG, Chromosomal aberrations, rat spermatogonia treated *in vivo*, spermatogonia observed	-		5.8×80 drinking-water	Smith *et al.* (1986)
CGG, Chromosomal aberrations, mouse spermatogonia treated *in vivo*, spermatogonia observed	-		150×1 ip	Adler *et al.* (1988)
DLM, Dominant lethal mutation, mouse	+		125×1 ip	Shelby *et al.* (1986)
DLM, Dominant lethal mutation, mouse	+		50×5 ip	Shelby *et al.* (1986)
DLM, Dominant lethal mutation, mouse	+		40×5 ip	Shelby *et al.* (1987)
DLM, Dominant lethal mutation, mouse	+		125×1 ip	Dobrzyńska *et al.* (1990)
DLM, Dominant lethal mutation, mouse	+		100×1 ip	Ehling & Neuhäuser-Klaus (1992)
DLM, Dominant lethal mutation, mouse	+		25×5 topical application	Gutierrez-Espeleta *et al.* (1992)
DLR, Dominant lethal mutation, rat	+		2.8×80 drinking-water	Smith *et al.* (1986)
DLR, Dominant lethal mutation, rat	+		30×5 gavage	Working *et al.* (1987)
MHT, Heritable translocation, mouse	+		40×5 ip	Shelby *et al.* (1987)
MHT, Heritable translocation, mouse	+		50×5 ip	Adler (1990)
AVA, Aneuploidy, mouse bone-marrow cells *in vivo*	?		60×3 wk diet	Shiraishi (1978)
AVA, Aneuploidy, mouse bone-marrow cells *in vivo*	?		100×1 ip	Shiraishi (1978)
AVA, Aneuploidy, mouse spermatogonia *in vivo*	?		60×3 wk diet	Shiraishi (1978)
AVA, Aneuploidy, mouse spermatogonia *in vivo*	?		100×1 ip	Shiraishi (1978)
AVA, Aneuploidy, mouse spermatogonia, hyperploidy	-		125×1 ip	Backer *et al.* (1989)
*, Aneuploidy, mouse spermatid micronuclei and kinetochore staining	(+)		50×1 ip	Collins *et al.* (1992)
AVA, Aneuploidy, mouse bone-marrow cells *in vivo* (mitotic delay)	-		120×1 ip	Adler *et al.* (1993b)
AVA, Aneuploidy, mouse spermatid, hyperploidy	+		120×1 ip	Adler *et al.* (1993b)
AVA, Aneuploidy, mouse spermatocytes, meiotic delay	+		120×1 ip	Adler *et al.* (1993b)
BIP, Binding (covalent) to neurofilaments and microtubule-associated proteins from rat neural cells *in vitro*	+		0.7	Lapadula *et al.* (1989)
BID, Binding (covalent) to 2′-deoxynucleosides *in vitro*	+		96000.0000	Solomon *et al.* (1985)

Table 9 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BID, Binding (covalent) to DNA from calf thymus *in vitro*	+		96000.0000	Solomon *et al.* (1985)
BVD, Binding (covalent) to DNA, mouse testis, skin, lung, liver, stomach *in vivo*	+		100×1 po	Carlson & Weaver (1985)
BVD, Binding (covalent) to DNA, mouse testis, skin, lung, liver, stomach *in vivo*	+		100×1 topical application	Carlson & Weaver (1985)
BVD, Binding (covalent) to DNA, mouse spermatocytes-spermatids *in vivo*	+		125 ip	Sega *et al.* (1989)
BVD, Binding (covalent) to DNA, mouse testis *in vivo*	+		46×1 ip	Sega *et al.* (1990)
BVD, Binding (covalent) to DNA, mouse liver *in vivo*	+		46×1 ip	Sega *et al.* (1990)
BVD, Binding (covalent) to DNA, rat liver, lung, kidney, spleen, brain and testis *in vivo*	+		50×1 ip	Segerbäck *et al.* (1994)
BVP, Binding (covalent) to RNA, mouse testis, skin, lung, liver, stomach *in vivo*	+		100×1 po	Carlson & Weaver (1985)
BVP, Binding (covalent) to RNA, mouse testis, skin, lung, liver, stomach *in vivo*	+		100×1 topical application	Carlson & Weaver (1985)
SPM, Sperm morphology, mouse *in vivo*	+		13×4 wk drinking-water	Sakamoto & Hashimoto (1986)
Protein binding				
BVP, Binding (covalent) to proteins, mouse testis, skin, lung, liver, stomach *in vivo*	+		100×1 po	Carlson & Weaver (1985)
BVP, Binding (covalent) to proteins, mouse testis, skin, lung, liver, stomach *in vivo*	+		100×1 topical application	Carlson & Weaver (1985)
BVP, Binding (covalent) to protamines, mouse spermatid-spermatozoa *in vivo*	+		125 ip	Sega *et al.* (1989)
BVP, Binding (covalent) to haemoglobin, rat *in vivo*	+		0.5×1 iv	Bailey *et al.* (1986)
BVP, Binding (covalent) to haemoglobin, rat *in vivo*	+		50×5 ip	Calleman *et al.* (1990)
BVP, Binding (covalent) to haemoglobin, rat *in vivo*	+		1×1 ip	Bergmark *et al.* (1991)

