

**NIOH and NIOSH basis for an occupational
health standard**

Acrylamide: a review of the literature



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
National Institute for Occupational Safety and Health



NIOH AND NIOSH BASIS FOR AN OCCUPATIONAL HEALTH STANDARD

Acrylamide: A Review of the Literature

by

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PREFACE

A memorandum of understanding has been signed by two government agencies in the United States and Sweden—the Division of Standards Development and Technology Transfer of the National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services (DSDTT/NIOSH), and the Criteria Group of Occupational Standard Setting, Research Department, National Institute of Occupational Health (NIOH) (formerly National Board of Occupational Safety and Health). The purpose of the memorandum is to exchange information and expertise in the area of occupational safety and health. One product of this agreement is the development of documents to provide the scientific basis for establishing recommended occupational exposure limits. These limits will be developed separately by the two countries according to their different national policies.

This document on the health effects of occupational exposure to acrylamide is the fourth product of that agreement. The document was written by Vlasta Molak, Ph.D., D.A.B.T. (DSDTT/NIOSH), and was reviewed by the Criteria Group and by DSDTT/NIOSH.

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1 BACKGROUND

1.1 CHEMICAL AND PHYSICAL PROPERTIES

Acrylamide is an odorless, white, crystalline solid used as a monomer or as a raw material in the production of polyacrylamides and other compounds. Chemical and physical properties of acrylamide are given in Table 1. Because most of the acrylamide monomer is produced and used as an aqueous solution, the physical properties of a 50% aqueous solution of acrylamide are also given (Table 2).

1.2 PRODUCTION AND USE OF ACRYLAMIDE

In 1985, 140 million lb (63,600 metric tons) of acrylamide were produced in the United States, and predictions in 1988 indicated that 164 million lb (74,500 metric tons) of this chemical would be produced in 1989 [EPA 1988a]. Total acrylamide production capacity in western Europe was 93.5 million lb (42,500 metric tons) in 1984, and the estimated production in Japan was 90 million lb (41,000 metric tons) in 1982 [WHO 1985]. Acrylamide is also used and produced in the Soviet Union and China, but production estimates have not been reported [He et al. 1989].

The reactive acrylamide monomer is used in the production of other compounds (mostly polymers of acrylamide) and as a grouting agent in the construction or rehabilitation of dams, buildings, sewers, tunnels, and other structures.

Acrylamide grouts are used predominantly as barriers against groundwater seepage into sewers. About 95% of the acrylamide produced in the United States is consumed in the production of other compounds and polyacrylamide products that are widely used as (1) flocculents in potable water and wastewater treatment, mineral ore processing, and sugar refining, (2) water flow control agents in oil well operations, and (3) adhesives in papermaking and construction [Davidson et al. 1980]. The remaining 5% is used as a monomer [CMR 1985].

1.3 POTENTIAL FOR OCCUPATIONAL EXPOSURE

Data on worldwide occupational exposures to acrylamide are unavailable. However, since exposure occurs during acrylamide production and use, the potential for occupational exposure exists in all countries that produce or import acrylamide. The National Institute for Occupational Safety and Health (NIOSH) estimates that more than 10,000 U.S. workers were potentially exposed to acrylamide monomer during the period 1981-83, either in acrylamide manufacturing and processing or in grouting operations (particularly in sewer grouting) [NIOSH 1983]. Other workers that are potentially exposed to variable and intermittent airborne concentrations and possible dermal contact with acrylamide are the researchers and technicians involved in the preparation of polyacrylamide

Acrylamide

Table 1. Identifying information and chemical and physical properties of acrylamide*

Item	Description
CAS registry number	79-06-1
RTECS number	AS3325000
Synonyms	Acrylamide monomer, acrylic amide, propenamide
Formula (structure)	C_3H_5NO ($CH_2=CHC(=O)NH_2$)
Molecular weight	71.08
Physical form	White, crystalline solid
Melting point	$84.5 \pm 0.3^\circ C$ ($184.1^\circ \pm 0.5^\circ F$)
Boiling point	$125^\circ C$ at 25 mm Hg (decomposes on boiling)
Density	1.122 g/cm^3 at $30^\circ C$ ($86^\circ F$)
Vapor pressure	0.007 mm Hg at $25^\circ C$ ($77^\circ F$), 0.033 mm Hg at $40^\circ C$ ($104^\circ F$), 0.070 mm Hg at $50^\circ C$ ($122^\circ F$)
Conversion factors	$1 \text{ ppm} \approx 2.9 \text{ mg/m}^3$ at $25^\circ C$, $1 \text{ mg/m}^3 \approx 0.34 \text{ ppm}$ at $25^\circ C$
Solubilities (in g/100 ml at $30^\circ C$):	
Acetone	63.1
Acetonitrile	39.6
Benzene	0.346
Carbon tetrachloride	0.038
Chloroform	2.66
1,2-Dichloroethane	1.50
Dimethylformamide	119
Dimethylsulfoxide	124
Dioxane	30
Ethanol	86.2
Ethylacetate	12.6
n-Heptane	0.007
Methanol	155
Pyridine	61.9
Water	215.5

*Adapted from American Cyanamid Company [1969] and NIOSH [1976].

Table 2. Physical properties of a 50% aqueous solution of acrylamide*

Property	Value
Assay (WT% acrylamide)	48-62
pH	5.0-6.5
Polymer, max %	0.05
Viscosity	2.71 centipoise at 25°C (77°F)
Specific gravity	1.0412 at 25°C (77°F)
Melting point	8°-13°C (47°-54°F)
Boiling point	99°-104°C (210°-220°F)
Vapor pressure	18 mm Hg at 23°C (73.4°F), 29.8 mm Hg at 31.2°C (88.2°F), 43.4 mm Hg at 37.8°C (100°F), 92.7 mm Hg at 52.7°C (126.9°F), 209.5 mm Hg at 70.5°C (158.9°F)
Specific heat (20°-50°C, range)	0.83 cal/(g-deg)
Heat of dilution to 20 WT%	1.1 cal/g of solution (or 2.0 Btu/lb of solution) [exothermic]
Heat of polymerization	20.4 kcal/g mole [exothermic]
Heat of melting (solution)	59.2 cal/g (106.5 Btu/lb)
Flammability	Nonflammable

* Adapted from GCA [1980].

