III. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

Acrylamide ($CH_2=CHCONH_2$), formula weight 71.08, is a white, crystalline solid which is assuming increasing industrial importance as a chemical intermediate and as a vinyl monomer that readily undergoes polymerization and copolymerization [1]. Acrylamide is highly soluble in water and is also moderately soluble in several other common solvents such as methanol, ethanol, and acetone. It is thermally stable, has a vapor pressure of 0.007 mmHg at 25 C, and sublimes at room temperature [1]. Some of the more important physical and chemical properties of acrylamide are shown in Table XII-1 [1]. Synonyms for acrylamide include propenamide, acrylic amide, and akrylamid [2]. Mixtures of acrylamide with small proportions of N,N'-methylenebisacrylamide have been marketed by American Cyanamid Company under the trademark AM-9 [3].

The acrylamide molecule consists of an amide and a vinyl group. It can undergo reactions both at the amide group and at the double bond (vinyl group) [1]. The double bond of the vinyl group can add halogens. The addition of bromine was the basis of a popular method of acrylamide analysis before gas-chromatographic and polarographic methods were used. Hydrolysis at the amide group with either acids or bases converts acrylamide to acrylic acid. Acrylamide molecules undergo homopolymerization and copolymerization by combining with anions in photochemical reactions; by exposure to ionizing radiation; and, lastly, in the most popular and commercially useful method, by the use of free radical

initiators of redox catalytic systems [1,4]. Molten acrylamide polymerizes vigorously with the evolution of heat [5].

Acrylonitrile is the major starting material used in all industrial methods for the manufacture of acrylamide [1]. The starting material is mixed with sulfuric acid, an exothermic reaction, and then diluted with water. Acrylamide is then prepared from acrylamide sulfate either by the lime process, ammonia process, carbonate process, or ion-exchange process. Acrylamide is difficult to recover at the aqueous stage since it may polymerize or undergo hydrolysis. Various processes have been devised by manufacturers to facilitate the recovery and to control the amount of heat generated in the procedure. Recently, a few manufacturers have developed pollution- and byproduct-free processes for direct production of acrylamide monomer via hydration of acrylonitrile over a catalyst [6].

Acrylamide monomer production has been estimated to have amounted to about 15-20 million pounds in 1966, 30 million in 1969, 32 million in 1972, 40 million in 1973, and approximately 70 million in 1974 [6]. During the past 20 years, the use of acrylamide polymers has also increased very rapidly with the increased production of acrylamide monomer [4]. In 1972, about 35 million pounds of acrylamide polymers were used in the United States alone. These are the latest years for which data are available.

The major use of acrylamide monomer is in the production of polymers [1]. Aqueous solutions of the monomers and a redox catalyst are used for soil stabilization. Polyacrylamides are very effective flocculants for finely divided solids in aqueous suspensions. AM-9 has found increasing application as a chemical grout. The largest market for acrylamide now is in the manufacture of polyacrylamides for waste and water treatment

flocculants, in products for aiding sewage dewatering, and in a variety of products for the water treatment industry. These uses consumed more than 40% of the acrylamide used in 1973 [6]. Acrylamide is also used to prepare polyacrylamides, which are used as strengtheners in papermaking and retention aids (to keep the fibers from being washed away). The pulp and paper industry accounted for about 20% of the acrylamide consumption in 1973. Some other uses of polyacrylamides are drilling-mud additives, textile treatment, and surface coatings. In very small quantities, acrylamide polymers are also used for flocculation of ores [4,7,8], mine tailings and coal, friction reduction [4], thickening [4,9], soil stabilization, gel chromatography and electrophoresis, photography, fog dissipation, breaking of oil-in-water emulsions, dyeing, ceramics, and clarification and treatment of potable water and foods [4].

Several other uses for monomeric acrylamide have been proposed by various investigators. Compounds such as N,N'-ethylene-bis-acrylamide and some bromo combinations have shown promise as antitumor agents in mice [10], in tomato plant tumors [11], and in plant tissue cultures [12].

NIOSH estimates that approximately 20,000 workers are potentially exposed to acrylamide in the United States. Table XII-2 [3] is a list of occupations with potential exposure to acrylamide monomer.

Historical Reports

Monomeric acrylamide was first made in Germany in 1893 and patented in the United States (Patent No. 2,021,763) by the Rohm and Haas Company in 1935. Interest was not shown in its preparation and properties until acrylonitrile became commercially available in 1940 [1]. It was first

offered by American Cyanamid Company for developmental consideration in 1952, and they began manufacture for the commercial market in 1954.

It was not until the advent of large-scale commercial production that some pharmacologic and toxicologic experiments were initiated by American Cyanamid Company at Hazleton Laboratories. After single large oral doses, death occurred as a consequence of convulsions and asphyxia in mice, rats, rabbits, and dogs. However, after repeated administration of acrylamide, a neurologic syndrome was characterized by postural and motor incoordination in these animals. The single-dose toxicity of monomeric acrylamide in animals was also reported by Druckrey et al [13] in 1953. The so-called average lethal dose by intraperitoneal (ip) injection was reported to be 120 mg/kg in rats which died within 1 or 2 days with severe pulmonary obstruction. A toxicologic study reported by Hamblin [14] in 1956 showed that the oral administration of acrylamide monomer produced neuropathy in mice, rats, and dogs.

Effects on Humans

In 1953, a new method of acrylamide production was undertaken at an American Cyanamid Company manufacturing plant [15] where acrylonitrile was hydrated by sulfuric acid to form acrylamide sulfate after which it was neutralized by ammonia, yielding free acrylamide. About 5 months after the new process was begun, a "handful" of the hundreds of potentially exposed plant workers noticed numbness and tingling of their hands and weakness of their hands and legs. Dermal contact of the workers was thought to have been limited because they wore protective clothing and gloves. The air in the plant was sampled and only traces of acrylamide were found. However,

by the use of methods (not described) of detection then in use, it was calculated that the maximum amount of acrylamide which could have been inhaled by one worker in a 5-month period was approximately 1.8 mg/kg. The whole manufacturing process was altered and the exposure of the workers to acrylamide was reduced or eliminated. Further details were not presented.

A total of 45 cases of intoxication from acrylamide have been reported in humans [16-22, DR Brinkley, written communication, June 1976] and up to 10 more have been suggested [15,16,19,20,23]. Of the 45, 3 were women (ages 17, 40, and 65), 2 children (a boy of 10 and a girl of 13), and 40 men (18-59 years old). All of the exposures were occupationally related, excepting a Japanese family of five who ingested and briefly used contaminated well water for bathing [22]. Monomeric acrylamide is a neurotoxin with an affinity for the peripheral ends of the spinal nerves in the extremities. Both motor and sensory nerves are affected but the sensory component usually more than the motor component. In some instances there was evidence of CNS involvement [20-22].

A pattern of reactions emerged when signs, symptoms, and results of neurologic examinations of the workers were compiled and compared (see Table III-1). Early reported symptoms typical of acrylamide intoxication in humans include, numbness of lower limbs [16,18,20-23], tingling of the fingers [18-22], tenderness to the touch [16,18], coldness [16,18], excessive sweating of feet and hands [16-21], bluish-red skin [16,20,21], peeling of the skin of the hands and less often of the feet [16-21], followed shortly by muscle weakness of the hands and feet (occasionally progressing to the inability to write or lift the feet when walking or climbing stairs) [16-23]. Later symptoms were loss of weight [17-20,23],

lassitude [16,18,21], sleepiness [20,22], and complete collapse (which occurred in two people after drinking alcoholic beverages) [20]. Still later, emotional changes [19,21-23] and finally, reactions typical of overt peripheral neuropathy, positive Romberg's sign [17,19-21,23,24], loss of vibration and position senses [17,19-21,23], weak or absent tendon reflexes such as the knee jerk [16-18,20-23], ataxic gait [16,20-23], foot drop [17,20,23], muscular atrophy (usually in the hand or thumb), and occasionally urinary and fecal retention [20,22] were observed. When patients were rested, it was found that conduction velocity was decreased in motor or sensory nerves or in both [21-23,25].

In hospitalized workers, clinical laboratory tests of blood, urine, cerebrospinal fluid (CSF), liver and kidney function, and electroencephalography were done [16,20,23]. Examination of the cardiovascular system, bones, and joints showed no pertinent deviations from normal [16,20,23]. These workers were only given symptomatic and supportive therapy. The length of time required for recovery, which was a few days to 2 years, was proportional to the severity of the reactions [16-21,23]. Only one affected worker [16] was reported to have returned to his occupation without further illness.

Some of the incidents of acrylamide intoxication which occurred in Japan were reported by Fujita et al [23]. They described in considerable detail the signs and symptoms which resulted from acrylamide exposure in 10 workers in one factory. Nine men and one woman ranging in age from 17 to 43 years were exposed at a pilot plant where manufacturing procedures were being developed. The length of employment in that plant varied from 3 to 12 months before signs and symptoms appeared. All of the workers had most

of the typical signs and symptoms of acrylamide intoxication relating more to the legs than to the arms. Of the three workers who were hospitalized because they were the most severly affected, one had been employed for 12 months and the other two for 3 months in their present jobs. Those three workers had, in addition to the typical reactions (tremor and numbness of the hands and feet, dizziness, loss of the knee jerk, heavy feeling of the legs, staggering, and positive Romberg's sign), emotional changes which were also somewhat similar to, but very much less severe than those of the family reported by Igisu et al [22], which is described in detail later. The symptoms were attributed to the presence of the peripheral neuropathy and the desquamation and sensitivity of the soles of the feet, which may also have been in contact with acrylamide. Fujita et al [23] also found sufficient signs and symptoms to postulate that the CNS and probably the cerebellum, in addition to peripheral nerves, were involved in acrylamide intoxication. All 10 workers improved during about 4 months of rest and supportive treatment.

