Overview of Acrylamide Toxicity and Metabolism

Prepared for

JIFSAN/NCFST Workshop on Acrylamide in Food

Toxicology and Metabolic Consequences Working Group

October 2002

This background document was developed prior to the workshop and was used by the working group in its discussion of the current state of knowledge regarding the toxicity and metabolic consequences of acrylamide in food and in identifying data gaps and conclusions regarding what research would fill those gaps and what research should occur first. Through the course of the working group's discussion of the state of knowledge, various hypotheses were raised to help formulate specific research projects that might fill the identified data gaps. Some modifications were made to the background document as a result of the working group's discussion and input, but the document should not be considered to necessarily represent the consensus views of the working group or of any individual participant in the working group.

1.0 Background

Recently, it has been reported that acrylamide monomer may form in certain foods cooked at high temperatures. The highest concentrations of acrylamide have been identified in potato and grain-based foods that are cooked at very high temperatures (e.g., frying, grilling or baking) (Tareke et al. 2002). Acrylamide levels as high as 3500 μg/kg have been reported in potato chips and French fries. Acrylamide is thought to form in food principally from the interaction of the amino acid asparagine with glucose or other carbohydrates.

Acrylamide has been extensively investigated and has a large database of very complex toxicity, pharmacokinetic and mode of action studies. The results of the animal toxicity studies indicate that acrylamide is carcinogenic in rodents and produces toxic effects on the reproductive and nervous systems. However, to date, only neurotoxicity has been demonstrated in humans. The purpose of this paper is to provide the reader with a broad overview of the toxicity and toxicokinetics of acrylamide and related issues rather than provide detailed information on each acrylamide study published in the literature. The studies cited in this report were considered the most appropriate for the characterization of the toxicity and issues related to the potential for acrylamide toxicity from the ingestion of acrylamide in food. It should be noted that biomarkers of exposure are discussed in another paper and will not be discussed here.

2.0 Physical Chemical Properties

Acrylamide is an odorless, white crystalline solid at room temperature, with a molecular formula of C_3H_5NO and weight of 71.08. Acrylamide is readily soluble in water (2155 g/l at 30°C) and polar solvents (e.g., acetone, methanol, and ethanol), but not in non-polar solvents (e.g. carbon tetrachloride). Acrylamide has a density of 1.27 g/l (25°C), a boiling point of 136°C at 3.3 kPa and a melting point of 84-85°C. Acrylamide contains an α,β-unsaturated amide system that reacts with nucleophilic compounds via a Michael addition. The major site of reaction is sulfhydryl groups contained on proteins and amino acids.

3.0 Toxicokinetics

A limited number of toxicokinetic studies with acrylamide were available. These studies were conducted with radiolabeled acrylamide, and the results reported are for total radioactivity, unless otherwise noted.

3.1 Absorption

Numerous studies, both *in vivo* and *in vitro*, have been conducted to evaluate the potential dermal absorption of acrylamide; however, no studies attempted to directly quantify absorption of acrylamide following oral or inhalation exposure. Results from distribution and excretion studies indicated that following oral administration in rats, acrylamide was readily absorbed (Miller et al. 1982; Marlowe et al. 1986; Ikeda et al. 1987). In a study conducted by Barber et al. (2001), rats received a single dose of acrylamide by gavage (20 mg/kg/day) or by intraperitoneal injection (50 mg/kg/day). Although no quantitative estimate of oral absorption can be made based on the results of this study, a comparison of the dose-adjusted area under the plasma concentration time curves (AUCs) between the two routes of exposure can provide

a qualitative estimate of oral absorption. Comparison of the dose-adjusted AUCs indicated a difference in absorption between the two routes that could not be explained by differences in dose (20 versus 50 mg/kg/day). This comparison indicated that systemic absorption following oral exposure appeared to be slightly less than observed following intraperitoneal administration of acrylamide, with greater conversion to glycidamide following oral exposure.

Results from *in vivo* studies conducted in rats indicated that dermal absorption ranged from approximately 14% to 61% of the applied dose (Ramsey et al. 1984; Frantz et al. 1995; Sumner et al. 2001). *In vitro* results obtained from rat, pig and human skin samples indicated 42 to 93%, 94%, and 27 to 33% of the applied acrylamide dose was absorbed for each species, respectively (Frantz et al. 1995; Diembeck et al. 1998; Marty 1998).

3.2 Distribution

Regardless of route of exposure, acrylamide appears to be rapidly distributed to tissues (Miller et al. 1982; Ramsey et al. 1984; Marlowe et al. 1986; Sumner et al. 2001). Following oral administration of 1 mg acrylamide/kg body weight, distribution of acrylamide in dogs and pigs was greatest to the muscle tissue, ranging from approximately 30 to 50% of the administered dose (Ikeda et al. 1987). In dogs, the greatest radioactivity after muscle tissue was observed in the liver (~14%), while in the pig it was observed in the gastrointestinal tract (~20%). The authors concluded that this may reflect slower absorption in the pig, compared to the dog. When fasted and pregnant female (days 13.5 and 17.5 days of gestation) Swiss Webster mice were administered 120 mg [2,3-^{14}C]-acrylamide/kg by gavage, fetuses examined at 13.5 days were uniformly labeled with the exception of slightly increased uptake in the fetal brain, which is typical of distribution of many compounds into fetuses at this stage of gestation (Marlowe et al. 1986). At 17.5 days, the distribution pattern resembled that in the maternal tissues, with the exception of high levels in the fetal skin.

At 24 hours following a 6-hour inhalation exposure to 3 ppm acrylamide, the majority of the absorbed dose in rats was found in the blood, followed by the skin, spleen and lung. In mice administered the same concentration via the same protocol, a different pattern of distribution was observed, with the highest fraction of absorbed dose reported in the skin, followed by the subcutaneous fat, testes, and blood (Sumner et al. 2001).

Studies in rats (Sumner et al. 2001) and mice (Carlson and Weaver 1985) evaluated the distribution of acrylamide following dermal application of 100 to 160 mg acrylamide/kg body weight. At 24 hours post-exposure, acrylamide levels were highest in the skin of both species. In the rat acrylamide concentrations of approximately 1 µmol/g tissue were reported in red blood cells. The only other location where the concentrations were higher was at the site of application (e.g., the skin: 4 µmol/g tissue) (Sumner et al. 2001). A similar pattern was observed in the mouse following dermal application of acrylamide although no measurement of acrylamide in the blood was conducted (Carlson and Weaver 1985).

Following intravenous administration of 10, 50, or 100 mg/kg acrylamide to rats, the majority of the administered acrylamide was found in the red blood cells at most time points (Hashimoto and Aldridge 1970; Miller et al. 1982; Ramsey et al. 1984). The distribution of administered acrylamide to the blood is related to the ability of both acrylamide and its metabolite glycidamide to bind to hemoglobin to form adducts (Calleman et al. 1990).

3.3 Metabolism

The major metabolite formed via the cytochrome P450 pathway is glycidamide (Miller et al. 1982). Species differences in the formation of this metabolite have been observed, with acrylamide converted to glycidamide to a greater extent in the mouse than in the rat, based on urinary metabolites (Sumner et al. 1997).

Once absorbed, acrylamide may be conjugated by glutathione-S-transferase (GST) to N-acetyl-S-(3-amino-3-oxopropyl) cysteine or it reacts with cytochrome P450 (CYP450) to produce glycidamide (Figure 1) (Dixit et al. 1980; Dixit et al. 1981c; Dixit et al. 1982; Miller et al. 1982; Calleman et al. 1990; Sumner et al. 1992). The major metabolite formed in both rat and mouse is N-acetyl-S-(3-amino-3-oxopropyl)cysteine, accounting for approximately 70% of the urinary metabolites observed in the rat and 40% of those observed in the mouse (Sumner et al. 1997). Several metabolic studies have been conducted that focused on the interaction of acrylamide with CYP450 and glutathione (GST) in the rat and the mouse (Dixit et al. 1980; Dixit et al. 1981c; Mukhtar et al. 1981; Das et al. 1982; Dixit et al. 1982). Results of these studies indicated that liver, kidney, brain and erythrocyte GST have significant binding capacity with acrylamide, with the liver GST being 3 times more efficient in conjugating acrylamide as compared to brain GST in the rat (Dixit et al. 1984). Additional studies in the rat indicate that acrylamide may inhibit GST, resulting in increased metabolism to glycidamide by the CYP450 pathway (Dixit et al. 1981b; Dixit et al. 1984).