Acrylamide

gels. The U.S. Environmental Protection Agency (EPA) [EPA 1988a] estimates that 100,000 to 200,000 of these U.S. workers have the potential for exposure.

Because acrylamide is produced by catalytic or sulfuric acid hydration of acrylonitrile (identified by the International Agency for Research on Cancer [IARC] as a probable human carcinogen [IARC 1987]), acrylamide production workers may also be exposed to acrylonitrile.

Evaluation of exposure data from industrial settings [Hills and Greife 1986] indicates that in all four U.S. acrylamide production plants, airborne concentrations were generally below 0.3 mg/m^3 —the occupational exposure limit set by many countries (see the Appendix).

Of the ninety 8-hr time-weighted average (TWA) personal samples collected in the four U.S. acrylamide production plants, only two samples from one of the plants were above 0.3 mg/m^3 (0.38 and 0.39 mg/m^3). However, many samples exceeded the current Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) of 0.03 mg/m^3 adopted in 1989 [54 Fed. Reg. 2332 (1989)].

Hills and Greife [1986] noted that of the cases they reviewed, most adverse health effects were caused by dermal or oral exposures to acrylamide. The major routes of occupational exposure appear to be dermal absorption of the acrylamide monomers from solution and inhalation of the dry monomer or aerosols of acrylamide solution [Hills and Greife 1986; EPA 1987; He et al. 1989]. An EPA study of sewer workers involved in grouting operations indicated high dermal exposure estimates ranging from 0.6 to 5.0 mg/hr [EPA 1987]. Studies evaluating occupational exposures to acrylamide monomer indicate that dermal exposures may be more important with regard to the total body intake than inhalation exposures [Hills and Greife 1986; EPA 1988a; He et al. 1989].

1.4 EXISTING STANDARDS AND RECOMMENDATIONS

Many international standards for workplace exposures to acrylamide are 0.3 mg/m^3 with a skin notation. The current American Conference of Governmental Hygienists (ACGIH) threshold limit value (TLV[®]) [ACGIH 1989] and the current OSHA PEL [29 CFR 1910.1000] are both 0.03 mg/m^3 . The current Swedish occupational exposure limit is 0.3 mg/m^3 with a skin notation and a short-term exposure limit (STEL) of 0.9 mg/m^3 . See the Appendix for a list of the occupational exposure limits for airborne acrylamide in various countries and a discussion of the bases for recommendations that depart from the frequently cited limit of 0.3 mg/m^3 .

2 TOXICOLOGY

2.1 METABOLISM

Acrylamide [$\text{CH}_2=\text{CHC}(=\text{O})\text{NH}_2$] is a small organic molecule that is very soluble in water and can react at both its amide group and its double-bond vinyl group. This reactivity and ease of transport may account for the toxic effects of acrylamide. Studies show that the toxicokinetics and tissue distribution of acrylamide are not significantly dependent on the dose and route of exposure. The parent compound is rapidly eliminated from the tissues. The uptake, distribution, biotransformation, and elimination, which are summarized below, have been discussed in detail by Dearfield et al. [1988].

2.1.1 Uptake

No studies were found on the absorption of acrylamide by inhalation. All animal studies involved either oral or dermal exposure, and intraperitoneal (i.p.) or intravenous (i.v.) dosing. The uptake of acrylamide through the gastrointestinal tract of rats was rapid and complete, as indicated by similar excretion profiles of the chemical whether it was administered by i.v. injection or by gavage [Miller et al. 1982]. Dermal absorption in the rat is less than complete. By comparing blood concentrations after i.v. or dermal administration of acrylamide, Ramsey et al. [1984] calculated that 25% of the applied doses (2 or 50 mg/kg) of acrylamide were absorbed through the skin of rats during the first 24 hr. Frantz et al. [1985] reported that 26% of a 0.5% aqueous solution of acrylamide was absorbed through the skin of rats in 24 hr and that, after the skin was washed, an additional 35% remained in the skin. The data from in vitro experiments were similar [Frantz et al. 1985]. These researchers used excised skin preparations to show that 67% of the applied acrylamide was either absorbed or available for absorption (that is, 54% was absorbed and 13% remained in the skin after washing).

2.1.2 Distribution and Toxicokinetics

After rats received various doses of acrylamide (0.5 to 100 mg/kg) by i.v. or oral administration, the ^{14}C -labeled chemical quickly distributed throughout the body [Hashimoto and Aldridge 1970; Edwards 1975; Miller et al. 1982; Ramsey et al. 1984]. Approximately 12% of the ^{14}C -labeled chemical rapidly accumulated in red blood cells [Hashimoto and Aldridge 1970; Miller et al. 1982], and high levels persisted for at least 10 days. This persistence has been postulated to result from the reaction of acrylamide with sulfhydryl moieties present in hemoglobin [Hashimoto and Aldridge 1970]. Miller et al. [1982] reported that high percentages of the ^{14}C -labeled chemical were found in muscle (48%), skin (15%), blood (12%), and liver (7%), whereas the neural tissues (brain, spinal

cord, and sciatic nerve) contained less than 1%. However, when the data were expressed as micromoles (μ moles) of acrylamide/g of tissue, the concentrations of acrylamide in the tissues were similar. In the neural tissues of rats there appears to be no preferential bioconcentration of acrylamide and/or its metabolites that can account for its neurotoxic effects [Miller et al. 1982].