Takahashi et al [21] described the reactions of 13 factory and 2 laboratory workers who were exposed to acrylamide from 2 months to 8 years. All of the workers were males aged 18-32 years. They were exposed during the polymerization of the monomer in the manufacture of papercoating materials. The described reactions conformed to the typical ones (numbness of lower limbs, ataxia, dizziness, gastrointestinal upset, and hand peeling). The authors [21] concluded that, although peripheral neuropathy was one of the most important effects in the patients, a few CNS effects also may have been present. When the work environment was changed to limit or prevent contact with acrylamide, the workers gradually recovered;

however, the authors [21] did not describe the controls used. Takahashi et al [21] also performed special studies in which motor and sensory nerve conduction velocities and action potentials were determined in some of the peripheral nerves in arms and legs of the affected workers. The motor nerves tested had essentially normal reactions, whereas the sensory nerves had decreased action potential amplitude. The authors [21] suggested that the defect in the action potential would precede decreases in conduction velocity and that this indicated sensory nerve injury.

Garland and Patterson [20] described six cases of acrylamide intoxication in workers in three factories in Great Britain where acrylamide flocculants were produced. The workers, all men and aged 19-59 years, had worked in the factories for periods varying from 1 to over 12 months. Although limited details of the medical histories and examinations were reported, the authors [20] suggested that what was recorded agreed with some of the signs and symptoms of the typical reactions (increased sweating of feet and hands, fatigue, muscle weakness and pain, hand peeling, sensory loss, and positive Romberg's sign). The authors stated that all of the men recovered after they were removed from exposure, although it took from 2 to 12 months. Garland and Patterson [20] interpreted that, because of the sleepiness of the men, the midbrain, as well as peripheral nerves, was involved. Fullerton [25] studied nerve conduction velocities in three of the patients reported by Garland and Patterson [20] while they were recovering from the ill effects of exposure. Maximal motor nerve conduction in the distal ends of median and ulnar nerves was found to be normal or slightly reduced and response to nerve stimulation in small muscles was dispersed irregularly. Fullerton

suggested that those changes were caused by degeneration and regeneration of the distal nerves (nerve endings near muscles). The action potentials of the sensory nerves were also decreased or absent; the author [25] indicated that the peripheral sensory nerves had been more severely damaged than their associated motor nerves. In addition to the determination of the neurophysiologic phenomena, Fullerton [25] microscopically examined biopsy specimens of nerves from the calf muscles from two of the three patients. The author [25] concluded that simultaneous nerve degeneration and regeneration occurred immediately after, and probably during acrylamide exposure, and that most probably nerve function was impaired before structural changes were evident.

Auld and Bedwell [16] in 1967 described in detail a mine worker's reaction to acrylamide exposure. The worker, a 21-year-old man, introduced a 10% aqueous solution of acrylamide monomer and the catalysts, 2-dimethylaminoproprionitrile (DMAPN) and ammonium persulfate, into holes previously drilled into the walls of a mine. He also loaded hoppers with the acrylamide solution. He did not avoid contact with the chemicals, which often splashed onto his hands and face. After about 2 weeks on the job, he noticed a red rash on his forearms. About 5 weeks after the rash began, he complained of leg weakness and soon after, of hand weakness. He also stumbled when walking and had difficulty climbing stairs, writing, and handling eating utensils. In about 2 more weeks, he noticed blueness and both a sensation of cold and profuse sweating of his arms, hands and fingers, as well as his lower legs, feet, and toes. He reported that his hands and feet felt "numb and tender when touched." With increasing weakness, stumbling gait, and excessive sweating, he was hospitalized 14

weeks after his first exposure to acrylamide. A general physical and neurologic examination corroborated his complaints, and the physician found, in addition, impairment of temperature, position and vibration senses, and absence of tendon reflexes (knee jerk, etc). His forearm and lower leg muscles were weak, and he was unsteady when standing or walking. Laboratory (clinical chemistry, hematology, urinalysis, and CSF) tests were performed, the results of which were within normal limits, except for elevated CSF protein which had decreased substantially at a second determination 2 weeks later. Therefore, the authors judged it was not significant. Removal from exposure, supportive therapy, and rest resulted in gradual and partial recovery in 6 weeks and in functionally complete recovery 14 weeks after hospitalization. The authors [16] stated that, because of a predisposition to the effects of acrylamide as described by Stokinger [26], the patient was strongly advised to avoid further contact with acrylamide. A coworker who had been simultaneously exposed to acrylamide had mild symptoms which disappeared spontaneously.

Two other incidents of acrylamide exposure similar to the case reported by Auld and Bedwell [16] occurred in France and were described by Graveleau et al [17] and Cavigneaux and Cabasson [18]. Exposure occurred while two workers were introducing monomeric acrylamide and catalyst into underground drilling operations. Signs and symptoms and results of neurologic examinations in both workers were typical of the described responses (numbness of the hands and feet, excessive sweating of the limbs, desquamation of the hands, and positive Romberg's sign). Morviller [19] described four cases of acrylamide exposure and implied that there were two more workers who were exposed to acrylamide and acrylonitrile during a

manufacturing project in a pilot plant. Adverse effects were similar to the typical effects (excessive sweating, desquamation of the hands, fatigue, weight loss, confusion, loss of reflexes, and positive Romberg's sign) of acrylamide intoxication. The author [19] also stated that the effects of acrylonitrile exposure were typical of those produced by acrylamide and that exposure for an undefined length of time to acrylonitrile had not resulted in similar adverse reactions in workers.

Igisu et al [22] reported five members of a Japanese family who were exposed to acrylamide through ingestion and external use of well water evidently contaminated by seepage from a waste system grouting operation. They began to show symptoms about 4 weeks after the grouting was done. Ten days after the onset of the symptoms, the well water was tested and found to contain 400 ppm of acrylamide and a trace of DMAPN. The mother, father, and grandmother were hospitalized, with strikingly similar symptoms 4-5 weeks after the well was grouted. They experienced marked rhinorrhea, coughing, dizziness, and irrational behavior. Mental changes consisting of poor orientation and memory, confusion, and severe hallucinations preceding unsteadiness in walking, sleepiness, and slurred speech occurred. General physical and neurologic examinations showed normal or hyperactive reflexes, normal cranial nerve reactions, ataxic gait (severe in both women), urinary and fecal retention (mother only), and ecchymoses of the extremities of both women. Electroencephalography showed excessive sleepiness patterns, but all other clinical laboratory tests performed were in the normal range. The only complaints referable to the skin, such as loss of touch, pain and sense of vibration, and a feeling of numbness in the extremities were made 2, 3, and 4 weeks after hospitalization by the mother, father, and

grandmother, respectively. They had mild dysesthesias of the fingers and feet. During the hospitalization period, the mother had no deep-tendon reflex response, and all three patients had slowed sensory but normal motor nerve conduction velocities. The two children of the family, who were away at school all day and presumably did not consume as much well water as the adults, had very mild sleep and gait disturbances (the boy) and mild personality changes (the girl). Both children recovered within 2 weeks. The father and grandmother recovered completely in 2 months and the mother in 4 months after hospitalization.

The Vistron Corporation (DR Brinkley, written communication, June 1976) supplied NIOSH with information concerning airborne acrylamide concentrations and occupational exposures. Eight-hour acrylamide samples were collected daily from stationary sites in the plant using an aqueous impinger technique and subsequently analyzed by a colorimetric procedure using a ferric chloride reagent. Limited personal monitoring was conducted. The stationary air monitoring site data were presented as weekly averages and ranged from 0.1 to 0.4 mg/cu m for the control room, 0.1 to 0.9 mg/cu m for the bagging room, and 0.1 to 0.4 mg/cu m for the second-floor processing area. Personal monitoring data showed that daily 8-hour average employee exposures ranged from a low of 0.1 to as high as 2.3 mg/cu m when stationary sites data taken on the same day showed concentrations ranging from 0.1 to only 0.3 mg/cu m.

Brinkley (written communication, June 1976) stated that attempts were made to maintain minimum employee contact with acrylamide by the installation of engineering controls and additional ventilation equipment with air-circulating fans. Emphasis was placed on personal hygiene as an

important factor in the prevention of acrylamide intoxication. Personal protective equipment, such as cartridge type respirators and dust masks and protective clothing, such as coveralls, safety glasses, caps, and gloves, were also provided. Despite these precautions, two employees experienced neurologic symptoms in May 1974. Prior to this date, no neurologic symptoms except finger tingling were noted in any employee. The initial symptoms of erythema and skin peeling were noted in almost every employee who was working in the acrylamide plant. If erythema or skin peeling or any of the following acrylamide-associated symptoms, such as increased sweating of feet and hands, muscular weakness or pain, abnormal skin sensations, sensory loss, absent reflexes, positive Romberg's sign, persisted after removal of the worker from the working environment, the employee would be transferred outside of the acrylamide unit. According to Brinkley, a statistical analysis of the stationary site data and the skin check records has suggested that the incidence of skin reactions could, at least to some degree, be explained by airborne concentrations of acrylamide. Because of the limited personal monitoring data, airborne acrylamide concentrations to which workers were actually exposed could not be correlated with skin reactions.

Epidemiologic Studies

No reports concerning epidemiologic studies of acrylamide monomer were found in the published literature.