Table 9 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Glycidamide				
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	25.0000	Hashimoto & Tanii (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	25.0000	Hashimoto & Tanii (1985)
KPF, *Klebsiella pneumoniae*, streptomycin resistance	-	0	3500.0000	Voogd *et al.* (1981)
G51, Gene mutation, mouse lymphoma L5178Y cells *in vitro*, *hprt* locus	+	-	218.0000	Barfknecht *et al.* (1988) (abstract)
URP, Unscheduled DNA synthesis, rat hepatocytes *in vitro*	-	0	348.0000	Barfknecht *et al.* (1988) (abstract)
URP, Unscheduled DNA synthesis, rat hepatocytes *in vitro*	+	0	70.0000	Butterworth *et al.* (1992)
UIH, Unscheduled DNA synthesis, human epithelial cells *in vitro*	+	0	70.0000	Butterworth *et al.* (1992)
UVM, Unscheduled DNA synthesis, mouse spermatids *in vivo*	+		0.0000	Sega *et al.* (1990)

*Not on profile

[a] +, positive; (+), weak positive; -, negative; 0, not tested; ?, inconclusive (variable response within several experiments within an adequate study)

[b] In-vitro tests, μg/ml; in-vivo tests, mg/kg bw

5.2 Human carcinogenicity data

Two cohort mortality studies were conducted among workers exposed to acrylamide. The first showed no significant excess of cancer but suffered from small size, short duration of exposure and short latency. In the other study, in one Dutch and three US plants, a nonsignificant increase was seen in deaths from pancreatic cancer, but there was no trend with increasing exposure.

5.3 Animal carcinogenicity data

Acrylamide was tested for carcinogenicity in one experiment in rats by oral administration. It increased the incidences of peritoneal mesotheliomas found in the region of the testis and of follicular adenomas of the thyroid in males and of thyroid follicular tumours, mammary tumours, glial tumours of the central nervous system, oral cavity papillomas, uterine adenocarcinomas and clitoral gland adenomas in females. In screening bioassays, acrylamide, given either orally or intraperitoneally, increased both the incidence and multiplicity of lung tumours in strain A mice.

Acrylamide was also tested as an initiating agent for skin carcinogenesis after oral, intraperitoneal and topical administration to mice of one strain and after oral administration to mice of another strain, followed by topical treatment with 12-*O*-tetradecanoylphorbol 13-acetate. It induced a dose-related increase in the incidence of squamous-cell papillomas and carcinomas of the skin in all four experiments.

5.4 Other relevant data

In occupational settings, acrylamide is taken up both through the skin and by inhalation. Damage to both the central and peripheral nervous systems has been reported on several occasions in exposed humans and has been thoroughly studied in animals.

Acrylamide is metabolized *in vitro* and *in vivo* in mice, rats and humans to the epoxide, glycidamide. Both substances are equally distributed throughout the tissues and have half-lives of about 5 h in rats; acrylamide itself has also been shown to be uniformly distributed between tissues in several other species. The conversion of acrylamide to glycidamide is saturable, ranging from 50% at very low doses to 13% at 100 mg/kg bw in treated rats. Both agents are detoxified by glutathione conjugation, and glycidamide is also detoxified by hydrolysis. Both agents react directly with haemoglobin *in vivo*, but DNA adducts result only from the formation of glycidamide.

The presence of haemoglobin adducts of acrylamide was correlated with neurotoxicity in a group of highly exposed workers.

Acrylamide was not teratogenic to rats or mice after oral treatment of dams with doses up to the toxic level. It causes testicular atrophy, with damage to spermatids and mature spermatozoa. Reduced sperm motility, impaired fertility and dominant lethal mutations at the spermatozoa stage have also been reported in mice and rats. A single study in rats provides evidence that the testicular damage is not secondary to neurotoxicity, since testicular damage but not neurotoxicity was induced by injection of the reactive epoxide, glycidamide.

The genotoxicity of acrylamide has been studied extensively. It induces gene mutation, structural chromosomal aberrations, sister chromatid exchange and mitotic disturbances in mammalian cells *in vitro* in the presence or absence of exogenous metabolic systems. It induces structural chromosomal aberrations *in vivo* in both somatic and germ-line cells. Chromosomal aberrations and micronuclei were induced in mouse bone marrow and in premeiotic and postmeiotic cells. Treatment with acrylamide *in vivo* also caused somatic mutation in the spot test, heritable translocation and specific locus mutations in mice and dominant lethal mutations in both mice and rats in several studies. Acrylamide induces unscheduled DNA synthesis in rat spermatocytes *in vivo* but apparently not in rat hepatocytes; glycidamide induced unscheduled DNA synthesis in rat hepatocytes in one study *in vitro*. Acrylamide induces transformation in cultured mammalian cells. It does not induce mutation in bacteria, but glycidamide does in the absence of an exogenous metabolic system. Acrylamide induces sex-linked recessive lethal and somatic mutations in *Drosophila*.

5.5 Evaluation[1]

There is *inadequate evidence* in humans for the carcinogenicity of acrylamide.

There is *sufficient evidence* in experimental animals for the carcinogenicity of acrylamide.

In making the overall evaluation, the Working Group took into consideration the following supporting evidence:

(i) Acrylamide and its metabolite glycidamide form covalent adducts with DNA in mice and rats.

(ii) Acrylamide and glycidamide form covalent adducts with haemoglobin in exposed humans and rats.

(iii) Acrylamide induces gene mutations and chromosomal aberrations in germ cells of mice and chromosomal aberrations in germ cells of rats and forms covalent adducts with protamines in germ cells of mice *in vivo*.

(iv) Acrylamide induces chromosomal aberrations in somatic cells of rodents *in vivo*.

(v) Acrylamide induces gene mutations and chromosomal aberrations in cultured cells *in vitro*.

(vi) Acrylamide induces cell transformation in mouse cell lines.

Overall evaluation

Acrylamide *is probably carcinogenic to humans (Group 2A)*.