Acrylamide has also been reported to distribute readily in the tissues of other animal species. For example, acrylamide was found in the blood, brain, heart, liver, kidneys, and lungs of miniature swine and beagle dogs, with the highest concentrations found in the liver and kidneys [Ikeda et al. 1985]. Because the authors did not analyze skin, muscle, and other tissues, it is difficult to compare the results with those reported by Miller et al. [1982]. In addition, autoradiographic studies have demonstrated a similar distribution of acrylamide in male and pregnant female mice [Marlowe et al. 1986]. Acrylamide was found to cross the placentas of rats, rabbits, dogs, pigs, and mice and to be uniformly distributed in those fetuses [Ikeda et al. 1983, 1985; Marlowe et al. 1986].

Ramsey et al. [1984] have examined the effects of multiple oral doses on tissue distribution. When rats were given acrylamide at 0.05 or 30 mg/kg for 13 consecutive days, the ratio of the 14 C-labeled chemical in the tissues at the two doses was proportional to the ratio of the doses administered (that is, 600) except in the red blood cells (304), blood plasma (1,089), and testes (934). These data demonstrate that multiple doses of acrylamide do not greatly alter its distribution except at those three sites.

2.1.3 Biotransformation and Elimination

Conjugation with glutathione (GSH) is the major route for the biotransformation of acrylamide. Several studies show that acrylamide depletes GSH [Hashimoto and Aldridge 1970; Edwards 1975; Srivastava et al. 1983] and that hepatic GSH-S-transferases catalyze the reaction of acrylamide with GSH [Dixit et al. 1981]. The major urinary metabolite of acrylamide is N-acetyl-S-(3-amino-3-oxypropyl) cysteine, and it accounts for approximately 50% of the applied dose in rats [Miller et al. 1982; Ramsey et al. 1984]. Three other unidentified, nonsulfhydryl urinary metabolites accounted for an additional 14% [Miller et al. 1982]. When acrylamide was labeled at the carbonyl carbon, it was found that 4% to 6% of the labeled chemical was eliminated as CO_2 [Hashimoto and Aldridge 1970; Ramsey et al. 1984]. Because 15% of the dose appeared in the bile within 6 hr but only 6% was excreted in the feces, it was concluded that acrylamide (or its metabolites) underwent enterohepatic circulation [Miller et al. 1982].

Miller et al. [1982] used rats in a detailed study of the pharmacokinetics and distribution of acrylamide, both as the parent compound and as the total 14 C-labeled chemical after an i.v. dose of 10 mg/kg. The elimination of the parent acrylamide was represented by a single compartment model. In the blood, the parent compound had a half-life of 1.7 hr, and the clearance of the unmetabolized acrylamide from all other tissues but the testes was similar. The testes showed a delay in the time necessary to reach peak concentration—an event attributed to their fat content. After the peak was attained, the parent acrylamide was cleared from the testes in a manner similar to that for other tissues [Miller et al. 1982].

The distribution and the elimination of the total 14 C-labeled chemical (representing acrylamide and its metabolites) was slower than that of the parent compound and was best represented by a biphasic curve [Miller et al. 1982]. In addition, four tissues (liver, kidney, fat, and testes)

demonstrated absorptive phases for the total ^{14}C -labeled chemical. Since no absorption phases were noted for the parent acrylamide in the liver and kidney, the increases in the ^{14}C -labeled chemical in these two tissues were attributed to metabolite accumulation. The higher lipid content of the fat and testes and the polar nature of acrylamide were reported by Miller et al. [1982] to have delayed the absorption in these two tissues. The initial portion (half-life of 5 hr) of the biphasic curve was attributed to the metabolism of acrylamide and the binding of its metabolites to biological macromolecules, since only 2% of the total dose of administered acrylamide was excreted as the parent compound. The terminal portion (half-life of 8 days) of the biphasic curve was thought to be the result of the release of acrylamide metabolites from tissue depots and the degradation of acrylamide-protein adducts [Miller et al. 1982]. Support for these suppositions was provided by Ramsey et al. [1984], who analyzed urine, plasma, and tissue samples in male Fisher 344 rats following either i.v., gavage, or dermal administration of ^{14}C -labeled acrylamide. Their data indicated that the initial phase was due to the loss of the parent compound and that the latter phase resulted from the clearance of acrylamide metabolites.

Because acrylamide affects reproduction, the very slow release of acrylamide or its metabolites from the testes is of particular interest. Although the fat content of the testes may have decreased the uptake of the parent acrylamide, it would not appear to account for its slow excretion. It is possible that acrylamide is metabolized and binds to constituents of the testes. Marlowe et al. [1986] reported that radioactivity appeared in the testes 1 hr after administration and migrated to the seminiferous tubules and head of the epididymis after 9 hr. After 9 days, radioactivity remained only in the tail of the epididymis and in the epithelium of the penis. Marlow et al. [1986] correlated this movement with that of the spermatids.

2.1.4 Factors That Can Affect Metabolism

Studies of the effects of altering microsomal mixed-function oxidase activity on the expression of acrylamide-induced neuropathy have given disparate results.

Pretreatment of rats with phenobarbital (PB) or DDT [Kaplan et al. 1973] or mice with PB [Hashimoto et al. 1981] reduced or delayed the neurological dysfunction caused by acrylamide administered by i.p. injection. However, Srivastava et al. [1985] found that pretreatment of rats with PB or DDT decreased the time necessary for onset of acrylamide-induced hind-limb paralysis. This disparity does not appear to be due to variations in experimental protocol, but it may be explained by differences in the animal strains.

The results of using drug metabolism inhibitors are difficult to interpret. For example, SKF 525A (which inhibits microsomal oxidation) has been reported to prevent acrylamide-induced enhancement of striatal dopamine receptor activity [Agrawal et al. 1981] and to increase the acute toxicity of acrylamide in rats [Kaplan et al. 1973], whereas cobalt chloride has been shown to cause a significant delay in the development of hindlimb paralysis in rats [Srivastava et al. 1985]. Presently, no correlation exists between the effects of modifying microsomal metabolism of acrylamide and expression of its toxic effects. Accordingly, identification of metabolic factors important in the toxic manifestations of acrylamide is not possible. Khanna et al. [1988] studied the role of protein deficiency on the neurobehavioral effects of acrylamide in rat pups exposed during pregnancy and early infancy. They demonstrated that acrylamide was more toxic in protein-deficient animals because it decreased the dopamine and benzodiazepam receptor binding and delayed the development of reflexes and other physical milestones in pups.