Animal Toxicity

(a) Oral Studies

Hamblin [14] reported that the single-dose oral LD50 for male albino mice given acrylamide as a 2 or 5% aqueous solution was 170 (130-220, 95% confidence limits) mg/kg. Toxic signs consisted of slight tremors, convulsions, labored respiration, and ataxia. The single-dose oral LD50 determined by Fullerton and Barnes [27] in 8-week-old female albino rats was 203 (166-249) mg/kg. The duration of the observation period was not specified in either of these studies. In a study by Keeler et al [28], the single-dose oral LD50 for acrylamide was 240 (184-316) mg/kg in female rats and 277 mg/kg in male rats (95% confidence limits not calculable). No information on rat strain, age, weight, or observation period was provided.

In a 1956 study reported by Hamblin [14], repeated oral administration of acrylamide, 50 or 100 mg/kg/day, by stomach tube to albino rats produced prostration and death. The 100-mg/kg/day level killed all six animals after three daily doses. The 50-mg/kg/day dose caused loss of weight, depression, and marked weakness of the extremities; death occurred within 5 days after the last (15th) daily dose. Barnes [29] obtained qualitatively similar results in adult Porton-strain albino rats. Fullerton and Barnes [27] also observed that acrylamide administered to rats at a single dose of 100 mg/kg by stomach tube produced only fine tremors, but when repeated in 24 hours killed most of the animals within 3 days. General weakness was also observed in the dying rats. When intubated with 50-mg/kg doses, 12 times over a 15-day period, all rats (number not specified) developed severe weakness and died within a few days after the final dose. At necropsy, many rats (both males and females) had

gross distention of the bladder. Ten-week-old rats given acrylamide orally at a dose of 25 mg/kg/day, 5 days/week, developed the first signs of weakness after the fourth week. A dose of 10 mg/kg/day, 5 days/week, given for about 5 months, produced no signs of toxicity in six female rats.

Fullerton and Barnes [27] also measured motor nerve conduction velocity in the fibers supplying the small muscles on the plantar surface of the hind paws in Porton-strain albino rats. Nerve conduction velocity in control animals was 56 (SD 5.8) meters/second compared with 44 (SD 2.2) meters/second in the 11 of the 15 rats fed acrylamide in their diets (200 ppm for 6 months or 400 ppm for 2-3 months) and showed severe neurologic abnormalities of the hindlimbs. The conduction velocities were normal in the remaining four rats recovering from the severe leg weakness.

The influence of age on acrylamide-induced leg weakness was investigated by Fullerton and Barnes [27] in groups of six rats (sex not specified) aged 5, 8, 26, and 52 weeks and fed 100 mg/kg of acrylamide at weekly intervals. After four doses, the youngest animals were only mildly affected, whereas those aged 26 weeks at the start of study were severely affected. The 52-week-old rats were severely affected after only three doses. The authors stated that, when acrylamide feeding was discontinued, the recovery in young animals, which had shown weakness for only a few weeks, was rapid and complete. For the older rats, in which weakness had been present for months, recovery was slow, and mild ataxia continued for some months.

The effects of orally administered acrylamide on dogs were briefly reported by Hamblin [14] in 1956, but recently have been studied in more detail by Thomann et al [30]. In the earlier investigation [14], groups of

two dogs each were given acrylamide at doses of 1 mg/kg/day for 19 weeks, 5 mg/kg/day for 5 weeks, or 8 mg/kg/day for 4 weeks without overt signs of toxicity. However, a dose of 10 mg/kg/day for 4-5 weeks produced incoordination, weakness of the extremities, and impaired righting reflex. A single dose of 100 mg/kg produced these same effects in 24 hours. In the 1974 report by Thomann et al [30], experiments were performed on 6- to 12-month-old beagles. The dogs were given acrylamide orally in gelatin capsules in daily doses of 5 or 15 mg/kg. The first group (three males and three females) received 5 mg/kg/day for 60 days; the second group (five males and five females) received 15 mg/kg/day for 22 days. After the first 3 weeks of the experiment, the dogs in the 5-mg/kg/day group were inactive and had muscular weakness, which was particularly noticeable in the jaw muscles. In addition to muscular weakness, the animals in the 15-mg/kg/day group had dilated pupils, conjunctivitis, salivation, difficulty in breathing, muscular stiffness of the hindlegs, muscle twitching, head shaking, and convulsions. One female beagle in the latter group (15 mg/kg) died on day 24, 2 days after the end of treatment. Microscopic examination of peripheral nerves from one of the surviving dogs showed swelling of the myelin sheaths and fragmentation of the axons. In general, the distal branches of the sciatic nerve were more severely involved than were the proximal parts. Occasional swelling and fragmentation of nerve fibers of the distal branches were found in three of the six dogs in the 5-mg/kg group after 60 days of treatment. The authors [30] reported no structural alterations in the white or gray matter of the lumbar spinal cord on microscopic examination.

Hopkins [31] studied the effects of acrylamide administered to five female and two male baboons (Papio hamadryas). The female baboons weighed between 12.1 and 15.4 kg and the two males weighed 9.4 and 10.3 kg. Acrylamide was administered in oranges or bananas as a 10% aqueous solution. Because the dosage schedule for one of the seven baboons was questionable, the data for this animal are excluded from the discussion that follows. According to Hopkins [31], this particular baboon was reluctant to eat all of the fruit into which the acrylamide had been injected. Of the remaining six baboons, one was treated with 20 mg/kg/day for 29 days, another with 15 mg/kg/day for 94 days, and four with 10 mg/kg/day for an average of 110 (89-137) days. The cumulative doses received by the baboons were thus 580, 1,410, and about 1,100 mg/kg, respectively. Incoordination and weakness of the hindquarters were first noticed on the 16th day in the 20-mg/kg/day animal, on the 42d day in the 15-mg/kg/day animal, and on an average of the 62d (42-82) day in the 10-mg/kg/day animals. Forelimb weakness first occurred on the 28th, 73d, and 82d (61-96) days, respectively. Recovery began within 2-12 days after feeding was discontinued. The baboon which received a total of 580 mg/kg recovered completely in 62 days; those which received an average of 1,100 mg/kg recovered in 110 days, and the baboon which received a total of 1,410 mg/kg recovered in 270 days. The author [31] concluded that the onset of limbs weakness and the progression of recovery were dose dependent.

The peripheral nerves from a baboon given acrylamide at 10 mg/kg/day for 118 days and biopsied 19 days after the last treatment were examined by light microscopy [31]. The main pathologic changes were of the Wallerian degenerative type (degeneration of a nerve fiber which has been severed

from its nutritive centers). Microscopic examination of transverse sections of nerves indicated that both motor and sensory nerves were affected and that there was a marked reduction in the number of myelinated fibers in the nerves. The author [31] reported that inspection of moderately affected nerves suggested the involvement of large-diameter myelinated fibers. The proportion of such myelinated fibers decreased with an increase in the duration of exposure. At necropsy, it was observed that long nerves to muscles were more severely affected than short nerves. Nerve fibers to the extremities were affected only in their distal parts, the proximal sciatic nerve and spinal roots remaining normal in baboons which showed a severe loss of myelinated fibers in nerves to muscles of the feet.

Hopkins and Gilliatt [32] examined nerve conduction velocities and muscle action potentials of baboons treated with acrylamide. It appears that this study reports additional results from the experiment described above by Hopkins [31]. Conduction velocity and ascending nerve action potentials were measured in the median and anterior tibial nerves of the left limbs; all specimens for microscopic examination were taken from the right limbs. Muscle action potentials were recorded from the abductor pollicis brevis and extensor digitorum brevis. At acrylamide doses of 10 or 15 mg/kg/day, the gradual development of limb weakness in the baboons was accompanied by a progressive reduction in the amplitudes of muscle and nerve action potentials. In the baboon receiving acrylamide at 15 mg/kg/day, there was a progressive decline in amplitudes of muscle and nerve action potentials with an increase in latency period. Acrylamide administration was stopped on day 94; however, the amplitudes of action

potentials did not return to normal until 1 year later. The percentage reduction in the amplitude of the action potential was greater in the muscle of the foot than in that of the hand. These results supported the clinical observation that the disease was usually more severe in the lower than in the upper limbs. Reductions in nerve conduction velocities and nerve action potential amplitudes were also observed in baboons treated with acrylamide at 10 mg/kg/day for an average of 110 days and measured within 3 weeks after the end of treatment. The percentage decreases in both velocity and amplitude were greater for sensory than for motor conduction in the median nerve. This deterioration sometimes continued for several weeks after acrylamide was discontinued.

Hopkins and Gilliatt [32] also studied the return of conduction velocity and action potential amplitude values to normal in one baboon treated with acrylamide at a dose of 15 mg/kg/day for 94 days and in two baboons given 10 mg/kg/day for 89 and 115 days. The baboon receiving 15 mg/kg/day was severely affected and, while the amplitude of the nerve action potential was little changed until the end of the first year, it had returned to 80% normal by the end of the second year. Baboons receiving acrylamide at 10 mg/kg/day were less severely affected and the amplitude of the muscle action potential began to return to normal within 2-3 months. Hopkins and Gilliatt [32] also observed that a baboon treated with acrylamide at 20 mg/kg/day for 29 days remained severely paralyzed for 2 days after treatment was stopped. Yet, the maximal conduction velocity in all the nerves examined was within the normal range and the action potential amplitude was only slightly reduced. The motor nerve conduction was normal at the same time that the animal was severely paralyzed

suggested to these workers [32] that damage to the CNS also had occurred.