6. References

Abou-Donia, M.B., Ibrahim, S.M., Corcoran, J.J., Lack, L., Friedman, M.A. & Lapadula, D.M. (1993) Neurotoxicity of glycidamide, an acrylamide metabolite, following intraperitoneal injections in rats. *J. Toxicol. environ. Health*, **39**, 447–464

[1]For definition of the italicized terms, see Preamble, pp. 27–30.

Adler, I.-D. (1990) Clastogenic effects of acrylamide in different germ-cell stages of male mice. In: Allen, J.W., Bridges, B.A., Lyon, M.F., Moses, M.J. & Russell, L.D., eds, *Biology of Mammalian Germ Cell Mutagenesis* (Banbury Report 34), Cold Spring Harbour, NY, CSH Press, pp. 115–131

Adler, I.-D., Ingwersen, I., Kliesch, U. & El Tarras, A. (1988) Clastogenic effects of acrylamide in mouse bone marrow cells. *Mutat. Res.*, **206**, 379–385

Adler, I.-D., Zouh, R. & Schmid, E. (1993a) Perturbation of cell division by acrylamide *in vitro* and *in vivo*. *Mutat. Res.*, **301**, 249–254

Adler, I.-D., Gassner, P., Schriever-Schwemmer, G. & Ru Min, Z. (1993b) Correlation between induction of meiotic delay and aneuploidy in male mouse germ cells. In: Vig, B.K., ed., *Chromosome Segregation and Aneuploidy* (NATO ASI Series, Vol. H 72), Berlin, Springer, pp. 297–308

Agrawal, A.K. & Squibb, R.E. (1981) Effects of acrylamide given during gestation on dopamine receptor binding in rat pups. *Toxicol. Lett.*, **7**, 233–238

Agrawal, A.K., Squibb, R.E. & Bondy, S.C. (1981) The effects of acrylamide treatment upon the dopamine receptor. *Toxicol. appl. Pharmacol.*, **58**, 89–99

American Conference of Governemental Industrial Hygienists (1993) *1993–1994 Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Limits*, Cincinnati, OH, p. 12

Anon. (1985) Chemical profile: acrylamide. *Chem. Mark. Rep.*, **227**, 64

Anon. (1988) Chemical profile: acrylamide. *Chem. Mark. Rep.*, **233**, 46

Anon. (1991) Chemical profile: acrylamide. *Chem. Mark. Rep.*, **239**, 42

Arbejdstilsynet [Labour Inspection] (1988) *Graensevaerdier for Stoffer og Materialer* [Limit Values for Compounds and Materials] (No. 3.1.0.2), Copenhagen, p. 10

Backer, L.C., Dearfield, K.L., Erexson, G.L., Campbell, J.A., Westbrook-Collins, B. & Allen, J.W. (1989) The effects of acrylamide on mouse germ-line and somatic cell chromosomes. *Environ. mol. Mutag.*, **13**, 218–226

Bailey, E., Farmer, P.B., Bird, I., Lamb, J.H. & Peal, J.A. (1986) Monitoring exposure to acrylamide by the determination of *S*-(2-carboxyethyl)cysteine in hydrolyzed hemoglobin by gas chromatography–mass spectrometry. *Anal. Biochem.*, **157**, 241–248

Bailey, E., Farmer, P.B. & Shuker, D.E.G. (1987) Estimation of exposure to alkylating carcinogens by the GC–MS determination of adducts to hemoglobin and nucleic acid bases in urine. *Arch. Toxicol.*, **60**, 187–191

Banerjee, S. & Segal, A. (1986) In vitro transformation of C3H/10T1/2 and NIH/3T3 cells by acrylonitrile and acrylamide. *Cancer Lett.*, **32**, 293–304

Barfnecht, T.R., Mecca, D.J. & Naismith, R.W. (1987) Evaluation of acrylamide in rodent hepatocyte DNA/repair assays (Abstract No. 23). *Environ. Mutag.*, **9** (Suppl. 8), 10–11

Barfnecht, T.R., Mecca, D.J. & Naismith, R.W. (1988) The genotoxic activity of acrylamide (Abstract No. 18). *Environ. mol. Mutag.*, **11** (Suppl. 11), 9

Batiste-Alentorn, M., Xamena, N., Creus, A. & Marcos, R. (1991) Genotoxicity studies with the unstable *zeste-white* (*UZ*) system of *Drosophila melanogaster*: results with ten carcinogenic compounds. *Environ. mol. Mutag.*, **18**, 120–125

Bergamini, C.M. & Signorini, M. (1990) In vivo inactivation of transglutaminase during the acute acrylamide toxic syndrome in the rat. *Experientia*, **46**, 278–281

Bergmark, E., Calleman, C.J. & Costa, L.G. (1991) Formation of hemoglobin adducts of acrylamide and its epoxide metabolite glycidamide in the rat. *Toxicol. appl. Pharmacol.*, **111**, 352–363

Bergmark, E., Calleman, C.J., He, F. & Costa, L.G. (1993) Determination of hemoglobin adducts in humans occupationally exposed to acrylamide. *Toxicol. appl. Pharmacol.*, **120**, 45–54

Brown, L., Rhead, M.M. & Bancroft, K.C.C. (1980) Case studies of acrylamide pollution resulting from the industrial use of polyacrylamides. *Water Pollut. Control*, **79**, 507–510

Budavari, S., ed. (1989) *The Merck Index*, 11th ed., Rahway, NJ, Merck & Co., p. 21

Bull, R.J., Robinson, M., Laurie, R.D., Stoner, G.D., Greisiger, E., Meier, J.R. & Stober, J. (1984a) Carcinogenic effects of acrylamide in SENCAR and A/J mice. *Cancer Res.*, **44**, 107–111