2.2 TOXICOLOGIC MECHANISMS

Because the most noticeable and earliest detectable effects of acrylamide exposures are neurological effects, numerous studies have attempted to find the mechanism of its action in neural tissues. Several mechanisms may account for the effects of acrylamide on the peripheral and central nervous systems. Biochemical studies indicate that acrylamide affects the proteins responsible for normal functioning of neural tissues. Berti-Mattera et al. [1986] found that acrylamide alters axonal protein phosphorylation and polyphosphoinositide metabolism. Another study revealed that acrylamide treatment *in vivo* induces changes in the composition of fast-transported proteins that are similar to those seen after axotomy [Bisby and Redshaw 1987]. *In vitro* treatment of PtK1 cells by acrylamide resulted in rapid dephosphorylation of keratins [Eckert and Yeagle 1988]. Many of these changes may be explained by altered phosphorylation of cytoskeletal proteins [Howland and Ali 1986]. This phosphorylation may result from the binding of acrylamide to proteins, which has been demonstrated *in vitro* with bovine serum albumin (BSA) [Dixit et al. 1986]. Husain et al. [1986, 1987] found that acrylamide inhibits succinic dehydrogenase (SDH) activity in mitochondrial preparations from rat brain and causes changes in levels of biogenic amines and activities of monoamine oxidase and acetylcholine esterase. Biochemical changes may be responsible for the functional changes observed in neural tissues—for example, changes in membrane capacitance and sodium permeability [Brismar et al. 1987], changes in primary afferent terminal function [De Rojas and Goldstein 1987], reduction of neurofilament transport in rat sciatic nerve [Gold 1987], and alterations in spinal cord reflexes [Goldstein and Fincher 1986].

In light of the reported genotoxicity and carcinogenicity of acrylamide (see Sections 4 and 5), information on the binding of this compound to DNA is of considerable interest. Binding of acrylamide to DNA has been demonstrated to occur in the lung, liver, testes, stomach, and skin of mice 6 hr after oral or dermal administration of acrylamide [Carlson and Weaver 1985]. Whether or not the parent acrylamide or a metabolite was responsible for the *in vivo* acrylamide-DNA adducts, it was demonstrated that acrylamide can alkylate DNA in an *in vitro* system [Solomon et al. 1985]. Because the yield of alkylated DNA after 40 days of incubation was low (approximately 10%), the relevance of these *in vitro* binding studies to the *in vivo* DNA-binding studies reported by Carlson and Weaver [1985] remains to be established. However, the fact that acrylamide has been shown to interact with genetic material may have implications for its genotoxic and carcinogenic effects.

2.3 TOXICITY IN HUMANS AND ANIMALS

Polyacrylamide products are generally considered nontoxic [McCollister et al. 1965]. Human health hazards related to acrylamide monomer exposures are neurotoxicity, developmental and reproductive effects, genotoxicity, and carcinogenicity.

2.3.1 Neurotoxic Effects

The neurologic effects of acrylamide exposures were extensively reviewed in the National Institute for Occupational Safety and Health (NIOSH) criteria document on acrylamide [NIOSH 1976].

Exposure to acrylamide can produce serious neurotoxic effects in humans and animals [Spencer and Schaumburg 1975; Tilson 1981; Miller and Spencer 1985; O'Donoghue 1985]. These include peripheral nerve damage (paresthesia of the hands and feet, muscle weakness, ataxia, and decreased tendon reflexes) and central nervous system (CNS) effects (drowsiness, tremors, slurred speech, and hallucinations). Although most of these effects ceased after the individual was removed from exposure, some severely affected persons did not recover completely, indicating irreversible effects [Miller and Spencer 1985].

Spencer and Schaumburg [1974] reviewed acrylamide neurotoxicity in humans. The earliest and most obvious signs of local acrylamide contact are erythema and peeling of the palms caused by contact with acrylamide solution. This contact dermatitis precedes neurological symptoms by several weeks. Shortly after the appearance of contact dermatitis, exposed individuals may notice excessive fatigue, weight loss, and somnolence [Garland and Patterson 1967]. The neurological symptoms occur after chronic exposures. Exposed individuals experience unsteadiness, muscle weakness, and paresthesia, with numbness in the hands and/or feet. Sensory symptoms frequently precede overt motor signs [Takahashi et al. 1971]. If individuals are removed from exposure sources, signs of peripheral neuropathy gradually disappear [He et al. 1989]. In less severely affected individuals, complete recovery may occur within 2 to 12 months [Garland and Patterson 1967].

A recent study described the neurological and electroneuromyographic assessment of the effects of acrylamide on occupationally exposed workers in China [He et al. 1989]. The authors reported that more than 90 cases of acrylamide intoxication have occurred in China in the past 5 years as a result of increased acrylamide exposures in a number of small township-run or village-owned industries. He et al. [1989] investigated occupational exposures and toxic effects of exposure to acrylamide in a factory that began acrylamide and polyacrylamide production in 1984. The initial air concentrations of acrylamide were measured at 5.6 to 9.0 mg/m³ during an exceptional increase in production from March to June 1985. After renovation of the factory in July 1985, the air concentrations dropped to an average of 0.03 mg/m³. Heavy skin contamination by aqueous acrylamide monomer was common among the exposed workers because of inadequate personal protection, lack of awareness of acrylamide toxicity, and unsatisfactory personal hygiene. Water in which three exposed workers washed their hands contained 410 mg acrylamide/liter.