3.4 Excretion

Excretion of acrylamide as the parent compound in rats is low (<2%) and occurs predominantly in the urine (Miller et al. 1982). In studies where radiolabeled acrylamide was administered via oral, dermal, or inhalation routes, approximately 40 to 70% of absorbed radiolabel was excreted in the urine, 5 to 6% in the feces, 6% in exhaled air, and 15% in the bile (Hashimoto and Aldridge 1970; Miller et al. 1982; Sumner et al. 1992; Sumner et al. 2001). The estimated plasma half-life for acrylamide and glycidamide following oral (20 mg/kg) or intraperitoneal (50 mg/kg) administration in male Sprague Dawley rats was approximately 2 hours for both acrylamide and glycidamide following single or repeated exposures (Barber et al. 2001).

3.5 DNA and Protein Adducts

3.5.1 Protein Adducts

Both acrylamide and glycidamide are electrophilic and can form adducts with sulfhydryl groups on hemoglobin and other proteins (Hashimoto and Aldridge 1970; Calleman et al. 1990; Bergmark et al. 1991; Barber et al. 2001). Acrylamide contains an α,β-unsaturated system that reacts with nucleophilic compounds via a Michael addition. The major site of reaction is cysteine with sulfhydryl groups, although acrylamide may also react with amino groups.

Hemoglobin adducts have been used as biomarkers of exposure and to estimate internal dose in occupationally exposed populations (Calleman et al. 1994; Hagmar et al. 2001). In a study of 41 workers employed at an acrylamide production plant, a neurotoxicity index specifically designed for acrylamide peripheral neuropathy correlated with acrylamide hemoglobin adduct levels (Calleman et al. 1994). In a study of 210 workers exposed to grouts containing acrylamide for approximately 2 months, there was a significant correlation between

hemoglobin adduct levels and exposure categories estimated by self-reporting (Hagmar et al. 2001). Hemoglobin adduct levels ranged from 0.02 nmol/g globin to 17.7 nmol/g globin.

When acrylamide was administered to rats, the conversion to glycidamide was 51% following the administration of 5 mg/kg and decreased to 13% after 100 mg/kg, based on the measurement of hemoglobin adducts (Bergmark et al. 1991). The conversion of acrylamide to glycidamide was higher following subchronic dosing as well. Acrylamide hemoglobin adduct formation was approximately 2- or 4.5-fold greater for oral (20 mg/kg/day for 15, 21, 34 or 47 days) or intraperitoneal (50 mg/kg/day for 11 days) dosing, respectively, than glycidamide adduct formation (Barber et al. 2001). Oral administration resulted in approximately 30% fewer acrylamide adducts than intraperitoneal dosing; however, more glycidamide adducts were formed after oral dosing. The authors suggested that the reason for these differences was due to a higher conversion of acrylamide to glycidamide following oral dosing, when compared with intraperitoneal dosing.

Dixit et al. (1986) conducted an *in vitro* study to evaluate the binding of acrylamide to bovine serum albumin (BSA), since acrylamide has shown reactivity with human serum proteins. The binding of acrylamide was measured using equilibrium dialysis, fluorescence studies, and ultraviolet spectroscopy. The results of the equilibrium dialysis indicated an increase in the amount of unbound acrylamide between 6 and 8 hours of dialysis. At this equilibrium point, more than 25% of the total acrylamide remained bound to protein. A concentration-dependent decrease in fluorescence of BSA was observed, as well as the fluorescence of sulfhydryl groups present on BSA, indicating a role of sulfhydryl groups in the binding of acrylamide.

3.5.2 DNA Adducts

There are limited data regarding the potential for acrylamide to form DNA adducts. When isolated nucleosides were incubated with acrylamide *in vitro*, the adduct yield and the rate of formation was low (Solomon et al. 1985). I*n vivo* studies in mice following an oral exposure to 100 mg/kg [^{14}C]-acrylamide, radiolabeled DNA was found in the tissues examined (stomach, liver, lung, skin and testes) (Carlson and Weaver 1985; Carlson et al. 1986). However, DNA adducts were not characterized so these results could be due to nonspecific binding, rather than the formation of adducts. Sega et al. (1990) reported that in mice that received a single intraperitoneal injection of 46 mg/kg of acrylamide, that alkylation increased in the testis and liver (the only tissues evaluated); however, as in the studies cited above, specific DNA adducts were not characterized and the authors attributed the observed results to glycidamide, rather than acrylamide. Segerback and co-workers (1995) identified N-7-(2-carbamoyl-2-hydroxyethyl)guanine adducts in the tissues examined (liver, lung, kidney, spleen, brain and testis) in rats and mice following a single intraperitoneal injection of acrylamide (46 mg/kg or 53 mg/kg, respectively). The formation of adducts in mice were generally higher than in rats.

3.6 Pharmacokinetic Models

An initial attempt at developing a physiologically-based pharmacokinetic (PBPK) model in the rat was conducted by (Kirman 2002). This model was developed using existing data from six published studies and provides a good description of the kinetics for both acrylamide and glycidamide. However, the authors noted that because of the uncertainty surrounding the mode of action by which acrylamide produces carcinogenic effects, a Monte

Carlo analysis was conducted to perform sensitivity analyses for each of four internal dose measures: genotoxicity, sulfhydryl reactivity, dopamine agonist, and glutathione depletion. The results indicated that the most important contributors to variation in dose metrics were the model parameters characterizing metabolism via cytochrome P450 and glutathione-S-transferase and tissue binding.

Calleman et al. (1992) developed a pharmacokinetic model in order to estimate tissue doses of acrylamide and glycidamide, specifically area under the blood concentration curve. Both a linear and a nonlinear model were developed, with the nonlinear model including saturable metabolism of acrylamide to glycidamide. The metabolic parameters (V_{max} and K_m) associated with the conversion of acrylamide to glycidamide were estimated by applying the models to hemoglobin adduct levels measured in rats following single injections of acrylamide ranging from 0.5 to 100 mg/kg body weight. In the linear model, first order elimination rates of 0.50 and 0.48 $(hr)^{-1}$ were estimated for acrylamide and glycidamide, respectively. For the nonlinear model, a V_{max} of 19.1 $(hr)^{-1}$ and a K_m of 66 µM was estimated for metabolic conversion of acrylamide to glycidamide, with first order rates of elimination of 0.21 $(hr)^{-1}$ and 0.48 $(hr)^{-1}$ estimated for acrylamide and glycidamide, respectively. The use of the models for high-to-low dose extrapolation could not be conducted due to a lack of information as to which of the two electrophilic agents, acrylamide or glycidamide, is primarily responsible for the observed toxicity.

4.0 Acute Toxicity

Based on the results of acute toxicity studies, the oral LD50 for acrylamide is approximately 100-150 mg/kg in mice, rabbits, guinea pigs and rats (McCollister et al. 1964; Paulet 1975; Hashimoto et al. 1981).

5.0 Genotoxicity

5.1 Mutagenicity

The genetic toxicity of acrylamide has been evaluated in a variety of *in vitro* and *in vivo* assays. The results of genotoxicity studies conducted in bacterial cell systems consistently demonstrated that acrylamide was not mutagenic in these systems, with or without metabolic activation (Zeiger et al. 1987; Knaap et al. 1988; Tsuda et al. 1993). Similarly, when acrylamide was incubated with mammalian cells *in vitro*, mutation frequency was not increased at non-cytotoxic concentrations (Knaap et al. 1988; Tsuda et al. 1993). For a discussion of the potential mutagenicity in germ cells, see Section 8.3.

5.2 Chromosomal Aberrations

In vitro addition of acrylamide to Chinese hamster V79 cells induced chromosomal aberrations at a concentration of ≥ 0.142 mg/mL after 20 hours of treatment, but not at lower concentrations (= 0.71 mg/mL) (Tsuda et al. 1993). In Chinese hamster lung cell lines DON:Wg3h and LUC2 p5, acrylamide induced alterations in cell divisions at concentrations of ≥ 0.5 mg/mL (Warr et al. 1990). Significant increases in the frequency of tetraploid cells were also observed in LUC2 p5 incubated with acrylamide at concentrations of 0.5 mg/mL, but not at lower concentrations (= 0.25 mg/mL). Polyploidy and spindle disturbances were observed in Chinese hamster V79 cells at concentrations of 0.071 mg/mL or 0.01 mg/mL, or greater, respectively (Adler et al. 1993; Tsuda et al. 1993).

An increase in the incidence of chromosomal aberrations in bone marrow cells was noted in mice exposed to acrylamide via intraperitoneal injection at doses of ≥ 50 mg/kg (Adler et al. 1988; Chiak and Vontorkova 1988). However, there were no significant increases in the frequency of chromosomal aberrations in lymphocytes of mice exposed intraperitoneally to acrylamide at doses up to 125 mg/kg (Backer et al. 1989) or in splenocytes from mice that received 100 mg/kg (Kligerman et al. 1991).