Bradley and Asbury [33] studied the effects of acrylamide added to the drinking water at a concentration of 250 ppm on 26 young adult female mice of BALB c Gn/J X SJL/J and SJL/J strains. Two mice from each strain were used as controls. Animals drank the treated water ad libitum for 45 days. Pairs of animals were selected at random and killed at 23, 30, 35, 40, 45, 50, 55, 60, 90, 120, or 165 days after the first exposure. The brain, spinal cord, sciatic nerve, and hamstring and gastrocnemius muscles of the mice were examined microscopically. No animals died during treatment with acrylamide at this dose. The first observed abnormality, difficulty in grasping and walking along the edge of the cage, appeared after 20 days of acrylamide administration, and by day 25, all animals either appeared unaware of the position of their hindlimbs or dragged their feet when walking. After 35 days, the mice had lost some weight and hair. Within 5 days of the withdrawal of acrylamide on day 45, the mice had gained some weight and their gait had improved. However, some signs of acrylamide poisoning persisted even 20 days after the withdrawal of acrylamide, as measured by the position of the lower limbs. Degenerating myelinated fibers were occasionally present in the sciatic nerve of mice killed 23 days after commencement of acrylamide and the proportion of such fibers increased with the duration of treatment. Multiple sections of the spinal cord showed no differences between acrylamide-treated and control mice. The results of microscopic studies of brain tissue were not mentioned. The hamstring and gastrocnemius muscles appeared wasted, although microscopic examination revealed only a slight increase in the number of areas of segmental necrosis and regeneration when compared with

control mice.

Hamblin [14] briefly described the effects on the growth of albino rats given acrylamide in the diet (10, 50, 100, or 300 ppm). No effects were reported at the 10- and 50-ppm levels. Diets containing 100 and 300 ppm of acrylamide produced growth retardation within 6 and 4 weeks, respectively.

Fullerton and Barnes [27] studied the effects of acrylamide on 6- to 8-week-old male albino rats. The animals were fed diets containing 100, 200, 300, or 400 ppm of acrylamide. According to the authors [27], these represented daily intakes of about 6-9, 10-14, 15-18, or 20-30 mg/kg, respectively. Control rats received a similar diet without acrylamide. Rats on the acrylamide diets developed slight leg weakness as follows: at 400 ppm after 3 weeks, at 300 ppm after 4 weeks, at 200 ppm after 12 weeks, and at 100 ppm after 40 weeks. Severe leg weakness developed in all except the 100-ppm rats on continuation of dietary treatment; the slight leg weakness observed at week 40 did not increase during the remaining 8 weeks of the experiment. The only macroscopic findings at necropsy were wasting of the hindlimb musculature and distended urinary bladders in all rats. Axons and myelin sheaths of the sciatic, tibial, median, and ulnar nerves examined microscopically at necropsy showed extensive degeneration in the peripheral nerves of all the clinically affected animals. Microscopic examination of kidney, spleen, pancreas, adrenal, lung, brain, and spinal cord tissues showed no abnormalities.

McCollister et al [34] studied the effects of acrylamide given at low concentrations in the diet. Groups of 10 male and 10 female 8-week-old rats of the Dow Wistar strain were maintained on diets containing 3, 9, or

30 ppm acrylamide for 90 days, and then killed and necropsied. As judged by their appearance, behavior, growth, mortality, organ weights, and microscopic examination of tissues, there was no evidence of adverse effects in either male or female rats. No signs of neurotoxicity were seen in two other groups of animals maintained on diets containing either 70 or 110 ppm of acrylamide for 189 days. In the same experiment, McCollister et al [34] also studied the effects on male and female rats of acrylamide given at high concentrations in the diet. At a 300-ppm acrylamide dietary level, the rats began to show loss of control of the hindquarters after 21 days. By day 42, all 10 males and 6 of 10 females were dead. Loss of hindquarter control was seen at 14 days in rats maintained at a diet containing 400 ppm of acrylamide. According to the authors [34], doses of 3, 9, 30, 70, 90, 110, 300, and 400 ppm of acrylamide in the diet were equivalent to 0.3, 0.9, 3, 7, 9, 11, 30, and 40 mg/kg/day, respectively.

In a 1-year feeding study on cats by McCollister et al [34], different concentrations of acrylamide were mixed with feed to deliver total calculated daily doses of 0.03, 0.1, 0.3, 1, 3, or 10 mg/kg. The acrylamide diets were fed to two cats for 5 days/week at each dose. Cats fed 10 mg/kg/day developed signs of neurotoxicity, definite weakness in the hindquarters by day 26, and were unable to stand by day 52. One of the two cats was then killed; the other, taken off the acrylamide diet, recovered completely 53 days later. The two cats receiving acrylamide at 3 mg/kg for 1 year survived to the end of treatment, but started showing twitching motions of the hindquarters by 26 days and signs of hindleg weakness by 68 days; the latter persisted for a further period of 299 days. One of two cats receiving the 1-mg/kg dose for 1 year showed some twitching in its

hindquarters by 26 days and signs of hindleg stretching when walking by 240 days. In the remaining three dosage groups (0.3, 0.1, and 0.03 mg/kg), five of the six cats died by day 106 from disease not attributable to acrylamide [34]. No toxic effects were seen in the only surviving cat (0.3 mg/kg) at the end of the study. Hematologic tests (not specified), blood clotting times, and blood cholinesterase activities in cats treated at 3, 1, or 0.3 mg/kg/day were not affected. Microscopic examination showed no evidence of adverse effects on central nervous tissues (brain and spinal cord) in any of the animals receiving acrylamide at any dose, including 10 mg/kg/day.

Leswing and Ribelin [35] studied physiologic and pathologic changes in 11 young adult cats administered acrylamide orally. Acrylamide was mixed in the diet to deliver a calculated dose of 20 mg/kg/day. Within 2-3 weeks, the cats showed slight weakness of the hindlimbs which progressed at variable rates to paralysis of the hindlimbs. Atrophy of the thigh and leg muscles was noticeable in severely paralyzed cats, and a few cats also had weakness of the forelimbs. The authors [35] reported that the cat cries became coarse, indicating possible involvement of the laryngeal nerves. Cats showed marked improvement when returned to their normal diet. Hindlimb strength was regained within 2-3 weeks, but complete recovery took several months and was directly related to the severity of the involvement. Microscopic examination of the nerves revealed degeneration of the myelin and axons of all four limbs. There was a suggestion of actual axon loss in the distal third or fourth portion of the tibial nerve fibers. Atrophy was evident grossly in nearly all muscles of the caudal limbs; however, microscopically, it was marked only in the digital muscles.

Leswing and Ribelin [35] also measured peripheral nerve conduction and nerve and muscle action potentials in cats and monkeys that had severe neurotoxic effects from acrylamide treatment. The cats were administered acrylamide at 20 mg/kg/day in their diet. Four monkeys were given acrylamide at 20 mg/kg/day, injected into bananas, for 8 weeks. The dose was then increased to 30 mg/kg/day. Acrylamide-treated animals required higher applied voltages to stimulate the nerve, and most had greatly reduced muscle and nerve spike amplitudes. Conduction velocity was reduced from preexposure levels in sciatic and tibial nerves by an average of about 29% in the cat and 24% in the monkey. "Substantial improvement" in conduction velocity was seen in both species on partial clinical recovery.

Schaumburg et al [36] studied ultrastructural changes in the nervous system of two cats given acrylamide at a dose of 3 mg/kg/day in drinking water. One cat received acrylamide for 252 days and the other for 294 days. Onset of gait disorder was noted after 70 days in one cat and after 163 days in the other cat. Hindfeet drop and distal muscle weakness were seen within 7 months. Tissues biopsied from the hindfeet after completion of the study showed a loss of all types of myelinated fibers in distal nerves. Only a few small and large myelinated nerve fibers were seen in plantar nerve twigs and most of the fibers were completely degenerated (bands of Bungner). Many unmyelinated nerve fibers were present. Most of the muscle fibers were vacuolated, shrunken, and had irregular borders--changes that the authors [36] considered secondary to denervation.

McCollister et al [34] also carried out a 1-year feeding study in female monkeys. One monkey was used at each dosage (0.03, 0.1, 1, 3, or 10 mg/kg/day, 5 days/week). Two monkeys received the 0.3-mg/kg dose and two

monkeys served as controls. The animals at the 10- and 3-mg/kg levels received their daily doses by intragastric administration of aqueous solutions of acrylamide. The other monkeys ate bananas injected with acrylamide given to them in the morning before any other food. The monkey on the 10-mg/kg dose showed some weakness of the hindquarters by day 48 and extreme weakness by day 69. The animal was transferred to a control diet on the 70th day and recovered completely 54 days after acrylamide was discontinued. The monkey fed 3 mg/kg of acrylamide did not show any loss in body weight. Neurologic examinations of this animal sometimes showed either knee jerk or pupillary reflexes that were somewhat sluggish when compared with the response of the controls. The authors [34] considered these responses as insignificant. "Terminal hematologic examinations" (unspecified) at 1 year showed no abnormalities. At autopsy, gross examination of the animal revealed no abnormalities. Liver and kidney weights were normal. Microscopic examination of the liver and kidney also showed no significant adverse effects on cells of these tissues. Microscopic examination of the brain and spinal cord showed no abnormalities attributable to acrylamide. The monkeys on other dose regimens showed no adverse effects as measured by growth, general appearance, behavior, periodic neurologic examinations, liver and kidney weights, gross necropsy, and microscopic examination of the tissues.