Seventy-one exposed workers (45 men and 26 women) were studied in October 1985 [He et al. 1989]. They had been exposed to variable concentrations of acrylamide for 1 to 18 months. Fifty-one unexposed blue collar workers from the same town (33 men and 18 women) served as a reference group; nobody in this group suffered from measurable neurological disease. All subjects were interviewed with the aid of a structured questionnaire to obtain information on demographic factors, occupational history, symptoms, past illnesses, and family history. Physical and neurological examinations, visual acuity and visual field testing, skin temperature measurements, electrocardiography, and electroencephalography were performed. The laboratory studies included routine blood and urine tests, liver function, serum hepatitis B surface antigen, serum glucuronidase, and immunoglobulins. Electroneuromyographic examinations were performed in 63 exposed and 48 unexposed workers. The exposed workers reported numerous symptoms that were nonexistent or rarely reported in the unexposed group. These included skin peeling from the hands, numbness in the hands and feet, lassitude, sleepiness, muscle weakness, clumsiness of the hands, anorexia, unsteady gait, coldness of the hands and feet, stumbling, falling, and difficulty in grasping. The initial symptoms were peeling of the skin and excessive sweating of the hands, which were mainly due to topical contamination by aqueous acrylamide. Muscle weakness of the legs and numbness and tingling of the hands and feet (indicating an involvement of the peripheral nervous system)

Acrylamide

appeared in one-fifth of the exposed workers after 3 to 10 months of exposure. Nine workers developed lassitude, sleepiness, anorexia, and loss of body weight 1 month after initial symptoms; the symptoms progressed to inability to hold things tightly, unsteadiness in walking, and difficulty in lifting legs when climbing stairs. Three of the nine were found to have horizontal nystagmus, truncal ataxia, and clumsiness of the hands when they were admitted to a hospital in May 1985. They showed improvement in cerebellar function a month later but began losing tendon reflexes and their sensation of vibration. When interviewed in October 1985, these individuals showed considerable recovery.

Electroneuromyographic studies of the 69 acrylamide workers showed a prolonged duration of motor unit potentials in 40 workers and an increase of polyphasic potentials in 29 workers. In 25 of these 40 workers, there were no signs of neuropathology, such as impairment of distal sensation or reflexes. This finding suggests that a partial denervation of the distal muscles caused by axonal degeneration of peripheral nerves could be a subclinical abnormality in workers exposed to acrylamide. He et al. [1989] suggested that electroneuromyographic changes could be used for early detection of acrylamide neurotoxicity. Most of the workers diagnosed as suffering from occupational acrylamide intoxication handled a 27% to 30% aqueous solution of acrylamide. Their exposure to aqueous solutions of acrylamide suggests that dermal contact may have been the main route of exposure.

Several subchronic and chronic animal studies have demonstrated that the neurotoxic effects of acrylamide exposures in animals are similar to the effects in humans [Schaumburg and Spencer 1979; Burek et al. 1980]. Hind-limb ataxia appears to be one of the first symptoms of neuropathy in dogs [Satchell and McLoad 1981; Hersch et al. 1989], cats [Spencer and Schaumburg 1977b], monkeys [Merigan et al. 1982], and rats [Spencer and Schaumburg 1976a, 1977b; Tilson et al. 1979].

2.3.2 Developmental and Reproductive Effects

Data on developmental and reproductive effects in animals are summarized in Tables 3 and 4. No such data are available for humans. Acrylamide exposure affected both fetal and postnatal development in mouse and rat offspring when dams were orally dosed during pregnancy [American Cyanamid Company 1980; Zenick et al. 1986]. Neurotoxic effects occurred in neonates when the dam drank water containing acrylamide concentrations that were not toxic to her [American Cyanamid Company 1980].

Reproduction is also affected by acrylamide exposure. Testosterone levels were depressed in rats [Ali et al. 1983], and fertility decreased in male mice after oral exposure to acrylamide in drinking water [Sakamoto and Hashimoto 1986]. Degeneration of testicular epithelial tissue in male mice dosed by gavage [Hashimoto et al. 1981] has been observed as well as dominant lethal effects in male rats exposed through drinking water [Smith et al. 1986] and mice treated i.p. [Shelby et al. 1986]. Oral exposure of male or female mice and rats to acrylamide in drinking water caused an increased resorption rate [Nalco Chemical Company 1987; Sakamoto and Hashimoto 1986].

Table 3. Reproductive effects of acrylamide exposure in female rats and mice and developmental effects in their offspring*

Type of study and reference	Species, route, and dosage	Maternal		Developmental		Observed effects	
		LOEL ⁺	NOEL ⁺	LOEL	NOEL	Maternal	Developmental
Developmental toxicity [Edwards 1976]	Porton rat; oral—diet; 0 ppm, 200 ppm, or 20 mg/kg per day, gd ⁺ 0–22; 400 ppm or 40 mg/kg per day, gd 0–20; 100 mg/kg (i.v.), gd 9	200 ppm ⁺ [20 mg/kg per day] ⁺	—	—	400 ppm [400 mg/kg per day]	Ataxia; abnormal gait	None; it was shown, however, that acrylamide readily crosses the placenta
Reproduction and fertility [Zenick et al. 1986]	Long Evans rat; oral—drinking water; 0, 25, 50, 100 ppm for 2 weeks before mating and throughout gestation and lactation	50 ppm	25 ppm	50 ppm	25 ppm	Decreased body weight (50 ppm) Decreased fluid intake (50 ppm) Increased hind-limb splaying (100 ppm)	Decreased birth weight Decreased body weight gain in pups through day 42
Developmental/neonatal [Walden et al. 1981]	Fischer 344 rat; oral—gavage; 20 mg/kg per day, gd 7–16	—	—	20 mg/kg per day	—	None reported	Changes in various intestinal enzyme concentrations measured in the neonate
Developmental toxicity (1-generation reproductive) [American Cyanamid Company, 1980]	Sprague-Dawley rat; oral—diet; 0, 25, 50 ppm or 0, 2.5, 5.0 mg/kg per day 3 weeks before mating, gd 1–19	50 ppm	25 ppm	Dose unspecified	Dose unspecified	Decreased body weight gain; slight alopecia Nerve fiber degeneration in sciatic and optic nerves	Wallerian degeneration of tibial nerve, unilateral optic nerve degeneration (dose unspecified)