Acrylamide increased the micronucleus frequency in bone marrow cells in mice following doses of ≥ 25 mg/kg (two doses) or a single dose of 50 mg/kg (Adler et al. 1988; Chiak and Vontorkova 1988; Knaap et al. 1988; Chihak and Vontorkova 1990). Significant increases in micronucleus frequency were also reported in reticulocytes and splenic lymphocytes from mice that were exposed to acrylamide at doses of 50 mg/kg or more via intraperitoneal injection (Backer et al. 1989; Kligerman et al. 1991; Russo et al. 1994). However, the micronucleus frequency in reticulocytes collected from rats that received a single dose of 100 mg/kg was not significantly different from the controls (Paulsson et al. 2002a).

Moore et al.(1987)reported an *in vitro* dose-response evaluation of the clastogenicity of acrylamide in L5178Y mouse lymphoma cells. In that study, acrylamide treatment promoted the formation of small-colony mutants, which represent chromosomal alterations to the chromosome carrying the tk (thymidine kinase) locus. In contrast, production of large-colony mutants, representing single-gene mutations, was not significantly increased. The dose-response curve for the small-colony mutants has a very shallow slope at low doses, with a significant point of inflection at which the dose-response curve becomes steep. This suggests the clastogenicity of acrylamide may be nonlinear, or have a very shallow slope that at low doses may be indistinguishable from the background response in this cell line. Moreover, the significant increases in small colony mutants occurred only at cytotoxic doses.

5.3 Sister Chromatid Exchange (SCE)

In *in vitro* studies, acrylamide increased the frequency of SCEs in Chinese hamster V79 cells at concentrations of ≥ 0.3 mg/mL without metabolic activation and ≥ 1 mg/mL in the presence of S9 metabolic activation (Knaap et al. 1988; Tsuda et al. 1993). *In vivo* SCE assays have indicated that acrylamide increased the frequency of SCE in splenocytes and splenic lymphocytes collected from mice that received a single intraperitoneal injection of ≥ 50 mg/kg (Backer et al. 1989; Kligerman et al. 1991). In a study by Knaap et al. (1988), it was reported that when incubated with S9 metabolic activation, a higher concentration of acrylamide was required to induce SCEs, than when acrylamide was incubated without S9 activation.

5.4 DNA Repair and Unscheduled DNA Synthesis

Acrylamide induced a dose-related increase in the percentage of cells in repair in a rat hepatocyte DNA repair test at a concentration of = 355.5 µg/ml (Barfknecht et al. 1988). However, Butterworth et al. (1992) reported that acrylamide did not yield a DNA repair response at higher concentrations (= 710.9 µg/ml), following *in vitr*o evaluation using primary rat hepatocytes. Acrylamide produced a marginal unscheduled DNA synthesis response at a concentration of 71.09 µg/ml in five different samples of human mammary epithelial cells (HMEC), but not in the HMEC strain 184 cell line (passage 9 cells). Acrylamide did not

induce DNA repair in rats that received a single dose of 100 mg/kg or repeated doses of 30 mg/kg for 5 days (Butterworth et al. 1992).

5.5 Cell Transformation

Acrylamide induced cell transformation in various cell lines; however, the requisite concentrations varied with each cell line. SHE cells and mouse BALB/c3T3 cells were transformed at higher concentrations (0.35 mg/mL and 0.71 mg/mL, respectively) than mouse C3H/10T1/2 clone 8 cells (0.05 mg/mL) or mouse NIH/3T3 cells (0.013 mg/mL) (Banerjee and Segal 1986; Tsuda et al. 1993; Park et al. 2002). Park et al. (2002) reported that co-treatment with 1-aminobenzotriazole (ABT), a nonspecific P450 inhibitor, had no effect on the transformation frequency of 0.5 or 0.7 mM acrylamide. Co-treatment with DL-buthionone-[S,R]-sulfoxime (BSO), a selective inhibitor of ?-glutamylcysteine synthetase, the rate-limiting step in GSH synthesis, resulted in a significant increase in the transformation frequency in acrylamide-treated cells. Both the parent and the metabolite are conjugated and eliminated by GSH. Acrylamide-induced cell transformation frequency was significantly decreased when co-treated with N-acetyl-L-cysteine, a thiol donor. Co-treatment with 17-β-estradiol resulted in a significant increase in transformation frequency. The authors noted that this increase was higher than additive. Based on these results, the authors concluded that acrylamide, rather than an oxidative metabolite, was responsible for SHE cell transformation observed. The authors suggested that the cell transformation may have been achieved following GSH depletion.

5.6 Genetic Toxicity of Glycidamide

A very limited number of studies that evaluated the potential genetic toxicity of glycidamide were available. Glycidamide was mutagenic in Salmonella strains TA100 and TA1535 ± S9 activation at 5000 µg/plate (Hashimoto and Tanii 1985). In an *in vitro* gene mutation assay conducted with mouse lymphoma L5178Y TK $^{+/-}$ cells, glycidamide was positive at a concentration of 2.5 mM without metabolic activation (Barfknecht et al. 1988). When tested in unscheduled DNA synthesis assays, glycidamide was negative in primary rat hepatocytes at a concentration of 4 mM (Barfknecht et al. 1988), but was positive in primary hepatocytes isolated from male Fischer 344 rats (1 mM) and in human mammary epithelial cells (Butterworth et al. 1992). In a recent study by Paulsson et al. (Paulsson et al. 2002b), the administration of glycidamide to mice resulted in dose-dependent increases in micronucleus frequency in CBA mice; however no response was observed in Sprague-Dawley rats.

5.7 Summary of Genotoxicity of Acrylamide

The results of the genetic toxicity studies with acrylamide provide evidence that acrylamide is not a direct acting mutagen in bacterial or mammalian cell assay systems. Acrylamide does, however, have weak clastogenic effects. The clastogenic effects of acrylamide are likely mediated by binding with sulfhydryl groups on proteins rather than effects on DNA (see discussion on germ cells below).

6.0 Carcinogenicity

6.1 Epidemiological Studies

Exposures to acrylamide may occur via the inhalation, oral or dermal routes. In the manufacture of polyacrylamides, workers may be exposed via inhalation. However,

exposures via the oral (food and water) and dermal (use of polyacrylamide-containing products) routes may also occur. Currently, OSHA PEL for acrylamide is 0.3 mg/m^3. The NIOSH REL and the ACGIH TLV for acrylamide are 0.03 mg/m^3.

There have been epidemiological studies that consist of cohort mortality studies in workers exposed to acrylamide. A mortality study of a cohort of 8,854 male acrylamide factory workers, 2293 of whom were exposed to acrylamide, was reported by Collins et al. (1989) and updated by (Marsh et al. 1999). Four factories were evaluated – three in the United States and one in the Netherlands. The cohort consisted of workers that were hired between January 1, 1925 and January 31, 1973. Smoking habits were unknown for most of the cohort. Exposure was estimated from available monitoring data and by recall of plant workers with knowledge of past processes. Exposure to acrylamide was defined as a cumulative exposure of greater than 0.001 mg/m^3-years. Mortality rates were compared to the expected rates for the United States and the Netherlands. The follow-up period extended through 1994, at which time the total number of person-years of follow-up was greater than 287,000 (Marsh et al. 1999). There were no statistically significant excesses of mortality due to cancer of any specific site in the study cohort when compared with the expected rates. The increase in respiratory tract cancer at one of the plants reported by Collins et al. (1989) remained increased in the follow-up (Marsh et al. 1999). However, this finding was again confined primarily to the workers exposed to muriatic acid. The results of an exposure-response analysis indicated an excess of deaths due to pancreatic cancer in workers with exposures to acrylamide at ambient concentrations greater than 0.30 mg/m^3-year, but not at the lower exposures. However, the authors noted that smoking histories were not available for each member of the cohort and that smoking is a major risk factor for the development of pancreatic cancer. The exposure-response analysis indicated no correlation between acrylamide exposure and mortality from cancer of the esophagus, rectum or kidney. Marsh et al. (1999) concluded that the results of this study provided little evidence of a relationship between acrylamide exposure and mortality due to cancer.

6.2 Carcinogenicity Bioassays

6.2.1 Studies in Rats

Two chronic bioassays have been conducted in which male and female Fischer 344 (F344) rats were administered acrylamide in drinking water for two years (Johnson et al. 1986; Friedman et al. 1995). In the bioassay conducted by Johnson et al. (1986), groups of Fischer 344 (F344) rats (60/sex/group) received acrylamide-treated drinking water that provided doses of 0, 0.01, 0.1, 0.5 or 2 mg acrylamide/kg/day for two years. Additional groups of 10 rats/sex/group were sacrificed after 6, 12 or 18 months of exposure. Food and water consumption and body weights were evaluated weekly for the initial 3 months of the study. Thereafter, body weights were collected monthly and food and water consumption were collected weekly. Blood was collected for hematological and clinical chemistry evaluation at 3, 6, 12, 18 and 24 months. At necropsy, organ weights were collected and the major organs were preserved for microscopic examination.