(b) Dermal and Eye Studies

Hashimoto and Ando [37] studied the dermal penetration of acrylamide in rabbits. A single application of acrylamide as a 10-30% aqueous solution penetrated the skin rapidly and appeared in the blood both as free compound and bound to proteins (mainly hemoglobin). Twenty-four hours

later, the concentrations of acrylamide in tissues (unspecified) were higher than in the blood. Seven successive applications of 30 minutes each day progressively increased the blood and tissue concentration of acrylamide. Autoradiographs showed that 14C-acrylamide concentrated around hair follicles. No quantitative data were given.

McCollister et al [34] investigated the possibility of skin irritation from acrylamide by applying an unspecified quantity of 10% aqueous solution to the ear and to the shaved intact abdominal skin of a rabbit. Applications to the ear and abdomen were repeated 10 times over a period of 2 weeks. In another experiment, an abraded area of the shaved belly was treated with a 10% aqueous solution of acrylamide for 3 consecutive days. No significant responses were observed except in the case of animals whose skins were abraded, which only showed a very slight reddening and slight edema that healed later. A 12.5% aqueous solution of acrylamide was applied to the skin of 12 rabbits and held in place with the aid of an impermeable sleeve for a 24-hour period. Two rabbits each received dermal applications of 0.063, 0.126, 0.5, and 1.0 g/kg of the acrylamide; four animals were treated with 0.252 g/kg. Only one rabbit that received the 1.0-g/kg application died within 2 days. Slight weight losses and reddening of the skin were noted in both rabbits treated with 0.5 g/kg of acrylamide. No other effects were observed in any of the other rabbits.

The effects of eye contact with 10 and 40% aqueous solutions of acrylamide were also studied by McCollister et al [34]. The solution was instilled into the right eye of a rabbit and washed within 30 seconds with a stream of water for 2 minutes. The left eye was treated with the same

amount of acrylamide solution but left unwashed. The eyes were examined with and without fluorescein staining, 2-3 minutes, 1 hour, and 24 hours later for conjunctival and corneal responses. The 10% aqueous solution caused signs of slight pain and slight conjunctival irritation immediately after contact, but the conjunctiva was completely normal within 24 hours. No injury to the cornea was reported. The application of the 40% aqueous solution of acrylamide to the unwashed eye caused signs of moderate pain, slight conjunctival irritation, and corneal injury. Although the conjunctival irritation was slow to heal, corneal healing was complete within 24 hours. Signs of moderate pain and slight conjunctival irritation were observed in the washed eye after administration of the 40% solution; however, there was no corneal injury, and the conjunctival irritation was nearly healed at 24 hours.

(c) Parenteral Studies

Hashimoto and Aldridge [38] studied the effects of acrylamide on body weights of male Porton-strain albino rats weighing about 200 g. Acrylamide was injected ip, twice a week, for 1 month. At 32 days after the first injection, there was a 28% reduction in body weight in rats injected with 50 mg/kg; those injected with 100 mg/kg showed a weight reduction of 63%. Rats in both groups were ataxic after 2 weeks.

Suzuki and Pfaff [39] studied the effects of acrylamide in white Osborne-Mendel strain suckling and adult rats. One group consisted of 30 suckling (1-day-old) rats weighing 5-8 g and the other of 28 adult rats weighing 150-300 g. The animals received ip injections of 50 mg/kg of acrylamide in saline, three times a week, for up to 18 injections; two additional adult rats each received a total of 26 injections. Controls

were injected with saline only. Suckling rats, both experimental and control, gained weight normally until their fifth or sixth injection, when weight gains of the acrylamide-injected animals slowed down. Slight weakness of the hindlimbs, noticeable in some of the young animals after five or six acrylamide injections, became more pronounced until the rats could no longer stand. In contrast to the results obtained in suckling rats, the body weights of the adults did not change. Weakness of the hindlimbs, noticeable after 7 or 8 injections, was followed by complete paralysis after 15-17 injections. At this time, wasting of the musculature of the hindlimbs was prominent. Weakness of the forelimbs was also noted in some rats. In the animals for which acrylamide treatment was terminated after the 16th injection, weakness of the extremities persisted for about 1 month but, in animals given 26 injections, it persisted for about 2 months. Animals with clinical signs of neuropathy showed prominent distention of the urinary bladder in pups and adults at autopsy. The other organs were congested, but otherwise normal.

Suzuki and Pfaff [39] observed on microscopic examination occasional myelin figures in Schwann cells and enlarged fibers within the sciatic nerve and their distal branches in acrylamide-treated suckling rats after the fourth injection. These changes became prominent after eight injections and myelin degeneration was observed after 12-14 injections. In adult rats, both myelin and axonal degeneration were prominent after 15 injections. According to the authors [39], rats which received 26 injections showed "severe axonal loss" in both the proximal and distal portions of the sciatic nerve, but this loss was more severe in the distal portions. In addition, the authors [39] reported an increased number of

Schwann cells, many of which had an abnormally high number of myelin figures in their cytoplasm. Light microscopic examination of sections of cerebrum, cerebellum, spinal cord, and brain stem showed degeneration of the spinal cord white matter and the presence of axonal spheroids in the cuneate nuclei of the medulla oblongata. No other CNS abnormalities were observed in adult rats. Hematoxylin and eosin-stained sections of lung, liver, spleen, pancreas, kidney, and adrenal showed no abnormalities.

With electron microscopy, the authors [39] found that the most common feature in suckling rats given nine injections of acrylamide was axons filled with fine filaments. Very few changes were noted in the adult rats killed after 10 or 12 injections. Many axons of the sciatic nerve were filled with neurofilaments; however, the myelin sheaths appeared normal. In addition to accumulations of neurofilaments, degeneration of axons and myelin sheaths was observed in adult rats receiving 15-18 injections. Sciatic nerves and their branches in adult rats which had received 26 injections had numerous Schwann cells and macrophages containing many myelin figures and fat droplets but very few myelinated fibers. There were many Schwann cells in the sections, but few of these sections contained axons. Microscopic examination of the nerves of adult rats killed 20 or 30 days after the last injection showed numerous axonal sprouts growing within the Schwann cells. Suzuki and Pfaff [39] concluded that, since degenerative changes of sciatic nerve axons seen only in adult rats in advanced stages of neuropathy were also frequently observed in suckling rats at the onset of paralysis, the peripheral nerves of suckling rats were more susceptible to acrylamide than were those of adults. The authors [39] suggested that the higher susceptibility of suckling rats could be a result

of the incomplete development of the barrier system of peripheral nerves.

Spencer and Schaumburg [40] examined the spatial-temporal distribution of hindlimb peripheral nerve degeneration in rats injected with acrylamide. Acrylamide, dissolved in saline, was administered daily by subcutaneous injection to 10 young-adult Spraque-Dawley rats in amounts of 10-60 mg/kg for 4-40 days. Sixteen age- and weight-matched rats were used as controls. The rats were killed before they developed signs of obvious hindlimb weakness. Nerve fibers separated from various sites in the sciatic, tibial, and plantar nerves were examined by light and electron microscopy. The large diameter fibers supplying the calf muscles and the long sensory fibers supplying the digits of the paws degenerated first. Degeneration of nerve fibers supplying the flexor digitorum brevis muscle occurred later. The findings from the electron microscopic examination showed accumulation of neurofilaments, abnormal mitochondria, and honeycombed, interdigitated Schwann cell-axon networks.

Kuperman [41] studied the neurotoxic effects of acrylamide on cats. Acrylamide was administered by various routes (iv, ip, im, oral, or subcutaneous) in daily doses of 1, 2, 5, 10, 15, 25, 40, or 50 mg/kg. Groups of 3-11 cats were used at each level. Effects of chronic poisoning from acrylamide, as measured by the development of ataxia, appeared at identical dose levels and after equivalent latent periods irrespective of the route of administration, whether iv, ip, im, subcutaneous, or oral. The length of the latent period from the start of the treatment was inversely related to the amount of the dose and varied between 125 days with 1 mg/kg and 2 days with 50 mg/kg. In this study [41], the average of the doses that killed eight cats was 320 mg/kg. Microscopic findings in

brain and spinal cord were normal.

McCollister et al [34] also studied the effects of acrylamide on one monkey given two ip injections 24 hours apart at doses of 100 mg/kg. Severe weakness was seen 24 hours after the second injection, and the monkey died on day 3. The findings from gross examination were congested lungs and kidneys and areas of necrosis in the liver. Microscopic examination of the kidneys showed degeneration of the convoluted tubular epithelium and of the glomeruli with albuminous material in the capsular space. Fatty degeneration of the liver was confirmed by the findings from the microscopic examinations.

Several investigators [36,40,42-46] have examined an acrylamide-induced degeneration of nerve fibers which begins in the distal portion of the fiber and proceeds slowly toward the cell body. This process, known as the "dying back" phenomenon, is a nonspecific type of nerve fiber degeneration that occurs simultaneously in the central and in the peripheral nervous systems. The nerves that are most commonly affected are those with the longest and largest axons. Sumner and Asbury [45] administered an acrylamide solution (100 mg/ml) by subcutaneous injection at a daily dose of 10 mg/kg to healthy adult cats of both sexes (number unspecified). Electrophysiologic measurements were carried out at various stages from 21 to 67 days after the start of the treatment. The first sign of neuropathy was slight hindleg ataxia, usually seen at about day 20 of treatment. The acrylamide-treated animals were divided into three groups, ie, A, B, and C. Group-A cats (21-34 days) had mild hindlimb ataxia. Group-B animals (38-44 days) had moderately severe hindlimb ataxia and depressed or absent Achilles tendon jerks. Group-C animals (47-67 days)

had extreme hindlimb ataxia that made walking very difficult. Achilles tendon jerks were absent in cats in this group. Conduction velocities were measured in a total of 1,001 afferent fibers isolated from animals in the three groups. All afferent fibers of the medial gastrocnemius nerve were conducted at 72-126 meters/second (Group-I velocity) or 24-72 meters/second (Group-II velocity). Maximal conduction velocities were similar in all three populations, but peak Group-I velocities were 108-114, 96-102, and 84-90 meters/second in groups A, B, and C, respectively. Many single fibers, when isolated, did not respond to electrophysiologic stimulation normally adequate for stretch receptors; these were termed nonresponsive units. In group-A animals, 10% of the fibers (38/366) were nonresponsive. In group B, the proportion of nonresponsive units increased to 68% (215/315). In group-C cats, 89% (285/320) of the fibers were nonresponsive. Sumner and Asbury [45] thus concluded that the number of nonresponsive single units isolated by systematic sampling of dorsal root filaments correlates well with the clinical severity of the neuropathy. They [45] also concluded from the similar maximal conduction velocities in all three groups that acrylamide produced no significant slowing of the conduction velocity in individual functioning fibers between the stimulating and recording electrodes.