Bull, R.J., Robinson, M. & Stober, J.A. (1984b) Carcinogenic activity of acrylamide in the skin and lung of Swiss-ICR mice. *Cancer Lett.*, **24**, 209–212

Butterworth, B.E., Eldridge, S.R., Sprankle, C.S., Working, P.K., Bentley, K.S. & Hurtt, M.E. (1992) Tissue-specific genotoxic effects of acrylamide and acrylonitrile. *Environ. mol. Mutag.*, **20**, 148–155

Calleman, C.J., Bergmark, E. & Costa, L.G. (1990) Acrylamide is metabolized to glycidamide in the rat: evidence from hemoglobin adduct formation. *Chem. Res. Toxicol.*, **3**, 406–412

Calleman, C.J., Stern, L.G., Bergmark, E. & Costa, L.G. (1992) Linear *versus* nonlinear models for hemoglobin adduct formation by acrylamide and its metabolite glycidamide: implications for risk estimation. *Cancer Epidemiol. Biomarkers Prev.*, **1**, 361–368

Calleman, C.J., Bergmark, E., Stern, L.G. & Costa, L.G. (1993) A nonlinear dosimetric model for hemoglobin adduct formation by the neurotoxic agent acrylamide and its genotoxic metabolite glycidamide. *Environ. Health Perspectives*, **99**, 221–223

Calleman, C.J., Wu, Y., He, F., Tian, G., Bergmark, E., Zhang, S., Deng, H., Wang, Y., Crofton, K.M., Fennell, T. & Costa, L.G. (1994) Relationship between biomarkers of exposure and neurological effects in a group of workers exposed to acrylamide. *Toxicol. appl. Pharmacol.*, **126**, 197–201

Cao, J., Beisker, W., Nüsse, M. & Adler, I.-D. (1993) Flow cytometric detection of micronuclei induced by chemicals in poly- and normochromatic erythrocytes of mouse peripheral blood. *Mutagenesis*, **8**, 533–541

Carlson, G.P. & Weaver, P.M. (1985) Distribution and binding of ^{14}C-acrylamide to macromolecules in SENCAR and BALB/c mice following oral and topical administration. *Toxicol. appl. Pharmacol.*, **79**, 307–313

Carlson, G.P., Fossa, A.A., Morse, M.A. & Weaver, P.M. (1986) Binding and distribution studies in the SENCAR mouse of compounds demonstrating a route-dependent tumorigenic effect. *Environ. Health Perspectives*, **68**, 53–60

Carrington, C.D., Lapadula, D.M., Dulak, L., Friedman, M. & Abou-Donia, M.B. (1991) In vivo binding of [^{14}C]acrylamide to proteins in the mouse nervous system. *Neurochem. int.*, **18**, 191–197

Chemical Information Services Ltd (1991) *Directory of World Chemical Producers 1992/93 Edition*, Dallas, TX, p. 13

Čihák, R. & Vontorková, M. (1988) Cytogenetic effects of acrylamide in the bone marrow of mice. *Mutat. Res.*, **209**, 91–94

Čihák, R. & Vontorková, M. (1990) Activity of acrylamide in single-, double- and triple-dose mouse bone marrow micronucleus assays. *Mutat. Res.*, **234**, 125–127

Collins, J.J., Swaen, G.M.H., Marsh, G.M., Utidjian, H.M.D., Caporossi, J.C. & Lucas, L.J. (1989) Mortality patterns among workers exposed to acrylamide. *J. occup. Med.*, **31**, 614–617

Collins, B.W., Howard, D.R. & Allen, J.W. (1992) Kinetochore-staining of spermatid micronuclei: studies of mice treated with X-radiation or acrylamide. *Mutat. Res.*, **281**, 287–294

Cook, W.A., ed. (1987) *Occupational Exposure Limits—Worldwide*, Akron, OH, American Industrial Hygiene Association, pp. 117, 127, 159

Costa, L.G., Deng, H., Gregotti, C., Manzo, L., Faustman, E.M., Bergmark, E. & Calleman, C.J. (1992) Comparative studies on the neuro- and reproductive toxicity of acrylamide and its epoxide metabolite glycidamide in the rat. *NeuroToxicology*, **13**, 219–224

Cummins, K., Morton, D., Lee, D., Cook, D. & Curtis, R. (1992) Exposure to acrylamide in chemical sewer grouting operations. *Appl. occup. environ. Hyg.*, **7**, 385–391

Dearfield, K.L., Abernathy, C.O., Ottley, M.S., Brantner, J.H. & Hayes, P.F. (1988) Acrylamide: its metabolism, developmental and reproductive effects, genotoxicity, and carcinogenicity. *Mutat. Res.*, **195**, 45–77

Denèfle, J.-P. & Zhu, Q.-L. (1989) Fibronectin network recovery in confluent PtK2 cells after acrylamide treatment. *Tissue Cell*, **21**, 647–651

Deng, H., He, F., Zhang, S., Calleman, C.J. & Costa, L.G. (1993) Quantitative measurements of vibration threshold in healthy adults and acrylamide workers. *Int. Arch. occup. environ. Health*, **65**, 53–56

Deutsche Forschungsgemeinschaft (1993) *MAK and BAT Values 1993* (Report No. 29), Weinheim, VCH Verlagsgesellschaft, p. 17

Direktoratet for Arbeidstilsynet [Directorate of Labour Inspection] (1990) *Administrative Normer for Forurensning i Arbeidsatmosfaere* [Administrative Norms for Pollution in Work Atmosphere], Oslo, p. 5