The study of Friedman and co-workers (1995) was similar with a few exceptions: fewer low-dose groups, a higher dose group for females, a larger number of animals per dose group, and inclusion of sentinel animals to screen for specific pathogens were used. In the Friedman et al. (1995) study, acrylamide was administered to groups of male F344 rats via the drinking water to provide doses of 0 (204 animals), 0.1 (204 animals), 0.5 (102 animals), or 2

(75 animals) mg/kg/day. Female rats received doses of 0 (100 animals), 1 (100 animals) or 3 (100 animals) mg/kg/day.

The primary observations of these studies were significant increases in the incidence of tumors primarily in hormonally-responsive organs, including tunica vaginalis mesotheliomas in male rats, mammary gland fibroadenomas in female rats and thyroid follicular cell adenomas in male and female rats (Tables 1 and 2). There was also a suggestion of an increase in the incidence of uterine adenocarcinomas, mammary gland adenocarcinomas, and oral papillomas in female rats and pheochromocytomas in male rats in the Johnson et al. study (1986), but not in the Friedman et al. study (1995). The incidence of glial cell tumors was increased in the Johnson et al. study (1986) study when the incidences of all glial tumors and glial cell proliferation were combined. This was not observed in the Friedman et al. study (1995) study.

Tunica vaginalis mesotheliomas. In the Johnson et al. study (1986), a statistically significant increase in the incidence of tunica vaginalis mesotheliomas was observed in male F344 rats that received 0.5 or 2 mg/kg/day. However, Friedman and co-workers (1995) only observed an increase in the incidence of tunica vaginalis mesotheliomas in male rats exposed to 2 mg/kg/day (Table 1). In the lower-dose groups in both studies, the incidence of tunica vaginalis mesotheliomas was comparable to the incidence reported in the respective control groups.

Mammary gland tumors. The incidence of mammary gland adenocarcinomas was not significantly increased in any acrylamide dose group in either study compared with respective controls (Table 2). There was a suggestion of a dose-response for mammary gland adenocarcinomas by Johnson and co-workers based on the results of a trend test (Table 2). However, the dose-response for mammary gland adenocarcinomas was relatively flat and incidence rates for each dose group were comparable to historical control rates, which range from about 2% in two-year bioassays (Goodman et al. 1979; Maekawa et al. 1983; Solleveld et al. 1984; Haseman et al. 1990) to 11% in full life-span studies (e.g., without a planned terminal sacrifice time) (Solleveld et al. 1984).

Dose-response curves for the incidence of mammary gland adenomas and mammary gland fibromas were also flat (Table 2). The incidence of adenomas noted in the Johnson et al. study at 0.5 and 2.0 mg/kg/day of acrylamide was not significantly increased compared to controls, nor was there a dose-related trend. Historical rates for adenomas in female F344 rats ranged from 1% to 4% (Goodman et al. 1979; Maekawa et al. 1983; Haseman et al. 1990). No adenomas were observed in the Friedman et al. study in the 1.0 or 3.0 mg/kg/day dose groups. There was no apparent dose-related increase in fibromas; however, the incidence of fibromas was significantly increased only in the 2.0 mg/kg/day dose group (from (Johnson et al. 1986)), when compared to the control group. This was not observed in the Friedman et al. study in the 3.0 mg/kg/day dose group. Consequently, it is likely that the observed incidence of adenomas and fibromas is representative of background events unrelated to treatment with acrylamide.

The incidence of fibroadenomas in the acrylamide-exposed groups from the Johnson et al. study was not statistically different from the response rate observed in the control. However, the incidence rates in the 1.0 and 3.0 mg/kg/day dose groups in the Friedman et al. study were significantly increased compared to their control groups. Although the incidence of fibroadenomas in all dose groups was within the historical control range reported for this response in female F344 rats [16% to 29% in two-year bioassays (Goodman et al. 1979;

Maekawa et al. 1983; Solleveld et al. 1984; Boorman et al. 1990) and up to 57% in full life-span studies (Solleveld et al. 1984)], the increase in the incidence of fibroadenomas may be related to acrylamide exposure.

Glial cell tumors. Johnson et al. (1986) reported an increase in the incidence of glial cell tumors in female rats that received 2 mg/kg/day of acrylamide, when the incidence of all glial cell tumors and glial cell proliferation were combined. The incidences of glial cell tumors are often combined, i.e., the incidence of astrocytoma may be combined with the incidence of oligodendrogliomas. However, oligodendrocytes are functionally distinguishable from astrocytes and the combination of the tumor incidence of these different cell types may be inappropriate. The incidence of glial cell proliferation was combined with the incidence of glial cell tumors in the Johnson et al. study. Use of a hyperplastic change in combination with tumor incidence should be evaluated very carefully, and it has been argued recently(Aschner 2002) that glial cell proliferation is not a preneoplastic change. Therefore, the incidence of glial cell proliferation was not combined with the tumor incidence data presented in Table 1 and 2. Because the incidence of oligodendroglioma was not related to dose, these tumors were not combined with the incidence of astrocytomas reported in Tables 1 and 2. However, these findings were not observed in the Friedman et al. study in a larger number of female rats. There was no statistically significantly increased incidence of glial cell tumors in male rats observed in the Johnson et al. or Friedman et al. studies.

Astrocytomas were observed in the brain and spinal cord of control and treated animals. However, the incidence of astrocytoma in the brain or the spinal cord was not statistically significantly increased in any treated group in either bioassay when compared to the respective controls. Only when both studies were considered was there a suggestion of a dose-response, although the response was at most a very weak one (Tables 1 and 2). The incidence of astrocytomas in the Friedman et al. and Johnson et al. studies was for the most part similar to the incidences reported in other chronic two-year bioassays in F344 rats in which the incidence rates ranged from approximately 0.1% to 4% (Goodman et al. 1979; Maekawa et al. 1983; Solleveld et al. 1984; Haseman et al. 1990; Haseman et al. 1998). Therefore, it is likely that the incidence of astrocytomas was not related to acrylamide treatment.

Thyroid tumors. In male rats, the incidence of thyroid follicular cell adenomas in both bioassays was significantly increased in the high-dose (2 mg/kg/day) groups (Table 1). However, when combined, the incidence of follicular cell adenoma/adenocarcinoma was significantly increased in the high-dose males of both bioassays.

In female rats, the incidence of follicular cell adenoma was significantly increased in the 1 and 3 mg/kg/day dose groups of the Friedman et al. bioassay (Table 2); however, the incidence of follicular cell adenoma was not significantly increased in female rats that received 2 mg/kg/day (Johnson et al. 1986). The incidence of follicular cell adenocarcinoma was not significantly increased in female rats in either bioassay. As observed in male rats, the combined incidence of adenoma/adenocarcinoma was significantly increased in the 1 and 3 mg/kg/day groups, but not in the 2 mg/kg/day group.

Other tissues. In the Johnson et al. study, significant increases in the incidence of papillomas of the oral cavity and uterine adenocarcinomas were reported in the high-dose (2 mg/kg/day) females. However, only one uterine adenocarcinoma was reported by Friedman et al., and there were no statistically significant increases in the incidence of papillomas of the oral cavity in any treated group (male or female). Because acrylamide is irritating, it is

possible that the oral cavity papillomas were the result of continued irritation. An increase in the incidence of pheochromocytoma was reported in the high-dose males in the Johnson et al. study; however, the incidence of these tumors was not increased in the Friedman et al. study or in female rats in either study. Given that the incidence of these tumors were observed in only one study and/or in one sex, the incidence of these tumors may have been biological variation and not related to acrylamide treatment.

6.2.2 Studies in Mice

In addition to the carcinogenicity bioassays conducted in rats (Johnson et al. 1986; Friedman et al. 1995), nonstandard carcinogenicity assays (e.g., mouse skin painting initiation/promotion studies) have been have been conducted in mice (Bull et al. 1984a; Bull et al. 1984b; Robinson et al. 1986). Increases in lung tumors were reported in the Bull et al. (1984a; 1984b) studies. However, the results of the studies by Bull et al. (Bull et al. 1984a; Bull et al. 1984b) are limited. In one study, the diagnosis of lung tumor was based on gross necropsy observations and was not confirmed microscopically (Bull et al. 1984a). In the second Bull et al. study (1984b), lung tumor yield was provided; however, the incidence of lung tumors was not included. In the study by Robinson et al. (1986), lung tumor incidence was increased only in SENCAR mice, a strain of mice genetically predisposed to the formation of tumors, and not in the BALB/c, A/J or ICR strains. However, the doses used in this study were lower than the doses used by Bull et al. In all studies, skin tumor yields were increased only following the application of a promoting agent, such as 12-O-tetradecanoylphorbol-13-acetate (TPA).