(Continued)

Table 3 (Continued). Reproductive effects of acrylamide exposure in female rats and mice and developmental effects in their offspring*

Type of study and reference	Species, route, and dosage	Maternal		Developmental		Observed effects	
		LOEL ⁺	NOEL ⁺	LOEL	NOEL	Maternal	Developmental
1-Generation reproductive [Nalco Chemical Co. 1987]	Fischer 344 rat; oral—drinking water; 0, 0.5, 2.0, 5.0 mg/kg per day for 10 weeks throughout gestation and lactation	2.0 mg/kg per day	0.5 mg/kg per day	5.0 mg/kg per day	2.0 mg/kg per day	Increased peripheral neuropathy Decreased body weight Decreased body weight gain Decreased number of litters (fecundity index) Increased pre-implantation loss (5.0 mg/kg per day)	Increased resorptions per litter Decreased litter size
Reproductive toxicity assay [Sakamoto and Hashimoto 1986]	ddY mouse, oral—drinking water; 0–5 mM for 4–6 weeks	1.2 mM ⁺ or 18.6 mg/kg per day			1.2 mM or 18.6 mg/kg per day	Increased number of resorptions per dam Slight hind-limb weakness	None reported

* Adapted from EPA [1987].

⁺ Abbreviations: gd = gestation day; LOEL = lowest-observable-effect level; NOEL = no-observable-effect level; mg/kg per day = milligrams per kilogram of body weight per day; mM = millimolar (concentration of acrylamide in drinking water); ppm = parts per million.

Table 4. Reproductive effects of acrylamide exposure in male rats and mice*

Type of study and reference	Species, route, and dosage	LOEL ⁺	NOEL ⁺	Observed effects
Dominant lethal and 1-generation reproductive [Nalco Chemical Co. 1987]	Fisher 344 rat; oral—drinking water; 0, 0.5, 2.0, 5.0 mg/kg per day for 10 weeks	2.0 mg/kg per day [†]	0.5 mg/kg per day	Increased peripheral neuropathy
				Decreased body weight
				Decreased body weight gain
				Decreased number of litters (fecundity index)
				Increased preimplantation loss (5.0 mg/kg per day)
Testicular effects [Hashimoto et al. 1981]	ddY mouse; oral—gavage; 0, 35.5 mg/kg 2×/wk for 8–10 weeks; avg. daily dose = 10.1 mg/kg	35.5 mg/kg per day	—	Degeneration of testicular epithelia
				Weakness and ataxia in hind limbs
Dominant lethal [Smith et al. 1986]	Long Evans rat; oral—drinking water; 0, 15, 30, 60 ppm ⁺ or 0, 1.5, 2.8, 5.8 mg/kg per day for 80 days	2.8 mg/kg per day	1.5 mg/kg per day	Increased preimplantation loss (highest dose only)
				Increased postimplantation loss
Reproductive toxicity assay [Sakamoto and Hashimoto 1986]	ddY mouse; oral—drinking water; 0.3, 0.6, 0.9, 1.2 mM for 4–6 weeks	0.6 mM ⁺ or 8.8 mg/kg per day	0.3 mM or 4.2 mg/kg per day	Decreased fertility rate
				Decreased number fetuses/dam
				Increased number resorptions/dam

(Continued)

Table 4 (Continued). Reproductive effects of acrylamide exposure in male rats and mice*

Type of study and reference	Species, route, and dosage	LOEL ⁺	NOEL ⁺	Observed effects
Reproduction and fertility [Zenick et al. 1986]	Long Evans rat; oral—drinking water; male: 0, 50, 100, 200 ppm or 0, 4.2, 7.9, 11.6 mg/kg per day for 10 weeks	100 ppm [7.9 mg/kg per day]	50 ppm [4.2 mg/kg per day]	Decreased copulatory performance Increased hind-limb splaying
Dominant lethal [Sublet et al. 1986]	Long Evans rat; oral—drinking water; 0, 5, 15, 30, 45, 60 mg/kg per day for 5 days	30 mg/kg per day	15 mg/kg per day	Increased preimplantation loss Increased postimplantation loss Effects seen primarily in weeks 1–3 post mating
Testosterone assay [Ali et al. 1983]	Fischer 344 rat; intraperitoneal; 0, 10, 20 mg/kg per day for 20 days	20 mg/kg per day	10 mg/kg per day	Dose-dependent decrease of testosterone and prolactin
Dominant lethal [Shelby et al. 1986]	C3H X101 hybrid mouse; i.p. 125 mg/kg or 5× 50 mg/kg per day	50 mg/kg per day	---	Increased postimplantation loss

* Adapted from EPA [1987].

⁺ Abbreviations: LOEL = lowest-observable-effect level; NOEL = no-observable-effect level; mg/kg per day = milligrams per kilogram of body weight per day; mM = millimolar (concentration of acrylamide in drinking water); ppm = parts per million.

2.4 GENOTOXIC EFFECTS

The major concern about the genotoxicity of acrylamide is its clastogenic activity (that is, chromosomal breakage and other chromosomal abnormalities). The clastogenic effect of acrylamide appears more pronounced in the germ cells compared with somatic cells. The interaction with germinal tissues suggests the possible heritability of acrylamide-induced DNA alterations. Acrylamide has been shown to be a clastogenic agent both in vivo and in vitro [Moore et al. 1987].