Dixit, R., Mukhtar, H., Seth, P.K. & Murti, C.R.K. (1981) Conjugation of acrylamide with glutathione catalyzed by glutathione-*S*-transferases of rat liver and brain. *Biochem. Pharmacol.*, **30**, 1739–1744

Dobrzyńska, M., Lenarczyk, M. & Gajewski, A.K. (1990) Induction of dominant lethal mutations by combined X-ray–acrylamide treatment in male mice. *Mutat. Res.*, **232**, 209–215

Dow Chemical Co. (1992) *Sales Specification: Aqueous Acrylamide*, Midland, MI

Ducatman, A.M. & Coumbis, J.J. (1991) Chemical hazards in the biotechnology industry. *Occup. Med. State Art Rev.*, **6**, 193–208

Eckert, B.S. (1986) Alteration of the distribution of intermediate filaments in PtK1 cells by acrylamide. II: Effect on the organization of cytoplasmic organelles. *Cell Motility Cytoskeleton*, **6**, 15–24

Edwards, P.M. (1975) The distribution and metabolism of acrylamide and its neurotoxic analogues in rats. *Biochem. Pharmacol.*, **24**, 1277–1282

Ehling, U.H. & Neuhäuser-Klaus, A. (1992) Reevaluation of the induction of specific-locus mutations in spermatogonia of the mouse by acrylamide. *Mutat. Res.*, **283**, 185–191

Farmer, P.B., Shuker, D.E.G. & Bailey, E. (1986) The monitoring of exposure to carcinogens by the GC–MS determination of alkylated amino acids in hemoglobin and of alkylated nucleic acid bases in urine. In: Kopfler, F.C. & Craun, G.F., eds, *Environmental Epidemiology*, Chelsea, MI, Lewis Publishers, pp. 29–39

Field, E.A., Price, C.J., Sleet, R.B., Marr, M.C., Schwetz, B.A. & Morrissey, R.E. (1990) Developmental toxicity evaluation of acrylamide in rats and mice. *Fundam. appl. Toxicol.*, **14**, 502–512

Frantz, S.W., Dryzga, M.D., Freshour, N.L. & Watanabe, P.G. (1985) In vivo/in vitro determination of cutaneous penetration of residual monomer from polyacrylamides (Abstract No. 39). *Toxicologist*, **5**, 10

Going, J.E. & Thomas, K. (1979) *Sampling and Analysis of Selected Toxic Substances. Task I: Acrylamide* (EPA Report No. EPA-560/13-79-013/US NTIS PB80-128150), Washington DC, US Environmental Protection Agency, Office of Toxic Substances

Gutierrez-Espeleta, G.A., Hughes, L.A., Piegorsch, W.W., Shelby, M.D. & Generoso, W.M. (1992) Acrylamide: dermal exposure produces genetic damage in male mouse germ cells. *Fundam. appl. Toxicol.*, **18**, 189–192

Habermann, C.E. (1991) Acrylamide. In: Kroschwitz, J.I. & Howe-Grant, M., eds, *Kirk-Othmer Encyclopedia of Chemical Technology*, 4th Ed., Vol. 1, New York, John Wiley & Sons, pp. 251–266

Harry, G.J. (1992) Acrylamide-induced alterations in axonal transport. Biochemical and autoradiographic studies. *Mol. Neurobiol.*, **6**, 203–216

Hashimoto, K. & Aldridge, W.N. (1970) Biochemical studies on acrylamide, a neurotoxic agent. *Biochem. Pharmacol.*, **19**, 2591–2604

Hashimoto, K. & Tanii, K. (1985) Mutagenicity of acrylamide and its analogues in *Salmonella typhimurium*. *Mutat. Res.*, **158**, 129–133

Hashimoto, K., Sakamoto, J. & Tanii, H. (1981) Neurotoxicity of acrylamide and related compounds and their effects on male gonads in mice. *Arch. Toxicol.*, **47**, 179–189

Hashimoto, K., Hayashi, M., Tanii, H. & Sakamoto, J. (1988) Toxicological aspect of acrylamide neuropathy. *J. UOEH* (Sangyo Ika Daigaku Zasshi), **10** (Suppl.), 209–218

He, F., Zhang, S., Wang, H., Li, G., Zhang, Z., Li, F., Dong, X. & Hu, F. (1989) Neurological and electroneuromyographic assessment of the adverse effects of acrylamide on occupationally exposed workers. *Scand. J. Work Environ. Health*, **15**, 125–129

Hills, B.W. & Greife, A.L. (1986) Evaluation of occupational acrylamide exposures. *Appl. ind. Hyg.*, **1**, 148–152

Hoekstra, E.J. & Weber, A. (1993) *Health Hazard Evaluation Report, Peabody No. 7 Mine Coal, Montcoal, W. Virginia* (HETA 92-313), Cincinnati, OH, National Institute for Occupational Safety and Health

Hoorn, A.J.W., Custer, L.L., Myhr, B.C., Brusick, D., Gossen, J. & Vijg, J. (1993) Detection of chemical mutagens using Muta^{R} mouse: a transgenic mouse model. *Mutagenesis*, **8**, 7–10

Howland, R.D., Vyas, I.L. & Lowndes, H.E. (1980a) The etiology of acrylamide neuropathy: possible involvement of neuron specific enolase. *Mutat. Res.*, **190**, 529–535

Howland, R.D., Vyas, I.L., Lowndes, H.E. & Argentieri, T.M. (1980b) The etiology of toxic peripheral neuropathies: in vitro effects of acrylamide and 2,5-hexanedione on brain enolase and other glycolytic enzymes. *Mutat. Res.*, **202**, 131–142

IARC (1986) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 39, *Some Chemicals Used in Plastics and Elastomers*, Lyon, pp. 41–66