7.0 Neurotoxicity

7.1 Epidemiological Studies

Multiple studies have been conducted to assess neurotoxic effects of acrylamide in occupationally-exposed workers in small factories manufacturing acrylamide or using acrylamide in production in China (He et al. 1989; Deng et al. 1993) and South Africa (Myers and Macun 1991; Bachmann et al. 1992). He et al. (1989) examined 71 workers (45 men and 26 women) between the ages of 17 and 41 exposed to acrylamide by industrial production facilities and had been employed from 1 to 18 months. Fifty-one unexposed workers (33 men and 18 women) served as the reference group. Approximately 73% of the workers exhibited symptoms of acrylamide poisoning. Early symptoms of acrylamide exposure were skin peeling from the hands followed by weak legs and numb hands and feet, and impairment of the vibration sensation in the toes and loss of ankle reflexes. Electroneuromyographic changes included a decrease in the sensory action potential amplitude, neurogenic abnormalities in electromyography and prolongation of the ankle tendon reflex latency. Most of the workers diagnosed with signs or symptoms had handled a 27 to 30% aqueous solution of acrylamide monomer.

A second group of Chinese workers were examined who had been exposed to acrylamide in a chemical factory at concentrations ranging from 0.20 to 1.58 mg/m^3 for 0.5 to 8 years (Deng et al. 1993). Vibration thresholds were significantly higher in acrylamide-exposed workers than those of the 105 healthy unexposed adults in the same age group.

In 1985 in a South African factory in which polyacrylamide flocculants were manufactured, five cases of peripheral neuropathy were diagnosed. These cases led to an industrial hygiene and neurologic evaluation of the remaining 66 exposed and unexposed

workers in that factory (Myers and Macun 1991). Acrylamide monomer concentrations in the factory ranged from 0.02 to 0.75 mg/m^3. The mean duration of exposure was 2 years. The overall prevalence of acrylamide-related abnormalities among the exposed group was approximately 67%, compared to approximately 14% in the unexposed group. Most of the subjects with abnormalities came from the two areas where the air concentration of acrylamide monomers was highest.

In a follow-up to the Myers et al. (1991)study by Bachmann et al. (1992), 75 workers from the same South African factory were examined. Significantly higher prevalences of numbness, limb pain, and peeling and sweating of hands were observed in exposed workers, compared to unexposed. No gross neurological abnormalities were reported and no association was found between vibration thresholds and exposure.

7.2 Neurotoxicity Studies in Animals

The neurotoxicity of acrylamide has been extensively studied in various animal models, including rats, mice, monkeys, dogs and cats, and by numerous dosing regimens and durations of dosing. Overt signs of neurotoxicity were consistent across species. However, while some studies evaluated only overt neurotoxicity (grip strength or leg splay), other studies examined only morphological or biochemical changes in nerve tissues. In some studies, overt signs of neurotoxicity were not accompanied by signs of morphological changes in key peripheral nerves. Few if any of studies examined the time course of acrylamide toxicity as manifested by both central and peripheral changes in biochemical function and morphology along with overt signs of peripheral neuropathy. For the purposes of this report, studies by the oral route of exposure are summarized below.

In general, cumulative dose is important when overt neurotoxicity is evaluated. In rats exposed via the oral route, single doses of ≥ 100 mg/kg were reported to result in alterations in grip strength and motor function (Fullerton and Barnes 1966; Tilson and Cabe 1979). With short-term multiple exposures, clinical signs of toxicity and deceased motor function were reported in rats following the administration of 25 mg/kg/day for 21 days (Dixit et al. 1981a; Aldous et al. 1983). In a subchronic toxicity studies, effects on rotorod performance were observed at doses ≥ 14.5 mg/kg (Tanii and Hashimoto 1981) and hindlimb splay was increased in rats that received ≥ 9 mg/kg (McCollister et al. 1964; Burek et al. 1980). Burek et al. (Burek et al. 1980) also reported increases in morphological changes following electron microscopic examinations of peripheral nerves of rats that received ≥ 1 mg/kg/day for 90 days (it should be noted that nerves of only 3 rats/dose group were examined with electron microscopy). These effects were reversible after 144 days of recovery, with the exception of the group that received 20 mg/kg/day of acrylamide. There were no changes observed following electron microscopic examination in rats that received 0.05 or 0.2 mg/kg/day. In two year chronic toxicity/carcinogenicity bioassays, no signs of overt neurotoxicity based on cage side observations were reported, but significant increases in the incidence of peripheral nerve degeneration was observed in rats that received ≥ 2 mg/kg/day for two years (Johnson et al. 1986; Friedman et al. 1995).

In mice, decreases in rotorod performance were reported following repeated administration of ≥ 9 mg/kg (Hashimoto et al. 1981; Gilbert and Maurissen 1982). The administration of 10 mg/kg/day to monkeys for approximately 45 to 60 days resulted in

evidence of loss of motor function (Maurissen et al. 1983; Eskin et al. 1985; Maurissen et al. 1990). Overt evidence of neurotoxicity and decreased nerve conduction velocity was reported in cats that received doses of 15 mg/kg daily for 4-16 weeks (Post and McLeod 1977). In dogs that were administered 5.7 or 7 mg/kg/day for 42 days, clinical signs of neurotoxicity (muscle weakness, hind limb ataxia) were reported (Satchell and McLeod 1981; Hersch et al. 1989).

Recent studies (LoPachin 2000) measuring multiple neurological parameters (gait, foot splay, grip strength and extensor thrust) across two intoxication schedules (21 and 50 mg/kg) have indicated that acrylamide produces cumulative neurotoxicity. Specifically, dose-rate did not determine final magnitude of neurological deficit (i.e., both dose-rates caused statistically similar maximal changes in each neurological parameter measured), instead dose-rate determined the time of onset and development of neurotoxicity (i.e., maximal neurological deficits were observed on day 11 of the 50 mg/kg dose-rate, whereas a similar level of neurotoxicity was achieved on day 40 of the 21 mg/kg exposure rate38. Research from other laboratories suggests that this cumulative phenomenon applies to dose-rates significantly lower than those used in the aforementioned study (Edwards 1977; Moser 1992; Shell 1992; Crofton et al. 1996).

Morphologic examinations (Leswing 1969; Prineas 1969; Hopkins 1970; Hopkins 1971; Suzuki 1973; Schaumburg 1974; Spencer 1974; Sumner 1974; Sumner 1975; Gold 1988) have revealed that low-dose (dose-rates reviewed in Barber et al. (2001)) subchronic induction of acrylamide neurotoxicity was associated with nerve damage in both the central and peripheral nervous systems. The morphologic hallmark of this toxic neuropathy was considered to be distal nerve terminal and preterminal axon swellings of the longest myelinated fibers (Prineas 1969; Suzuki 1973; Schaumburg 1974). As exposure continued, progressive retrograde degeneration of these distal axon regions ensued with preservation of more proximal segments (reviewed in Spencer and Schaumberg (1974)). Early morphologic and electrophysiologic research (Schaumburg 1974; Sumner 1974; Sumner 1975) suggested that sensory axons and their receptors (i.e., Pacinian corpuscle, annulospiral terminal, Golgi tendon) developed neuropathic changes before motor neurons. However, other studies indicated that both sensory and motor systems were equally vulnerable to acrylamide-induced damage (Fullerton and Barnes 1966; Prineas 1969; Hopkins 1971; Tsujihata 1974; Lowndes 1976).

Although the relative vulnerabilities of the sensory vs. motor systems were debated, the pattern of neuropathological expression induced by acrylamide (i.e., initial nerve terminal damage and subsequent retrograde axon degeneration) was consistent with the theory of toxic "dying-back" neuropathies proposed by Cavanagh (Cavanagh 1964; Cavanagh 1979; Calleman 1996). However, other evidence implied that the dying-back label did not adequately describe the neuropathy caused by acrylamide. For example, Spencer and Schaumburg (Schaumburg 1974; Spencer 1977b; Spencer 1977a) reported that degeneration did not start at the nerve terminal and move rostrally in a seriatim fashion as stipulated by the dying-back hypothesis. Instead, degeneration "bloomed" simultaneously at multifocal sites along distal preterminal axons. Work by Jennekins et al., (1979) indicated that nerve terminals of long axons were not preferentially damaged by acrylamide intoxication as was predicted by the dying-back theory. Considerable other evidence indicated that the "dying back" was not a primary effect (Fullerton and Barnes 1966; Morgan-Hughes 1974; Griffin

1977; Cavanagh 1982; Sterman and Sposito 1985; DeGrandchamp 1990a; DeGrandchamp 1990b; Myall et al. 1990; Gold 1991).

Spencer and Schaumburg (1976) formulated a hypothesis that emphasized direct axonal injury. They proposed that large diameter axons in the CNS and PNS were most sensitive to development of simultaneous, multifocal paranodal axon swellings in distal regions and that these swellings served as initiation points for subsequent degeneration. In the PNS, acrylamide preferentially affected axons in tibial nerve branches supplying calf muscles, plantar sensory nerves innervating the digits and plantar nerve branches supplying the flexor digitorum brevis muscle (Spencer 1977a). Axon swelling and degeneration were noted in certain CNS regions; e.g., dorsal spinocerebellar tract, gracile fasciculus, cerebellar white matter (Prineas 1969; Ghetti 1973; Spencer 1977a). The characteristic spatiotemporal pattern of axon damage in the central and peripheral nervous systems lead Spencer and Schaumburg (1976) to classify acrylamide neuropathy as a "central-peripheral distal axonopathy".