Reports suggest that acrylamide binds to DNA [Carlson and Weaver 1985], induces DNA damage and repair effects, and causes in vitro cell transformation [Banerjee and Segal 1986]. In a recent study by Backer et al. [1989], various cytogenetic endpoints in both somatic and germ cells from acrylamide-treated male C57BL/6J mice were evaluated. Sister chromatid exchanges and micronuclei (but not chromosomal aberrations) were induced in spleen cells; synaptonemal complex irregularities (asynopsis) (but not chromosomal aberrations) were induced in germ cells.

2.4.1 Gene Mutation Assays

Acrylamide does not appear to induce gene mutations in the three types of gene mutation assays examined: the *Salmonella*/mammalian activation assay [Bull et al. 1984a; Lijinsky and Andrews 1980; Hashimoto and Tanii 1985], the CHO/HPRT mutation assay [American Cyanamid 1985], and the *Drosophila* sex-linked recessive lethal assay [American Cyanamid 1985]. The mouse lymphoma mutation data suggest that acrylamide may induce mutations in an eukaryotic gene mutation assay [Moore et al. 1987], but these data may reflect a clastogenic event associated with the predominant formation of small colonies. It may be relevant that the CHO subclone generally used in the CHO/HPRT mutation assay does not appear to be sensitive to clastogens [Hsie et al. 1986].

2.4.2 Chromosomal Assays

Studies that have examined the chromosomal effects of acrylamide have confirmed its clastogenic potential. This effect appears to be more pronounced in the germ cells than in somatic cells. The suggestive reciprocal translocation results raise the possibility that acrylamide-induced alterations to DNA may be transmissible to future generations. A study of heritable translocation in mice [Shelby et al. 1987] demonstrated that acrylamide is an effective inducer of translocations in postmeiotic germ cells. This result demonstrates that acrylamide may be capable of affecting heritable germ cells in a mammalian system.

In vivo and in vitro results suggest that acrylamide may induce aneuploidy. It is uncertain whether metabolic activation is required for acrylamide to exert its genotoxic effects, because most of the clastogenic activity was noted in in vivo studies. In vitro studies with human lymphocytes in which clastogenicity was observed were performed without activation [IHE 1985]. However, the metabolic activating capability of lymphocytes has not been totally explored, and it is known that lymphocytes are capable of activating chemicals such as cyclophosphamide to genotoxic forms [Waalkens et al. 1981]. The mouse lymphoma results demonstrate that acrylamide induces aberrations without activation [Moore et al. 1987].

2.5 CARCINOGENIC EFFECTS

2.5.1 Epidemiologic Findings

Collins et al. [1989] studied the mortality of workers in acrylamide production plants. This study was performed by scientists at major production plants. Mortality was tracked for a cohort of 8,854 males from 1925 to 1983. This cohort (2,293 of whom were exposed to acrylamide) consisted of four plant populations in the United States and the Netherlands. Followup was completed for 95% of the cohort. At the end of the study 2,148 individuals were deceased, and death certificates were obtained for 95%. The underlying cause of death was coded by a nosologist. Exposure estimates were developed for all jobs at each of the four plants using exposure data collected at each plant and information obtained from plant personnel who had knowledge of past jobs and processes. Exposure to acrylamide was defined as a cumulative exposure greater than 0.001 mg/m³-years, which is approximately equivalent to a 1-day exposure to an average concentration of 0.3 mg/m³. Data on smoking history were available for only a third of the cohort. Standardized mortality ratios (SMRs) and internally standardized rate ratios were employed to estimate the risk.

According to the authors, a particular strength of their study was the ability to detect a 25% increase in total cancer, a 50% increase in respiratory cancer, and a threefold increase in brain and other central nervous system cancers based on a two-tailed, 5% significance level with a power of 80%.

The SMRs for all causes for both exposed and unexposed groups were significantly less than expected, which may be the result of both initial selection and the ongoing benefits of employment (i.e., the healthy worker effect). An excess of total cancer deaths was not observed among the exposed workers. A slight increase in cancer of the respiratory system (30 observed versus 26.3 expected) was not statistically significant. Analyses of trends by cumulative exposure showed no increased risk of mortality with increasing exposure. Because smoking history was obtained for only a third of the cohort, it was impossible to determine whether smoking habits had confounded the results.

Sobel et al. [1986] examined mortality in a group of 371 workers assigned to acrylamide monomer or polyacrylamide operations. Exposure to acrylamide was categorized on the basis of a review of job classifications. Before 1957, personal TWA exposures to acrylamide in the monomer production areas were 0.1 to 1.0 mg/m³. The data from 1957 to 1970 indicated that exposures to acrylamide were 0.1 to 0.6 mg/m³. After 1970, personal exposures to acrylamide were <0.1 mg/m³ for all job classifications. Associated with the production of acrylamide monomer is the potential for exposure to acrylonitrile. The possible confounding effect of this exposure was not formally addressed in this study; but in a previous epidemiologic study [Ott et al. 1980], no effects could be directly related to acrylonitrile under the conditions of exposure. The total number of deaths from malignancies was slightly elevated (11 observed versus 7.9 expected [$p < 0.05$]), but this result was apparently due to the excess in a subgroup that was also exposed to organic dyes [Sobel et al. 1986]. Overall, the results did not indicate an excess of cancers from exposures to acrylamide. However, these results may be due to the small cohort size, limited followup, and short duration of exposure (274 workers were exposed for less than 5 years). Given the size of the total cohort and the period of observation, the study had only an 80% likelihood (power) of detecting a twofold or greater increase in total cancer incidence.