Ikeda, G.J., Miller, E., Sapienza, P.P., Michel, T.C., King, M.T., Turner, V.A., Blumenthal, H., Jackson, W.E. & Levin, S. (1983) Distribution of ^{14}C-labelled acrylamide and betaine in foetuses of rats, rabbits, beagle dogs and miniature pigs. *Food chem. Toxicol.*, **21**, 49–58

Ikeda, G.J., Miller, E., Sapienza, P.P., Michel, T.C., King, M.T. & Sager, A.O. (1985) Maternal–foetal distribution studies in late pregnancy. II. Distribution of [1-^{14}C]acrylamide in tissues of beagle dogs and miniature pigs. *Food chem. Toxicol.*, **23**, 757–761

Ikeda, G.J., Miller, E., Sapienza, P.P., Michel, T.C. & Inskeep, P.B. (1987) Comparative tissue distribution and excretion of [1-^{14}C]acrylamide in beagle dogs and miniature pigs. *Food chem. toxicol.*, **25**, 871–875

Institut National de Recherche et de Sécurité (1993) *Limit Values for the Concentrations of Dangerous Substances in the Air of Work Places*, Paris (in French)

International Labour Office (1991) *Occupational Exposure Limits for Airborne Toxic Substances: Values of Selected Countries* (Occupational Safety and Health Series No. 37), 3rd Ed., Geneva, pp. 6–7 (Substance No. 23)

Japan Petrochemical Industry Association (1993) *Petrochemical Industry of Japan*, Tokyo, p. 27

Johnson, K.A., Gorzinski, S.J., Bodner, K.M., Campbell, R.A., Wolf, C.H., Friedman, M.A. & Mast, R.W. (1986) Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicol. appl. Pharmacol.*, **85**, 154–168

Khanna, V.K., Husain, R. & Seth, P.K. (1988) Low protein diet modifies acrylamide neurotoxicity. *Toxicology*, **49**, 395–401

Khanna, V.K., Husain, R. & Seth, P.K. (1992) Protein malnourishment: a predisposing factor in acrylamide toxicity in pregnant rats. *J. Toxicol. environ. Health*, **36**, 293–305

King, D.J. & Noss, R.R. (1989) Toxicity of polyacrylamide and acrylamide monomer. *Res. environ. Health*, **8**, 3–16

Kliesch, U., El-tarras, A., Ingwersen, I. & Adler, I.-D. (1989) Clastogenicity of acrylamide in somatic and germinal cells of mice (Abstract No. 43). *Mutagenesis*, **4**, 319–320

Knaap, A.G.A.C., Kramers, P.G.N., Voogd, C.E., Bergkamp, W.G.M., Groot, M.G., Langebroek, P.G., Mout, H.C.A., van der Stel, J.J. & Verharen, H.W. (1988) Mutagenic activity of acrylamide in eukaryotic systems but not in bacteria. *Mutagenesis*, **3**, 263–268

Lapadula, D.M., Bowe, M., Carrington, C.D., Dulak, L., Friedman, M. & Abou-Donia, M.B. (1989) In vitro binding of [^{14}C]acrylamide to neurofilament and microtubule proteins of rats. *Brain Res.*, **481**, 157–161

Lide, D.R., ed. (1991) *CRC Handbook of Chemistry and Physics*, 72nd Ed., Boca Raton, FL, CRC Press, p. 3–27

Lijinsky, W. & Andrews, A.W. (1980) Mutagenicity of vinyl compounds in *Salmonella typhimurium*. *Teratog. Carcinog. Mutag.*, **1**, 259–267

Mallevialle, J., Bruchet, A. & Fiessinger, F. (1984) How safe are organic polymers in water treatment? *J. Am. Water Works Assoc.*, **76**, 87–93

Mannsville Chemical Products Corp. (1985) *Chemical Products Synopsis: Acrylamide*, Cortland, NY

Marlowe, C., Clark, M.J., Mast, R.W., Friedman, M.A. & Waddell, W.J. (1986) The distribution of [^{14}C]acrylamide in male and pregnant Swiss-Webster mice studied by whole-body autoradiography. *Toxicol. appl. Pharmacol.*, **86**, 457–465

Matsuoka, M., Igisu, H., Lin, J. & Inoue, N. (1990) Effects of acrylamide and *N,N'*-methylene-bis-acrylamide on creatine kinase activity. *Brain Res.*, **507**, 351–353

Miller, M.J. & McQueen C.A. (1986) The effect of acrylamide on hepatocellular DNA repair. *Environ. Mutag.*, **8**, 99–108

Miller, M.S. & Spencer, P.S. (1985) The mechanism of acrylamide axonopathy. *Ann. Rev. Pharmacol. Toxicol.*, **25**, 643–666

Miller, M.J., Carter, D.E. & Sipes, I.G. (1982) Pharmacokinetics of acrylamide in Fischer-344 rats. *Toxicol. appl. Pharmacol.*, **63**, 36–44

Molak, V. (1991) *NIOH (National Institute of Occupational Health, Solna, Sweden) and NIOSH Basis for an Occupational Health Standard. Acrylamide: A Review of the Literature* (DHHS (NIOSH) Publ. No. 91-115), Cincinnati, OH, National Institute for Occupational Safety and Health

Moore, M.M., Amtower, A., Doerr, C., Brock, K.H. & Dearfield, K.L. (1987) Mutagenicity and clastogenicity of acrylamide in L5178Y mouse lymphoma cells. *Environ. Mutag.*, **9**, 261–267

Myers, J.E. & Macun, I. (1991) Acrylamide neuropathy in a South African factory: an epidemiologic investigation. *Am. J. ind. Med.*, **19**, 487–493