Other morphological evidence has indicated that early nerve terminal damage might be importantly involved in the pathophysiological process leading to acrylamide neurotoxicity (Prineas 1969; Tsujihata 1974; Cavanagh 1982; DeGrandchamp 1990a; DeGrandchamp 1990b). Electrophysiological studies by Goldstein and Lowndes (Lowndes 1976; Lowndes 1978b; Lowndes 1978a; Goldstein 1979; Goldstein 1981; Goldstein 1985; DeRojas 1987) showed that neurotransmission was impaired at spinal cord primary afferent nerve terminals as an early consequence of acrylamide intoxication of cats (see also Tsujihata et al. (1974)). Recent quantitative morphometric research showed that peripheral nerve axon degeneration was not linked to the expression of neurological deficits, but rather appeared to be an exclusive product of lower acrylamide dose-rates (< 21 mg/kg/d); i.e., intoxication at a higher dose-rate (50 mg/kg/d) did not produce peripheral axon degeneration (Lehning et al. 1994; Lehning et al. 1998). This suggested that the axonopathic effect of acrylamide was an epiphenomenon and that the neurotoxicologically significant site of action was elsewhere (reviewed in (LoPachin 2000; LoPachin et al. 2002)). Based on evidence of early structural and functional damage, LoPachin et al. (2002) suggested that nerve terminals were the primary site of acrylamide action and that synaptic dysfunction and subsequent degeneration were necessary and sufficient steps for production of acrylamide neurotoxicity. Corroborative research using the de Olmos silver stain method to detect neurodegeneration has shown that higher dose-rate intoxication (50 mg/kg/day) produced a selective terminalopathy characterized by very early, widespread nerve terminal degeneration in rat PNS (NMJ) and CNS (brain and spinal cord) (Lehning 2002c; Lehning 2002a; Lehning 2002b). Intoxication of rats at a lower dose-rate (21 mg/kg/day) caused initial nerve terminal degeneration in PNS and CNS, which was followed by axon degeneration. Together, these data (Lehning et al. 1998; Lehning 2002c; Lehning 2002a; Lehning 2002b) suggest that, regardless of dose-rate, acrylamide causes initial, generalized nerve terminal degeneration. The early appearance of this effect suggests that nerve terminals are a primary site of action. In contrast, axon degeneration in PNS and CNS was a delayed event and occurred only during lower dose-rate intoxication (Lehning et al. 1998; Lehning 2002c; Lehning 2002a; Lehning 2002b). Because full neurotoxicity can develop at the higher and lower dose-rate used in these studies (LoPachin 2001), the conditional, dose-rate dependent expression of axon degeneration indicates that this effect is not a significant neurotoxicological event (LoPachin 2000; LoPachin et al. 2002). The axon orientation of formative morphological investigations (e.g., Spencer and Schaumburg (1977b; 1977a)) is likely due to a focus on subchronic acrylamide

dosing schedules (reviewed in (LoPachin et al. 2002)). Recent quantitative morphometric studies of PNS and silver stain analysis of CNS (see above discussion) have confirmed that axon degeneration is associated with low dose-rate acrylamide intoxication. However, these studies also demonstrate that intoxication at higher dose-rates does not induce axon degeneration. A growing awareness of dose-rate impact on neurotoxicological expression (reviewed in LoPachin 2000) and advances in computer-assisted quantitative morphometrics and histochemical techniques (de Olmos silver stain) have contributed to the changing view of axon and nerve terminal damage in acrylamide neurotoxicity.

An alternative mechanism proposed by Sickles et al. (1996; 2002) is the inhibition of kinesin, the motor protein for anterograde axonal transport, and the resultant decreases in anterograde axonal transport are critical components in the neurotoxicity of acrylamide (reviewed in Sickles et al. (2002)). In a microtubule motility assay, acrylamide (0.1mM) inhibited kinesin. Several studies (see Table 1 in Sickles et al. (2002)), including *in vivo* studies in rats and mice and *in vitro* studies in primary cultured neurons have reported significant reduction in fast axonal transport with acrylamide exposure. The inhibition of axonal transport produces deficient delivery of macromolecules to the distal axons and nodes of Ranvier, which has been associated with decreases in a fast-transported form of acetylcholinesterase into muscle; decreases in GAP-43 within distal neuritis of primary cultured neurons; decreased quantity of synaptic vesicles and mitochondria within neuromuscular junctions; axonal accumulation of fast transported material; progressive decrease in synaptophysin in neuromuscular junctions with high and low dosing rates; decreases in Na and K channels in nodes of Ranvier that are greater in the distal tibial nerve than the more proximal sciatic nerve and occur with either low or high dosing rates. In addition, similarities of acrylamide intoxication and Drosophila models of kinesis mutants have been reported.

This hypothesis has been challenged by the observations that with high dosing rates, acrylamide produced neurological deficits but did not produce changes in axonal elemental composition, Na/K ATPase activity, sensitivity to anoxia and degeneration. Since these functions are supported by fast axonal transport, it is reasonable to expect a reduction. However, it is also reasonable to expect a differential sensitivity to acrylamide intoxication. The changes in total elements (not ions), Na/K ATPase activity, etc., are correlated with degeneration and therefore may be expressed only under severe conditions, just prior to, or during, regeneration. The mechanistic explanation for differential reductions may be based upon differential effects on kinesin superfamily members, turnover rates of proteins, quantities of each protein, thresholds for functional loss, or safety factors in delivery.

8.0 Effects on the Reproductive System

8.1 Epidemiological Studies

No studies were located that evaluated the potential effects of acrylamide on the human reproductive system.

8.2 Reproductive Toxicity Studies in Animals

Acrylamide has been evaluated for reproductive toxicity in multigenerational studies in rats (Tyl et al. 2000a) and mice (Chapin et al. 1995) and in cross-over breeding studies in rats (Zenick et al. 1986; Tyl et al. 2000b) and mice (Sakamoto and Hashimoto 1986; Chapin et al. 1995). The results of all of the above studies are highly consistent. Acrylamide

administered in drinking water or by gavage to rats or mice at doses equal to or greater than 5 mg/kg/day resulted in significant increases in both pre-implantation and post-implantation losses with resulting significant decreases in the number of live pups per litter (Chapin et al. 1995; Tyl et al. 2000a). These effects were seen in both the F_0 and F_1 generations, with the percentage decrease in the number of live pups per litter similar in the F_0 and F_1 generations in rats (approximately 34% versus 23% in F_0 and F_1, respectively). In mice the effects were more pronounced in the F_1 than the F_0 generation (11% compared to 47%). However, doses of acrylamide less than 15 mg/kg/day did not result in significant changes in:

- clinical signs of toxicity (some reduction in body weight in rats);
- overt signs of neurotoxicity (as measured by heat tilt or leg splay);
- indices of reproductive performance (mating, pregnancy, fertility) or the number of corpora lutea;
- organs weights or signs of histological or neuropathology changes;
- sperm parameters (concentration, motility, morphology); or
- estrus cyclicity (only mice evaluated).

At higher doses (15 mg/kg/day or greater), signs of neurotoxicity and changes in copulatory behavior were noted as well as effects on sperm motility and morphology. At these doses in both mice and rats, acrylamide resulted in decreases in fertility, in addition to changes in pre- and post-implantation loses (Zenick et al. 1986; Tyl et al. 2000b).

Cross-over matings of treated males with untreated females demonstrated that pre- and post-implantation losses, with resulting decreases in the number of live pups/fetuses per litter, could be attributed to effects on males (Sakamoto and Hashimoto 1986; Zenick et al. 1986; Chapin et al. 1995). In a continuous dosing/breeding protocol in mice (4 to 5 litters per breeding pair), the mean number of live pups per litter across litters from the first to the last litter were similar, indicating the absence of progressive reproductive toxicity (Chapin et al. 1995).

Some data suggests that exposure of females may contribute to reproductive toxicity. A delay in vaginal patency was noted in offspring of treated females (14 mg/kg/day, 70 days) mated to untreated male (Zenick et al. 1986) suggesting that perinatal exposure of female pups might result in subtle alterations in the estrogen/progesterone balance. Further, a slight, but significantly higher number of resorptions was seen at gestation day 13 in treated females (19 mg/kg/day, 42 days); however, the number of live pups per litter was comparable to controls (Sakamoto and Hashimoto 1986). Lastly, Tyl et al. (2000a) noted that post-implantation loss at the F0 mating (34.3%) was higher than that seen in the dominant lethal mating (24.9%) when administered the same dose (5 mg/kg/day), suggesting the possibility that the female was contributing to this decrease. Any increase in fetal loss from gestation days 13 to 21 likely reflects the continued influence of male dominant lethal effects (see below). Also, the percentage post-implantation loss was similar in the F_1 generation (23.1%).