2.5.2 Evidence of Carcinogenicity in Animals

Studies in rats and mice indicate that acrylamide is an animal carcinogen [Bull et al. 1984a, 1984b; Johnson et al. 1986]. Acrylamide was tested for skin-tumor-initiating activity in Sencar mice and for the ability to induce lung adenomas in A/J mice [Bull et al. 1984a]. In a mouse skin initiation-promotion assay, 6- to 8-week old female Sencar mice were divided into groups of 40 and treated topically, by gavage, or by i.p. injection with acrylamide dissolved in water. Acrylamide was tested at doses of 12.5, 25, and 50 mg/kg for six applications by the three routes over a 2-week period, resulting in total doses of 75, 150, and 300 mg/kg. Ethyl carbamate, a known initiator, was used for a comparison in doses of 30, 100, and 300 mg/kg in one application administered topically, by gavage, or i.p. A tumor promotion regimen was started 2 weeks after the last of the tumor-initiating doses of acrylamide. One microgram of 12-*o*-tetradecanoyl-phorbol-13-acetate (TPA), a known tumor promoter, was applied topically 3 times/week for 20 weeks. Tumor incidences were charted from weekly observation. Surviving animals were sacrificed at 52 weeks and histopathological evaluations were done on all gross lesions. A highly significant ($p < 0.01$) dose-response relationship existed for time to first tumor and for the total number of tumors in treated animals. The effect was more pronounced with i.p. and gavage treatment than with topical application. However, without application of TPA promotion, no increase occurred in tumor yield, either with acrylamide or ethyl carbamate treatment. In this assay system, acrylamide was therefore confirmed as being a tumor initiator.

In a bioassay for lung adenoma in mice, groups of 40 male and 40 female, 8-week-old A/J mice received doses of 6.25, 12.5, or 25 mg/kg of acrylamide by gavage in an aqueous solution three times/week for 8 weeks [Bull et al. 1984a]. Animals were sacrificed at 7 months of age. Acrylamide increased the yield of lung adenomas in both sexes in a dose-related manner; the average number of tumors per animal increased from 0.2 to 1.4. The dose-response relationship was statistically significant ($p < 0.01$) when both the number of animals with tumors and the multiplicity of tumors in each dose group were tested using logit regression model analysis. In the same investigation, groups of 16 male and 16 female, 8-week-old A/J mice received i.p. injections of 1, 3, 10, 30, or 60 mg/kg acrylamide three times/week for 8 weeks. The average number of tumors per animal increased from 0.4 to 2.2. The number of lung adenomas increased with the dose up to 30 mg/kg. The dose-response relationship was statistically significant ($p < 0.01$) by the logit regression model. Because acrylamide increased the lung tumor yield by two routes of administration in the absence of a promoter, the authors concluded that acrylamide acted as a complete carcinogen in this test system.

In a subsequent study [Bull et al. 1984b], groups of 40 female Swiss-ICR mice were administered an oral dose of 0, 12.5, 25, or 50 mg/kg acrylamide six times in a 2-week period. Ethyl carbamate served as a positive control and was administered in the same way at concentrations of 50 mg/kg. Two weeks after the last dose, a tumor promotion regimen was started. The regimen consisted of 3 weekly applications of 2.5 μg TPA dissolved in acetone to the shaved back of each animal. Another group of mice treated with 50 mg/kg acrylamide did not receive promotion treatment. The appearance of skin tumors was observed weekly, and after 52 weeks of study, the surviving animals were sacrificed and evaluated histologically for skin and lung tumors. When TPA treatment followed, acrylamide produced a dose-related increase in the number of animals that bore skin tumors (33% in the high-dose group versus none in the control group). Without TPA treatment there was no significant increase. The number of lung tumors (adenoma and carcinoma) was increased by acrylamide treatment in a dose-related manner (4 tumors in the control group and 11 tumors in the 50-mg/kg group). This increase was also observed in the absence of TPA (14 tumors

Acrylamide

in the 50 mg/kg group). This study confirms the initiating effects of acrylamide on the skin of Sencar mice and the carcinogenic effects of acrylamide on the lungs of A/J mice.

Acrylamide administered in drinking water to female and male F344 rats for 2 years caused a statistically significant increase in the incidence of benign and malignant tumors at several sites in both sexes [Johnson et al. 1986]. Groups of 90 male and 90 female rats were maintained on drinking water providing acrylamide intakes of 0, 0.01, 0.1, 0.5, or 2.0 mg/kg per day. Ten rats per sex per treatment group were randomly selected for examination after 6, 12, or 18 months of study. Cumulative mortality showed no apparent dose relationship until the 21st month of study. From that time to the conclusion of the study at 24 months, mortality increased in the group receiving 2.0 mg/kg per day. No overt signs of neurotoxicity or other effects were attributable to acrylamide treatment. Rats in the group receiving the highest dose had an increased number of palpable masses, subsequently identified as subcutaneous or skin tumors. The number of tumors by site is listed in Table 5. In females, tumor incidence increased in the mammary glands, CNS, thyroid gland follicular epithelium, oral tissues, uterus, and clitoral gland. In males, tumor incidence increased in the CNS, the thyroid gland follicular epithelium, and the scrotal mesothelium. These increases occurred in the group receiving the highest dose (2.0 mg/kg per day). The only statistically significant increases ($p < 0.05$) at the 0.5-mg/kg per day dose were the incidence of scrotal mesotheliomas in male rats and the incidence of combined mammary and clitoral gland tumors in female rats [Johnson et al. 1986].

Table 5. Pooled tumor incidence data in F344 rats*

Rat	Dose of acrylamide (mg/kg per day)				
	0.0	0.01	0.1	0.5	2.0
Males:					
Number of animals with tumors ⁺ (testes, thyroid, adrenal [§])	7/57	8/53	13/57	14/53	22/54
Number of animals with malignant tumors (testes)	3/57	0/53	7/57	11/53	10/54
Females:					
Number of animals with tumors ⁺ (thyroid, mammary, CNS ^{**} , oral, uterus)	13/60	18/60	14/60	21/60	46/60
Number of animals with malignant tumors (thyroid, mammary, CNS, uterus)	4/60	5/60	3/60	4/60	20/60

* Adapted from Johnson et al. [1986] study of rats exposed to acrylamide in drinking water for 24 months.

⁺ Benign or malignant. Site must be statistically significant at the high dose (treated versus control) for tumors to be considered.

[§] Includes only adrenal benign tumors.

^{**} Tumors only (no "proliferations").