Neuhäuser-Klaus, A. & Schmahl, W. (1989) Mutagenic and teratogenic effects of acrylamide in the mammalian spot test. *Mutat. Res.*, **226**, 157–162

Norppa, H. & Tursi, F. (1984) Erythrocyte-mediated metabolic activation detected by SCE. *Basic Life Sci.*, **29B**, 547–559

O'Shaughnessy, D.J. & Losos, G.J. (1986) Comparison of central and peripheral nervous system lesions caused by high-dose short-term and low-dose subchronic acrylamide treatment in rats. *Toxicol. Pathol.*, **14**, 389–394

Rohwein, C.E. (1991) *Occupational Exposure Assessment of Laboratory Workers to Acrylamide Monomer*, MSc Thesis, University of Washington

Russell, L.B., Hunsicker, P.R., Cacheiro, N.L.A. & Generoso, W.M. (1991) Induction of specific-locus mutations in male germ cells of the mouse by acrylamide monomer. *Mutat. Res.*, **262**, 101–107

Sadtler Research Laboratories (1991) *Sadtler Standard Spectra. 1981–1991 Supplementary Index*, Philadelphia, PA

Sager, P.R. & Matheson, D.W. (1988) Mechanisms of neurotoxicity related to selective disruption of microtubules and intermediate filaments. *Toxicology*, **49**, 479–492

Sakamoto, J., Kurosaka, Y. & Hashimoto, K. (1988) Histological changes of acrylamide-induced testicular lesions in mice. *Exp. mol. Pathol.*, **48**, 324–334

Sangster, J. (1989) Octanol–water partition coefficients of simple organic compounds. *J. phys. chem. Ref. Data*, **18**, 1111–1229

Sega, G.A. & Generoso, E.E. (1990) Measurement of DNA breakage in specific germ-cell stages of male mice exposed to acrylamide, using an alkaline-elution procedure. *Mutat. Res.*, **242**, 79–87

Sega, G.A., Valdivia Alcota, R.P., Tancongco, C.P. & Brimer, P.A. (1989) Acrylamide binding to the DNA and protamine of spermiogenic stages in the mouse and its relationship to genetic damage. *Mutat. Res.*, **216**, 221–230

Sega, G.A., Generoso, E.E. & Brimer, P.A. (1990) Acrylamide exposure induces a delayed unscheduled DNA synthesis in germ cells of male mice that is correlated with the temporal pattern of adduct formation in testis DNA. *Environ. mol. Mutagenesis*, **16**, 137–142

Segerbäck, D., Calleman, C.J., Schroeder, J.L., Costa, L.G. & Faustman, E.M. (1994) Formation of *N*-7-(2-carbamoyl-2-hydroxyethyl)guanine in DNA of the mouse and rat following intraperitoneal administration of [^{14}C]acrylamide. *Cancer Res.* (in press)

Shelby, M.D., Cain, K.T., Hughes, L.A., Braden, P.W. & Generoso, W.M. (1986) Dominant lethal effects of acrylamide in male mice. *Mutat. Res.*, **173**, 35–40

Shelby, M.D., Cain, K.T., Cornett, C.V. & Generoso, W.M. (1987) Acrylamide: induction of heritable translocations in male mice. *Environ. Mutag.*, **9**, 363–368

Shiraishi, Y. (1978) Chromosome aberrations induced by monomeric acrylamide in bone marrow and germ cells of mice. *Mutat. Res.*, **57**, 313–324

Shiver, T.M., Sackett, D.L., Knipling, L. & Wolff, J. (1992) Intermediate filaments and steroidogenesis in adrenal Y-1 cells: acrylamide stimulation of steroid production. *Endocrinology*, **131**, 201–207

Skelly, N.E. & Husser, E.R. (1978) Determination of acrylamide monomer in polyacrylamide and in environmental samples by high performance liquid chromatography. *Anal. Chem.*, **50**, 1959–1962

Smith, E.A. & Oehme, F.W. (1991) Acrylamide and polyacrylamide: a review of production, use, environmental fate and neurotoxicity. *Rev. environ. Health*, **9**, 215–228

Smith, M.K., Zenick, H., Preston, R.J., George, E.L. & Long, R.E. (1986) Dominant lethal effects of subchronic acrylamide administration in the male Long-Evans rat. *Mutat. Res.*, **173**, 273–277

Sobel, W., Bond, G.G., Parsons, T.W. & Brenner, F.E. (1986) Acrylamide cohort mortality study. *Br. J. ind. Med.*, **43**, 785–788

Sofuni, T., Hayashi, M., Matsuoka, A., Sawada, M., Hatanaka, M. & Ishidate, M., Jr (1985) Mutagenicity tests on organic chemical contaminants in city water and related compounds. II. Chromosome aberration tests in cultured mammalian cells. *Eisei Shikensho Hokoku*, **103**, 64–75 (in Japanese)

Solomon, J.J., Fedyk, J., Mukai, F. & Segal, A. (1985) Direct alkylation of 2′-deoxynucleosides and DNA following in vitro reaction with acrylamide. *Cancer Res.*, **45**, 3465–3470

Spencer, P.S. & Schaumburg, H.H. (1977) Ultrastructural studies of the dying-back process. IV. Differential vulnerability of PNS and CNS fibers in experimental central–peripheral distal axonopathies. *J. Neuropathol. exp. Neurol.*, **36**, 300–320

Spencer, P.S., Sabri, M.I., Schaumburg, H.H. & Moore, C.L. (1979) Does a defect of energy metabolism in the nerve fiber underlie axonal degeneration in polyneuropathies? *Ann. Neurol.*, **5**, 501–507