At the end of each experiment and before the animal was killed, the medial gastrocnemius muscle and nerve were dissected free and fixed for microscopic examination. Microscopically, no nerve fibers were observed to have been lost or to have broken down in the medial gastrocnemius nerve before 55 days of acrylamide treatment; however, after 55 days, nerve breakdown ranged from 0 to 55% or more. The authors [45], in agreement

with Schaumberg et al [36], found that the muscle spindle nerve terminal was vulnerable to acrylamide. According to the authors [45], the results presented in this study indicated that interruption of nerve function may have been well advanced before any nerve fiber degeneration had occurred in nerve trunks. Hence, determination of conduction velocities would not have shown any abnormalities until nerve fiber degeneration had proceeded toward the cell body to the point at which the stimulating electrode had been placed.

Kaplan and Murphy [47] studied the influence of age on rotarod performance of male Holtzman rats treated with acrylamide. The rotarod test procedure involved the use of a partitioned enclosure containing a floor that could be electrified and a rod which turned at 8 rpm. Rats were trained to maintain their balance on the rod throughout three 2-minute trials. A rat that fell during any two of the three trials failed the test. Four groups of 12 rats each (5, 7, 11, and 14 weeks of age) were administered acrylamide ip at a dose of 50 mg/kg/day until all the rats in the respective age group failed the test. The onset of, duration of, and recovery from acrylamide poisoning were measured by rotarod performance; body weights were also recorded. Small reductions in body weights or slower-than-expected growth rates were observed during the administration of acrylamide for 18-22 days. The means for the onset to failure were 7.3, 6.4, 5.5, and 5.3 days, and the means recovery were 19.2, 15.6, 13.8, and 14.8 days for 5-, 7-, 11-, and 14-week-old rats, respectively. Thus, the delay in onset to failure was longer in younger animals; however, once impaired, they required a longer time to recover.

Prineas [42] employed light and electron microscopy in his observations on the tissues of cats treated with acrylamide. Acrylamide, 10 mg/kg, was administered daily by subcutaneous injection to five cats of both sexes weighing between 2.5 and 4 kg. One cat was killed 11 days after commencement of the injections but before the onset of neurologic signs. The animals developed neurologic signs between days 17 and 22 and were killed 22-49 days after injections were begun. The finding from light microscopic examination of nerves from the cat killed on day 22 were fragmented axons and myelin-ovoid formations in the intramuscular nerves and in the tibial nerve. Specially prepared sections of the upper cervical cord from cats examined at 32 or more days after commencing the injections showed degenerating myelin sheaths in the spinocerebellar tracts, in the gracile tracts, and in the white matter next to the anterior fissure; also, electron microscopy of occasional fibers in the tibial nerve showed increased numbers of neurofilaments. By day 22, however, a majority of the large-diameter fibers showed an increase in the number of neurofilaments. Myelin degeneration was usually evident by 49 days, first appearing at the nodes of Ranvier. In all animals treated with acrylamide for 32 days, the cell bodies in the dorsal root ganglia showed some loss of the normal parallel arrangement of granular endoplasmic reticulum, breakdown of polyribosomes with release of ribosomes into the cytoplasm, and an increase in the amount of electron-dense material in the cytoplasm. Similar changes were also found in the anterior horn cells. Other changes included striking changes in nerve fibers and terminal buttons in the anterior spinal gray matter at the S1 level. Small myelinated fibers frequently contained excessive numbers of neurofilaments which appeared to distend the

fiber in some instances. Between 5 and 15% of the terminal buttons were enlarged and contained excessive numbers of neurofilaments. Thus, this study [42] demonstrated structural damage in the distal CNS tracts of cats with signs of acrylamide poisoning.

Schaumburg et al [36], using techniques similar to those of Prineas [42], examined the early pathologic events in terminals of sensory and motor nerve fibers in the paws of cats treated ip with acrylamide. Two groups of animals were used for these parenteral studies. Five cats were injected with 10 mg/kg/day of acrylamide in an aqueous solution for 7-32 days; another five cats received 10 mg/kg/day, alternating with 20 or 40 mg/kg/day, either in interrupted or steady sequences. Six cats were used as controls. Tissues of acrylamide-intoxicated cats were obtained before and after total body perfusion with fixatives. With the cats under general anesthesia, biopsies of hindfeet toepad pacinian corpuscles, plantar lumbrical muscle, flexor digitorum brevis muscle, and twigs of the medial plantar nerve were performed and the tissues were examined under light and electron microscopy. The authors [36] observed that cats receiving 10 mg/kg/day developed a weaving gait, thought to be secondary to hindlimb unsteadiness, within 13 to 15 days. This evolved into a gross truncal ataxia. In the second group of animals, who received acrylamide at more than 10 mg/kg/day, a rapid and irregular head tremor was noted occasionally. After 28 days on the 10-mg/kg/day dose or 15 days on the higher doses, the cats were barely able to walk; however, foot drop and muscle wasting were not observed at this time. The authors [36] reported that the axons of pacinian corpuscles began to degenerate before sensory nerve terminals supplying muscle spindles. Pacinian corpuscles are

important to the animal's sense of position and they degenerated before the adjacent motor nerve terminals. These authors [36] concluded that sensory changes precede motor changes in the cat and that pathologic findings precede the occurence of clinical signs. They [36] also concluded that, in the dying-back phenomenon produced by acrylamide, changes occurred first in the distal parts of axons but not necessarily at the nerve terminal.

(d) Mechanism of Action

Various theories are provided in the literature explaining the pathogenesis of selective axonal lesions from acrylamide. One explanation is that acrylamide interferes with the metabolic pathways of the nerve cell body which gradually fail in their functions to provide nutrient material for the axon [48]. This leads to a depletion in the amount of material reaching the distal regions of axons where degeneration begins. Another hypothesis suggests that acrylamide interferes with the intracellular transport system by which substances, assembled in the neuron cell body, are transported along the axon [49]. A third theory [44,50] notes that acrylamide may have local toxic effects along the entire axon and that axons are more vulnerable than the cell bodies [42].

Interruption of transport of proteins along axons could result in the breakdown of the axons. To test this hypothesis, Pleasure et al [49] measured flowrates of newly synthesized proteins within sensory and motor axons of 12 healthy cats for comparison with those in 9 cats showing neuropathies induced by acrylamide at a dose of 20 mg/kg orally for 5 days/week. Axonal degeneration, predominantly distal, was found in hindlimb nerves after neurologic signs had appeared but no alterations in the cell bodies of motor or of sensory neurons were evident. The flowrates

of axonal proteins in motor and in sensory nerves were determined in nine cats, including two controls, at intervals from 4 hours to 2 weeks after an ip injection of 3H-L-leucine. One day after injection of tritiated leucine, the radioactivity in the ventral roots of cats in both the control and acrylamide-treated groups was maximal adjacent to the spinal cord, while maximal radioactivity in the dorsal root bordered the ganglion. In acrylamide-treated cats killed 2 or more days after receiving the isotope, maximal radioactivity in all seven cats remained at the border of the ganglion in the dorsal root and in five of the seven at the edge of the spinal cord in the ventral root. Pleasure et al [49] demonstrated the existence of a protein fraction moving along axons from motor and from sensory neurons at about 1.5 mm/day. Evidence of such transport was absent in most of the cats made neuropathic by acrylamide. The authors [49] indicated that the absence of a migrating protein peak in acrylamide-induced neuropathy may have been from inhibition of protein synthesis or a defect in the transport mechanism. The accumulation of radioactivity close to the cell bodies in cats treated with acrylamide suggested that protein synthesis had occurred and that the defect was in the transport process itself.

In a subsequent report, Bradley and Williams [51] studied both the fast and slow components of axonal transport from dorsal root ganglia along the proximal regions of the sciatic nerve of cats with mild-to-moderate degrees of acrylamide neuropathy. Young cats of mixed breed, 1.5-4.25 kg in weight, were given acrylamide orally at a dose of 20 mg/kg/day, 5 days/week. The dose of acrylamide was adjusted to induce mild-to-moderate incordination and weakness of the hindlimbs in 2-6 weeks. Cats were

injected with 3H-L-leucine in the seventh lumbar dorsal root ganglion only on one side. Control animals were injected with 3H-L-leucine only. Three control and three acrylamide-treated animals were killed 6 hours, 10 days, or 30 days after injection of the tritiated leucine. Contrary to the findings of Pleasure et al [49], the authors [51] found no difference in slow axonal transport between acrylamide-intoxicated and control cats. There was a decrease in the velocity of the crest of fast axon transport in the acrylamide-treated cats, but they [51] suggested that this reduction was unlikely to be responsible for the degeneration present in the distal portions of the axon.