8.3.1 Germ Cell Mutagenicity Studies

8.3.2 Germ Cell UDS, DNA Strand Breaks, Chromosomal Aberrations, Micronuclei, SCE, and Protamine Alkylation

In germ cells studies, serial recovery of sperm from the caudate epididymides that were at the early spermatocyte (pre-meiotic/meiotic) stage to the spermatozoa (post-meiotic) stage at the time of treatment showed no increase in unscheduled DNA synthesis (UDS); these stages correspond to those associated with dominant lethality (Sega et al. 1990). In contrast,

DNA strand breaks were increased in these sensitive stages. DNA strand breakage in sperm in earlier stages of development at the time of treatment decrease with time, likely due to DNA repair, prior to becoming functional spermatozoa. Slight increases in DNA strand breaks in isolated human testicular cells have been reported; however, there was considerable variation and no dose response even at the highest concentration 1000 μM (Bjorge et al. 1996). Further, chromosomal aberrations and sister chromatid exchanges were not found in spermatogonia or early spermatocytes in contrast to positive results in late spermatids and spermatozoa (Adler et al. 1988; Backer et al. 1989). In a micronucleus test, weak clastogenic activity was noted in rat primary spermatocytes (100 mg/kg) (Xiao and Tates 1994). Further studies of DNA, total sperm head, and sperm protamine alkylations suggest that the stage specificity can be explained by preferential binding to cysteine sulfhydryl groups in sperm protamine (Sega et al. 1989). In mid-to late spermatid stages, chromosomal histones are replaced by protamines that are relatively rich in arginine and cysteine. Alkylation of free sulfhydryl groups of cysteine in the "immature" protamine of late spermatids and early spermatozoa might prevent normal chromatin condensation leading to stress in the chromatin structure and strand breakage (Sega et al. 1989).

8.3.2 Dominant Lethal Mutation Studies

Eight dominant lethal studies have been conducted in male rats (Smith et al. 1986; Working et al.; Sublet et al. 1989; Tyl et al. 2000a) and mice (Shelby et al. 1987; Bishop et al. 1991; Adler et al. 2000) by the oral route of administration and in male mice by the intraperitoneal injection and dermal routes (Gutierrez-Espeleta et al. 1992). All studies report the induction of dominant lethal mutations by acrylamide. In the oral studies, significant decreases in fertility and increases in dominant lethality were observed in the first week post-dosing (15 mg/kg/day) up to 4 weeks post-dosing (60 mg/kg/day)(Sublet et al. 1989). No significant changes in any parameter were noted in dams that were mated with treated males more than 4 weeks post-dosing (Shelby et al. 1986; Working et al. 1987; Sublet et al. 1989). In the dermal study, significant decreases in the numbers of live fetuses per litter were noted for males receiving 5 dermal applications of 50 mg/kg or greater (up to 125 mg/kg). In males given 5 dermal applications of 25 mg/kg, the number of live embryos per female was not elevated but the per cent of dead implants per female was significantly increased. (Gutierrez-Espeleta et al. 1992). These effects consistently show that acrylamide induces dominant lethal mutations in mid-late spermatids and spermatozoa. Further, acrylamide's effects on fertility and sperm parameters following a single intraperitoneal injection of 125 mg/kg acrylamide were not blocked by pre-administration of aminobenzotriazole (ABT – an inhibitor of P-450 isoform 2E1); however, the dominant lethal effects were completely or partially blocked (depending on the ABT post-administration time of evaluation) by ABT (Adler et al. 2000). This suggests that acrylamide's dominant lethal effects are likely due to the major metabolite, glycidamide, but that the effects on fertility and sperm motility are not. This finding, along with the report by Sega et al. (1989) that dominant lethal mutations correlate with the binding of acrylamide to protamines in spermatids and spermatozoa leaves open the question of the mechanism by which dominant lethals are induced. Generoso et al (1996) reported that a single i.p. injection of 125 mg/kg glycidamide in male mice induced dominant lethal mutations in the same germ cell stages and at somewhat higher frequencies as acrylamide.

8.3.3 Heritable Translocations

Because of positive results of dominant lethal studies, the potential for acrylamide to induce heritable translocations in male mouse germ cells has been evaluated in three studies (Shelby et al. 1987; Adler et al. 1994; Marchetti et al. 1997). All three studies reported the induction of translocations. In the study by Shelby et al. (1987), male mice received 5 daily doses of 40 or 50 mg acrylamide/kg by intraperitoneal injection. The frequencies of translocation carriers among F1 males conceived 7-10 days post-treatment was 24% (40 mg) and 39% (50 mg) compared to an historical control frequency of 0.2%. Adler et al. (1994) treated male mice with a single intraperitoneal dose of 50 or 100 mg/kg. The frequencies of translocation carriers among F1 males and females conceived 7-16 days post-treatment were 0.6% (50mg/kg) and 2.7% (100 mg/kg) compared to an historical control frequency of 0.04%. However, heritable translocations were not significantly increased in male rats given up to 8 mg/kg/day for 80 days (Adler 1990). Generoso et al. (1996) reported the induction of translocations with a single i.p. injection of 100 mg/kg glycidamide, a metabolite of acrylamide. Among male offspring conceived 3.5-7.5 days post-treatment, the frequency of translocation carriers was 20.18%, compared to an historical control frequency of 0.06%. As with the dominant lethal studies, this suggests but does not confirm that the genetic damage induced in mouse germ cells following acrylamide exposure may ultimately be the result of glycidamide rather than the parent compound.

8.3.4 Specific-Locus Mutations

Two specific-locus mutation studies have been conducted to determine if acrylamide induces gene mutations in male mouse germ cells. In the first study (Russell et al. 1991), males received intraperitoneal injections of 50 mg/kg on 5 consecutive days and were then mated for up to 182 days. Five mutant offspring were recovered in the first 7 weeks of matings from among 28,971 offspring, a frequency that is significantly above the historical control. No mutant offspring were recovered from spermatogonial stem cells, i.e., from matings occurring 8 or more weeks post-treatment. This finding suggested that there was no concern for long-term genetic risks associated with mutations induced in the spermatogonial stem cell population. However, a subsequent study reported the induction of mutations in mouse spermatogonial stem cells by acrylamide (Ehling and Neuheuser-Klaus 1992). These authors confirmed that, in male mice receiving a single intraperitoneal injection of 100 mg/kg acrylamide, an increase in specific-focus mutations was induced in post-meiotic germ cell stages. But, contrary to the earlier study, they also observed an increase in mutations in spermatogonial stem cells (6 mutants out of 23489 off-spring compared to 22 in 248,413 in the historical control). These 6 mutant animals were conceived 43 days or more post-treatment (one each on mating days 43, 70, 181, 201, 234, and 439). Based on data from these two studies, it is clear that acrylamide induces gene mutations in post-meiotic male germ cells and the latter study reports mutations induced in spermatogonial stem cells.

8.3.5 Developmental Toxicity Studies

Three developmental toxicity studies are reported in rats, including one study of developmental neurotoxicity. No significant treatment-related effects on embryo/fetal viability, growth, or development or dose-related increases in fetal malformations were found

in off-spring of female rats administered up to 15 mg/kg/day on gestational days 6 to 20 (Field et al. 1990) or of female mice administered up to 15 mg/kg/day on gestational days 6 to 17 (Field et al. 1990). In the off-spring of female rats treated from gestational day 6 to lactational day 10, there were no clinical signs of toxicity and pup survival was not affected at birth or up to 21 days at maternal doses of 10 mg/kg/day or lower (Wise et al. 1995). In this study, pup body weight (preweaning) at a maternal dose of 15 mg/kg/day and reduced pup survival at birth and for days 1 to 3 at a maternal dose of 20 mg/kg/day were observed; however, maternal toxicity and signs of neurotoxicity were significant at these doses (Wise et al. 1995). Developmental neurotoxicity has been assessed in rats (Wise et al. 1995). At doses equal to or less than 10 mg/kg/day during gestation, no effects in behavioral tests (open-field motor activity, auditory startle habituation, or passive avoidance) were noted in offspring when tested either within 21 days post-natal or as adults. Decreases in catecholamine (dopamine) levels in cerebellum, pons medulla, and mid-brain but not the hypothalamus or basal ganglia were noted and were more pronounced in younger rats (12, 15, or 21 days old) than older rats (60 days) when exposed directly to acrylamide (25 mg/kg/day for 5 days) at various ages (12, 15, 21, or 60 days post-partum) (Husain et al. 1987).