Srivastava, S.P., Das, M. & Seth, P.K. (1983) Enhancement of lipid peroxidation in rat liver on acute exposure to styrene and acrylamide a consequence of glutathione depletion. *Chem.-biol. Interactions*, **45**, 373–380

Sublet, V.H., Zenick, H. & Smith, M.K. (1989) Factors associated with reduced fertility and implantation rates in females mated to acrylamide-treated rats. *Toxicology*, **55**, 53–67

Sumner, S.C.J., MacNeela, J.P. & Fennell, T.R. (1992) Characterization and quantitation of urinary metabolites of [1,2,3-^{13}C]acrylamide in rats and mice using ^{13}C nuclear magnetic resonance spectroscopy. *Chem. Res. Toxicol.*, **5**, 81–89

Tanii, H. & Hashimoto, K. (1985) Effect of acrylamide and related compounds on glycolytic enzymes of rat brain. *Toxicol. Lett.*, **26**, 76–84

Tanii, H., Miki, N., Hayashi, M. & Hashimoto, K. (1988) Cytotoxicity of acrylamide and related compounds to mouse neuroblastoma and rat schwannoma cells. *Arch. Toxicol.*, **61**, 298–305

Tilson, H.A. (1981) The neurotoxicity of acrylamide: an overview. *Neurobehav. Toxicol. Teratol.*, **3**, 445–461

Tripathy, N.K., Patnaik, K.K. & Nabi, M.J. (1991) Acrylamide is genotoxic to the somatic and germ cells of *Drosophila melanogaster*. *Mutat. Res.*, **259**, 21–27

Tsuda, H., Shimizu, C.S., Taketomi, M.K., Hasegawa, M.M., Hamada, A., Kawata, K.M. & Inui, N. (1993) Acrylamide: induction of DNA damage, chromosomal aberrations and cell transformation without gene mutations. *Mutagenesis*, **8**, 23–29

Työministeriö [Ministry of Labour] (1993) *Limit Values 1993*, Helsinki, p. 8

UNEP (1993) *IRPTC Data Profile on Acrylamide*, Geneva

US Environmental Protection Agency (1986) Method 8015. Nonhalogenated volatile organics. In: *Test Methods for Evaluating Solid Waste—Physical/Chemical Methods*, 3rd Ed. (US EPA No. SW-846), Washington DC, Office of Solid Waste and Emergency Response, pp. 8015-1–8015-8

US Environmental Protection Agency (1987) *Final Draft for Drinking Water Criteria Document on Acrylamide*, Washington DC, Office of Drinking Water

US Environmental Protection Agency (1990) *Assessment of Health Risks from Exposure to Acrylamide*, Washington DC, Office of Toxic Substances

US Food and Drug Administration (1993) Food and drugs. *US Code fed. Regul.*, **Title 21**, Parts 172.255, 173.5, 173.310, 173.315, 175.105, 175.300, 176.110, 176.170, 176.180, 177.1010, 178.3520, pp. 36, 104–105, 119–121, 129–144, 147–167, 170–200, 208–213, 355–356

US National Institute for Occupational Safety and Health (1976) *Criteria for a Recommended Standard for Occupational Exposure to Acrylamide* (DHEW (NIOSH) Pub. No. 77-112), Washington DC, US Department of Health, Education, and Welfore

US National Institute for Occupational Safety and Health (1992) *Recommendations for Occupational Safety and Health: Compendium of Policy Documents and Statements* (DHHS (NIOSH) Publ. No. 92-100), Cincinnati, OH

US National Institute for Occupational Safety and Health (1993) *National Occupational Exposure Survey (1981–1983)*, Cincinnati, OH

US National Library of Medicine (1993) *Toxic Chemical Release Inventory (TRI) Data Banks* (TRI87, TRI88, TRI89, TRI90, TRI91), Bethesda, MD

US Occupational Safety and Health Administration (1993) Air contaminants. *US Code fed. Regul.*, **Title 29**, Part 1910.1000, pp. 6–34

Vanhorick, M. & Moens, W. (1983) Carcinogen-mediated induction of SV40 DNA amplification is enhanced by acrylamide in Chinese hamster C060 cells. *Carcinogenesis*, **4**, 1459–1463

Voogd, C.E., van der Stel, J.J. & Jacobs, J.J.J.A.A. (1981) The mutagenic action of aliphatic epoxides. *Mutat. Res.*, **89**, 269–282

Walum, E., Odland, L., Romert, L., Ekblad-Sekund, G., Nilsson, M., Calleman, C.J., Bergmark, E. & Costa, L.G. (1992) Biochemical approaches to neurotoxicology *in vitro*: attempts to develop a test system for chemically induced axonopathy. In: *In Vitro Methods in Toxicology: An Alternative to Animal Testing*, London, Academic Press, pp. 237–251

Warr, T.J., Parry, J.M., Callander, R.D. & Ashby, J. (1990) Methyl vinyl sulphone: a new class of Michael-type genotoxin. *Mutat. Res.*, **245**, 191–199

Weast, R.C. & Astle, M.J. (1985) *CRC Handbook of Data on Organic Compounds*, Vol. I, Boca Raton, FL, CRC Press, p. 42

WHO (1985) *Acrylamide* (Environmental Health Criteria 49), Geneva

Working, P.K., Bentley, K.S., Hurtt, M.E. & Mohr, K.L. (1987) Comparison of the dominant lethal effects of acrylonitrile and acrylamide in male Fischer 344 rats. *Mutagenesis*, **2**, 215–220

Zenick, H., Hope, E. & Smith, M.K. (1986) Reproductive toxicity associated with acrylamide treatment in male and female rats. *J. Toxicol. environ. Health*, **17**, 457–472