Hashimoto and Ando [52] examined the effects of acrylamide on the in vitro incorporation of radioactive amino acids into the proteins of brain, spinal cord, sciatic nerve, and liver tissues. Eight-week-old male Sprague-Dawley rats were fed a diet containing 500 ppm of acrylamide for 4 weeks and no acrylamide for the next 4 weeks. The control group was fed the untreated diet only. The animals given acrylamide began to show weakness of the hindlimbs at 2 weeks, slight disturbances in walking at 3 weeks, and paralysis at 4 weeks. After removal of acrylamide from the diet, the paralyzed animals recovered in 5-6 weeks. The incorporation of 14C-lysine into tissue proteins was studied at 1, 2, 3, 4, 6, and 8 weeks after acrylamide feeding was begun. No differences in the incorporation in the brain and liver slices of the control and acrylamide-treated rats were seen at any of these intervals. However, more radioactivity from labeled lysine was counted in proteins of the spinal cords of treated than of control rats after 4 weeks of feeding and the difference continued to increase, particularly in the lower cord, until 6 or 8 weeks. In the

sciatic nerve, a slight decrease in radioactivity was noted after 2 or 3 weeks, but was followed by a larger increase beginning at 4 weeks.

The effects of acrylamide on the incorporation of 35S-methionine into proteins were studied at weekly intervals from weeks 2 to 6 of feeding [52]. The incorporation by the controls was highest at all times in the sciatic nerve followed by the liver, brain cortex, and spinal cord. A significant increase in incorporation was demonstrated at 6 weeks after start of the feeding in the spinal cord and sciatic nerve, but not in the brain or liver. In contrast to the results obtained with lysine, when methionine was used, no decrease in radioactivity was seen in the sciatic nerve protein at the early stages. The early decrease in 14C-lysine incorporation in the sciatic nerve may have been associated with the biochemical mechanism of neuropathy, such as interruption of the protein axoplasmic flow in nerve roots as postulated by Pleasure et al [49], and the decreased metabolism of proteins in the axons and Schwann cells. The increased incorporation of amino acids into the spinal cord during the recovery period may have been because of increased protein metabolism in the anterior horn cells, in which a large number of silver grains from 14C-lysine were visible in the autoradiograph. The increased incorporation of amino acids into the sciatic nerve during the later stages of neuropathy might have been related to the proliferation and increased metabolism of Schwann cells, in which many silver grains were demonstrated. Since amino acid incorporation in the brain and in the liver was not affected by acrylamide, these authors [52] suggested that the compound had a specific effect on the spinal cord and peripheral nerves.

Hollinger and Rossiter [53], in their study on Wallerian degeneration of peripheral nerves, found that beta-glucuronidase activity in the sciatic nerve of 56 cats markedly increased during the regenerative phase of the injury. Kaplan and Murphy [47] measured sciatic nerve beta-glucuronidase activity in 24 acrylamide-treated rats. Male Holtzman rats, weighing 200-300 g, were administered acrylamide ip at a dose of 50 mg/kg/day for 8 days. There was a slight but significant rise in beta-glucuronidase activity of sciatic nerves 1 week after the last dose of acrylamide; the enzyme activity continued to increase, reaching a peak of 340% of the control values 3 weeks after the last injection. The greatest increase in enzyme activity occurred after apparent recovery from the neuromuscular impairment produced by acrylamide. The investigators [47] suggested that, if the acrylamide-induced increase of beta-glucuronidase activity in the peripheral nerve reflected peripheral nerve regeneration and hence incomplete healing, an increased sensitivity to acrylamide might have been anticipated during this period. Indeed, at 30 days, when beta-glucuronidase activity in the sciatic nerve of cats was 320% of the control values, rats were significantly more susceptible to acrylamide. The mean days of failure, as measured by a rotarod performance test, for rats retreated with 50 mg/kg/day of acrylamide and their age-matched controls were 2.5 and 4 days, respectively. Conversely, at 90 days, when beta-glucuronidase activity of the sciatic nerve was 155% of control values, no differences in rotarod performance between retreated rats and their age-matched controls were evident. If the increased beta-glucuronidase activity in the sciatic nerve observed after acrylamide-induced neuropathy reflects peripheral nerve regeneration, then the

increased susceptibility of rats to retreatment with acrylamide suggests the addition of a new injury to a preexisting injury that is not functionally apparent.

Hashimoto and Aldridge [38] studied the distribution and excretion of acrylamide in rats. Six male Porton-strain albino rats weighing about 200 g were injected iv with radioactive acrylamide at a dose of 100 mg/kg. The 14C-radioactivity was measured in the expired air and in the urine. About 6% of the injected dose was exhaled as carbon dioxide in the first 8 hours; excretion was very low after that (reaching only slightly more than 6% in 24 hours). Urinary excretion of the 14C-radioactive material was very rapid, 40% of the injected dose excreted over the first day and a maximum of about 65% reached by day 4; the excreted metabolites were not identified. The distribution of 14C-radioactivity was studied in whole blood, plasma, brain, spinal cord, sciatic nerve, liver, and kidney at 1, 4, and 14 days after injection. At each of these intervals, the radioactive material was found in all tissues examined, with high counts in the blood. A considerable amount of radioactivity was present at 14 days; most of it was not extractable by 5% trichloroacetic acid and so was presumably protein-bound.

Edwards [54] recently reported on the half-life of acrylamide in the blood of male Porton-strain rats weighing 200 g. Acrylamide dissolved in 0.9% saline was injected iv at a dose of 100 mg/kg. The drop in the blood concentration of acrylamide was exponential and its half-life was 1.9 hours.

Kaplan et al [55] studied the effects of hepatic microsomal enzyme inducers on the functional neuronal deficit produced by acrylamide. The

authors observed that pretreatment with either DDT or phenobarbital noticeably delayed the onset of neurologic deficit. The total dose of acrylamide required for 100% failure of control rats in rotarod performance tests was 360 mg/kg; it was 520 and 600 mg/kg for the DDT- and phenobarbital-pretreated animals, respectively.

Edwards [56] also investigated the effects of DDT and phenobarbital on acrylamide-induced neurotoxicity; however, the results obtained in this study were different from those reported by Kaplan et al [55]. Animals were fed a diet containing 500 ppm of acrylamide. Slight ataxia developed in the controls (receiving acrylamide only) and in both experimental groups after 8-10 days on the acrylamide-treated diet. All rats recovered at the same time after treatment. There were two major differences in the experimental procedures used by Kaplan et al [55] and by Edwards [56]. In the former study, acrylamide was given daily by single ip dose; in the latter, it was given in the diet. However, the calculated daily dose of acrylamide in the study by Edwards [56] was similar to that given by Kaplan et al [55]. The second major difference in the two studies was the method of assessing neurotoxicity. In the study by Edwards [56], it was subjective and depended upon personal observations, whereas Kaplan et al [55] used the rotarod performance test which they claimed was more sensitive than subjective analysis.

Correlation of Exposure and Effect

Humans have been exposed to the monomeric form of acrylamide both in occupational [16-21,23] and nonoccupational [22] situations. Workers have been exposed in the manufacture of the acrylamide monomer from

acrylonitrile acid hydrolysis [23]; in the handling of a 10% aqueous solution in a mine [16]; in the production of flocculators from the monomer [20]; in the use of a resin mixture that apparently contained residual monomer in sealing processes [17,18]; and in the production of polymers while manufacturing papercoating materials [21]. The exposure of a Japanese family to acrylamide-contaminated well water, which they drank, cooked with, and bathed in (the latter for a few days only) was the single nonoccupational incident [22].

In all of the occupational incidents [16-21,23], the dermal route of exposure was predominant, with some respiratory exposure and with slight oral ingestion possible through hand contamination [16-21,23]. An example of dermal exposure was one worker who repeatedly splashed a 10% aqueous solution of acrylamide on his unprotected hands, forearms, and face [16]. Other workers who were filling pumps or working in areas where there were leaks in the pressurized delivery system were splashed with a resin containing an unknown amount of monomers. In contrast to those exposures, workers in a flocculator plant where all skin contact was avoided exhibited no signs of intoxication [20]. The authors [20] also stated that the crystalline monomer was heavy (large particle size) and did not form stable aerosols. The vapor pressure of solid monomeric acrylamide is 0.007 mmHg at 25 C [1], equivalent to a saturation concentration of 27 mg/cu m, so acrylamide vapor may pose a hazard in confined or poorly ventilated spaces. The more likely inhalation hazard from acrylamide solution is from aerosolization of the solution.

Although signs and symptoms that developed in some workers who were exposed dermally to monomeric acrylamide have been well documented [19-

21,23], the exact time of the appearance of symptoms after dermal exposure was not. The times of onset that were reported varied from 4 weeks [20] to about 24 months [21] except for one worker [21] who was employed for approximately 8 years before symptoms appeared. Not all of the exposed workers had recognized adverse effects [16,19-21,23]. Initial symptoms after skin exposure were numbness [16,18,20-22], tingling (paresthesia) [18-22], and "tenderness to the touch," followed in days (or 1-2 weeks) by coldness of the hands and fingers, and less often of the feet and toes [16,18]. Concurrently, or occasionally somewhat later, excessive sweating [16-21], bluish-red skin on the hands [18-21,23], and peeling of the skin of the hands and less often the feet [16-21] were followed by fatigue and marked weakness of the limb muscles [16,19,20].

Skin absorption in mammals has been confirmed by Hashimoto and Ando [37], who found that absorption of 14C-acrylamide in rabbits was rapid. The 14C-acrylamide was found free in the blood and bound to protein, mainly hemoglobin, within hours of skin application, and in unidentified tissues in 24 hours. Successive dermal applications increased the blood and tissue levels. McCollister et al [34] also found that the rabbit skin absorbed acrylamide. They made dermal applications of 1 g/kg which killed one of two rabbits.

Many of the effects reported in humans have been confirmed in animals. Muscular weakness also occurred in rats [14,27], dogs [14], cats [34], monkeys [34], and baboons [31]. Weight loss was noted in humans [16,18-20], especially those who handled 10% aqueous solutions of acrylamide [16], rats [14,38,39], monkeys [34], and mice [33]. Sleepiness and lassitude preceded death in rats [14]; however, these effects were more

evident in those humans who ingested acrylamide-contaminated water [22] than in those dermally exposed. Another manifestation, which occurred in adults who ingested acrylamide, was retention of feces and urine that resulted in constipation and overflow urinary incontinence [20,22]. Distended urinary bladders were also reported in animals [27,34]. Reddened skin from dermal contact with acrylamide has been reported in humans [17] and in rabbits [34].

It is notable and in marked contrast to the reactions of those people who were occupationally exposed (mainly dermally) to acrylamide [17-20,23] that the initial complaints of those exposed by ingestion [22] were not directed toward the extremities or the skin. When symptoms of mild dysesthesia did occur by oral ingestion and probably also by dermal absorption, the three adults [22] had been hospitalized for emotional problems for 2-4 weeks.

Electromyography and nerve conduction studies were performed on humans before [21,23] and during [25] recovery from acrylamide intoxication. Muscle response to nerve stimulation was abnormal, indicating damage of distal nerve terminals [21]. Conduction velocity was affected in only a few units of each motor nerve [21,25]. Structural abnormalities were also found in the distal portions of the long nerves [25]. Because both the action potentials and conduction times in the sensory nerves were more extensively affected than in the motor nerves, the author [25] concluded that the sensory fibers were damaged earlier and more severely than the motor fibers.

Microscopic examination by Fullerton [25] of biopsied sensory nerves from two patients in the Garland and Patterson [20] study showed the

presence of simultaneous degeneration and regeneration of the sensory nerves which seemed to have occurred before the onset of symptoms. Neuroanatomic and physiologic studies [39,41,42,47,49,52,53,55] on animals, performed on a much more extensive scale than in humans, confirmed these results. Excretion of 14C-acrylamide and the effects of hepatic microsomal enzyme inducers on the toxicity of acrylamide also have been studied. While these results have added to the information concerning the effects of acrylamide on various life processes, they do not describe the initial process whereby acrylamide produces peripheral neuropathy in humans and animals.

Other important and diagnostic manifestations of the acrylamide effect on humans are: dizziness [17,20,21,23]; vertigo [23]; positive Romberg's sign and inability to stand on one leg [17,19-23]; slurring of speech [20]; confusion, insecurity, and bizarre behavior [22]; poor memory and orientation [22]; writing inability or difficulty [19]; muscle pain [18,20,21]; adiadochokinesis [23,34]; gastrointestinal disturbance and dysphagia [18,23]; loss of temperature and touch and vibration senses [16,20-22]; dysarthria [20]; and paresthesia, dysesthesia, and hyperesthesia [21,22].

In summary, in the absence of pertinent exposure data, no useful correlation can be made between the type and extent of exposure and the degree of human intoxication produced by acrylamide in the industrial environment [16-21,23-25]. However, the signs and symptoms, results of general medical and neurologic examinations, and the treatment and cure or regression of reactions reported in humans [15-23], including the nerve conduction and microscopy studies [21,25], when compiled and summarized,

are very valuable for the recognition of the sequence and characterization of adverse effects produced on humans by exposure to monomeric acrylamide. The animal studies also are pertinent in understanding human effects since they are very similar. In the single report of a nonoccupational episode [22] found, three of five family members were hospitalized after ingesting well water containing an acrylamide concentration of 400 ppm. Just how much each ingested is unknown. It is evident that effects on the CNS, rhinorrhea, and coughing were the first symptoms which were noticed by the people who ingested acrylamide, while those dermally exposed first noticed paresthesias, skin changes, and muscle weakness of the extremities. In all known recorded human cases of and in all types of exposures to acrylamide, persons recovered in 2 weeks-2 years (latter associated with peripheral nerve defects); most persons recovered in 1-12 months after cessation of exposure to acrylamide.

Carcinogenicity, Mutagenicity, and Teratogenicity

No reports which address the subject of possible carcinogenic, mutagenic, or teratogenic properties of acrylamide monomer were found.

Summary Tables of Exposure and Effect

The effects of dermal and oral exposures on humans to acrylamide that were presented in Chapter III are summarized in Table III-1. The effects of short- and long-term exposures on animals to acrylamide are summarized in Table III-2.

TABLE III-1

SUMMARY OF EFFECTS OF ACRYLAMIDE EXPOSURE ON HUMANS

Number of Subjects	Duration and Route of Exposure	Observed Effects	References
6	3 - 24 mo dermal and possible inhalation	Erythema, excessive sweating, muscular weakness	16-18, Brinkley*
8	3 - 13 mo dermal and possible inhalation	Loss of weight, anorexia	17-20, 23
6	4 - 7 mo dermal and possible inhalation	Eye irritation, skin rash, fatigue, confusion	16,19
7	2 mo - 8 yr dermal and possible inhalation	Gastrointestinal upset	21
5	1 mo ingestion	Rhinorrhea, urinary and fecal retention, ecchymoses	22
9	7 - 12 mo dermal and possible inhalation	Vertigo, abnormal reflexes, emotional changes	16,17, 19,23
4	3 - 24 mo dermal and possible inhalation	Ataxia, hypoesthesia	16-18

TABLE III-1 (CONTINUED)

SUMMARY OF EFFECTS OF ACRYLAMIDE EXPOSURE ON HUMANS

Number of Subjects	Duration and Route of Exposure	Observed Effects	References
17	1 mo - 8 yr dermal and possible inhalation	Pain, tremor, desquamation, sensory loss	16,17, 20,21
10	1 - 15 mo dermal and possible inhalation	Positive Romberg's sign	17,19, 20,23

*From DR Brinkley (written communication, June 1976)

TABLE III-2

SUMMARY OF EFFECTS OF ACRYLAMIDE EXPOSURE ON ANIMALS

Routes of Exposure	Species	Dose and Duration	Observed Effects	References
Dermal	Rabbits	0.5 - 1.0 g/kg 24 hr	Edema, death 1/5	34
"	"	10% solution 3 d	On abraded skin, slight reddening, edema	34
"	"	10% solution 2 wk	On shaved skin, no effects	34
Ocular	"	10 and 40% 24 - 48 hr	Pain, conjunctival irritation	41
Oral	Rats	203 - 277 mg/kg 1 dose	LD50, death 5/5	27,28, 34
"	"	50 - 126 mg/kg 1 - 15 d	Lethargy, weakness, bladder distension	29
"	"	100 mg/kg 2 - 3 d	Death of most animals	14,27, 29
"	"	0.3 - 11 mg/kg 55 - 189 d	No effects	27,34
"	"	200 - 400 ppm 1 - 6 mo	Loss of motor control, ataxia, leg weakness, progressive paralysis	14,27, 29,34, 56
"	"	100 ppm 6 - 40 wk	Growth retardation, leg weakness	27,34
"	"	10 - 50 ppm 6 wk	No effects	14

TABLE III-2 (CONTINUED)

SUMMARY OF EFFECTS OF ACRYLAMIDE EXPOSURE ON ANIMALS

Routes of Exposure	Species	Dose and Duration	Observed Effects	References
Oral	Mice	170 mg/kg 1 dose	LD50	14
"	"	250 ppm 45 d	Weight loss, ataxic gait	33
"	Rabbits	252 mg/kg 1 dose	Death 4/4	34
"	"	126 mg/kg 1 dose	Death 1/4; tremors, pupil dilation	34
"	Guinea pigs	252 mg/kg 1 dose	Death 4/4	34
"	"	126 mg/kg 1 dose	No deaths, slight weight loss	34
"	Cats	1 - 20 mg/kg 53 - 367 d	Weakness, paralysis, twitching	35
"	"	0.03 - 0.3 mg/kg 367 d	No effects	34
"	Dogs	5 - 100 mg/kg 4 - 5 wk	Ataxia, sedation, weakness	14,21
"	"	1 - 8 mg/kg 4 - 19 wk	No effects	14
"	Monkeys	10 - 30 mg/kg 8 - 10 wk	Weakness, decreased nerve conduction velocity, myelin and axonal degeneration	34,35
"	"	0.03 - 3 mg/kg 51 wk	No effects	34
ip	Rats	50 - 100 mg/kg 4 - 6 wk	Weight loss, paralysis, bladder distension, myelin and axonal destruction	38,39

TABLE III-2 (CONTINUED)

SUMMARY OF EFFECTS OF ACRYLAMIDE EXPOSURE ON ANIMALS

Routes of Exposure	Species	Dose and Duration	Observed Effects	References
ip	Monkey	100 mg/kg 2 d	Lung and kidney congestion, liver necrosis, severe weakness, death	34
iv	"	50 mg/kg 4 d	Death within 4 d	34
Subcutaneous	Cats	10 mg/kg 21 - 61 d	Ataxia, absent Achilles tendon jerks, interruption of nerve function	45
Oral ip iv im Subcutaneous	"	1 - 50 mg/kg (duration not specified)	Ataxia, progressive weakness, gradual blood-pressure drop to shock level; death of some animals	41