ACKNOWLEDGEMENTS

This publication was made possible by support from the ILSI North America (ILSI N.A.) Project Committee on Acrylamide, whose members include: Archer Daniels Midland Company; Cargill, Incorporated; Hershey Foods Corporation; H.J. Heinz Company; A.E. Staley Manufacturing Company; Gerber Products Company; Masterfoods USA; Nestlé USA, Inc.; E.I. du Pont de Nemours and Company; Frito-Lay, Inc.; General Mills, Inc.; Kellogg Company; Kraft Foods NA; and The Procter & Gamble Company.

The Project Committee on Acrylamide is under the umbrella of the Board of Trustees of ILSI N.A. ILSI policy mandates that ILSI and ILSI branch Boards of Trustees must be composed of at least 50% public sector scientists; the remaining trustees represent ILSI's member companies. For a list of ILSI N.A. Trustees, or for more information about ILSI N.A., visit the website at www.ilsi.org or email ilsi@ilsi.org.

Table 1

Bioassay Data - Males

Study	Dose (mg/kg/day)				
	0	0.01	0.1	0.5	2
Tunica vaginalis mesothelioma					
(Johnson et al. 1986)	3/60	0/60	7/60	11/60*	10/60*
(Friedman et al. 1995)	8/204	_	9/204	8/102	13/75*
Astrocytoma					
(Johnson et al. 1986)	4/60	0/60	0/60	2/60	5/60
(Friedman et al. 1995)	1/204	_	1/98	0/50	3/75
Thyroid follicular cell adenoma/adenocarcinoma					
(Johnson et al. 1986)	1/60	0/58	2/59	1/59	7/59*
(Friedman et al. 1995)	6/202	_	12/203	5/101	17/75*
Oral cavity papilloma					
(Johnson et al. 1986)	4/60	7/60	0/60	5/60	4/60
Pheochromocytoma					
(Johnson et al. 1986)	3/60	7/59	7/60	5/60	10/60*

* Statistically different from control using Fischer's Exact Test, a = 0.05

Table 2

Bioassay Data – Females

Study	Dose (mg/kg/day)						
	0.0	0.01	0.1	0.5	1	2	3
Mammary gland adenoma							
(Johnson et al. 1986)	0/60	1/60	0/60	3/58	–	2/61	–
(Friedman et al. 1995)	0/96	–	–	–	0/94	–	0/95
Mammary gland adenocarcinoma							
(Johnson et al. 1986)	2/60	1/60	1/60	2/58	–	6/61#	–
(Friedman et al. 1995)	2/96	–	–	–	2/94	–	4/95
Mammary gland fibroma							
(Johnson et al. 1986)	0/60	0/60	0/60	0/58	–	5/61*	–
(Friedman et al. 1995)	0/96	–	–	–	0/94	–	0/95
Mammary gland fibroadenoma							
(Johnson et al. 1986)	10/60	11/60	9/60	17/58	_	16/61	_
(Friedman	9/96	–	–	–	20/94*	_	26/95*

et al. 1995)							
Astrocytoma							
(Johnson et al. 1986)	1/60	1/60	0/60	0/60	_	6/61	_
(Friedman et al. 1995)	0/100	_	_	_	2/100	_	3/100
Thyroid follicular cell adenoma/adenocarcinoma							
(Johnson et al. 1986)	1/58	0/59	0/59	0/58	-	5/60	_
(Friedman et al. 1995)	2/100	_	_	_	10/100*	_	23/100*
Oral cavity papilloma							
(Johnson et al. 1986)	0/60	3/60	2/60	1/60	_	7/61*	_
Uterine adenocarcinoma							
(Johnson et al. 1986)	1/60	2/60	1/60	0/59	_	5/60*	

* Statistically different from control using Fisher's Exact Test, a = 0.05

Linear trend by Mantel Hanszel extension of Cochran-Armitage test, $\alpha = 0.05$.

References

Adler, I, P Reitmeir, R Schmoller, et al. (1994). "Dose response for heritable translocations induced by acrylamide in spermatids of mice." Mutat Res 309: 285-291.

Adler, I., I. Ingwersen, U. Kliesch, et al. (1988). "Clastogenic effects of acrylamide in mouse bone marrow cells." Mutat Res 206: 379-385.

Adler, I., R. Zouh and E. Schmid (1993). "Perturbation of cell division by acrylamide in vitro and in vivo." Mutat Res 301: 249-254.

Adler, I.D. (1990). Clastogenic effects of acrylamide in different germ-cell stages of male mice. Banbury Report 34: Biology of Mammalian Germ Cell Mutagensis. Cold Spring Harbor NY, Cold Spring Harbor Laboratory**:** 115-131.

Adler, I-D, A Baumgartner, H Gonda, et al. (2000). "1-Aminobenzotriazole inhibits acrylamide- induced Dominant Lethal Effects in Spermatids of male mice." Mutagenesis 15(2): 133-136.

Aldous, C, C Farr and R Sharma (1983). "Evaluation of acrylamide treatment on levels of major brain biogenic amines, their turnover rates, and." Fundam Appl Toxicol 3: 182-186.

Aschner, M (2002). Glial Cells. A. Shipp. Washington, D.C.

Bachmann, M, J Myers and B Bezuidenhout (1992). "Acrylamide monomer and peripheral neuropathy in chemical workers." Am. J. Ind. Med 21(2): 217-222.

Backer, L, K Dearfield, G Erexson, et al. (1989). "The effects of acrylamide on mouse germ-line and somatic cell chromosomes." Environmental and Molecular Mutagenesis13: 218-226.

Banerjee, S and A Segal (1986). "In vitro transformation of C31VIOT'/2 and NHV3T3 cells by acrylonitrile and acrylamide." Canc. Lett 32: 293-304.

Barber, DS, JR Hunt, MR Ehrich, et al. (2001). "Metabolism, toxicokinetics and hemoglobin adduct formation in rats following subacute and subchronic acrylamide dosing." NeuroToxicology 22: 341-353.

Barfknecht, T, D Mecca and R Naismith (1988). "The genotoxic activity of acrylamide." Environmental and Molecular Mutagenesis 11(Supp l): 9.

Bergmark, E, CJ Calleman and LG Costa (1991). "Formation of hemoglobin adducts of acrylamide and its epoxide metabolite glycidamide in the rat." Toxicol Appl Pharmacol 111(2): 352-363.

Bishop, J., R. Chapin, P. Fail, et al. (1991). "Acrylamide induced dominant lethality in mice following low dose chronic administration in drinking water." Environ. Mol. Mutagen. 17(Suppl.9): A25 (abstract). (As cited in EU, 1995).

Bjorge, C., G. Brunborg, R. Wiger, et al. (1996). "A comparative study of chemically induced DNA damage in isolated human and rat testicular cells." Reprod Toxicol 10(6): 509-19.

Boorman, GA, JT Wilson, MJ van Zwieten, et al. (1990). Mammary gland. Pathology of the Fischer Rat. G. Boorman, S. Eustis, M. Elwell and e. al. New York, Academic Press**:** 295-313.

Bull, RJ, M Robinson, RD Laurie, et al. (1984a). "Carcinogenic effects of acrylamide in Sencar and A/J mice." Cancer Res 44(1): 7-11.

Bull, RJ, M Robinson and JA Stober (1984b). "Carcinogenic activity of acrylamide in the skin and lung of Swiss-ICR mice." Cancer Lett 24: 209-212.

Burek, J.D., R.R. Albee, J.E. Beyer, et al. (1980). "Subchronic toxicity of acrylamide administered to rats in the drinking water followed by up to 144 days of recovery." J. Path. Tox. 4: 157-182.

Butterworth, B. E., S. R. Eldridge, C. S. Sprankle, et al. (1992). "Tissue-specific genotoxic effects of acrylamide and acrylonitrile." Environ Mol Mutagen 20(3): 148-155.

Calleman, C (1996). "The metabolism and pharmacokinetics of acrylamide: implications for mechanisms of toxicity and human risk estimation." Drug Metab Rev 28: 527-590.

Calleman, C., L. Stern, E. Bergmark, et al. (1992). "Linear versus nonlinear models for hemoglobin adduct formation by acrylamide and its metabolite glycidamide: implications for risk estimation." Cancer Epidemiol. Biomarkers Prev. 1: 361-368.

Calleman, CJ, E Bergmark and L. Costa (1990). "Acrylamide is metabolized to glycidamide in the rat: evidence from hemoglobin adduct formation." Chem Res Toxicol 3(5): 406-12.

Calleman, CJ, Y Wu, F He, et al. (1994). "Relationship between biomarkers of exposure and neurological effects in a group of workers exposed to acrylamide." Toxicology and Applied Pharmacology 126: 361-371.

Carlson, G, A Fossa, M Morse, et al. (1986). "Binding and distribution studies in the SENCAR mouse of compounds demonstrating a route-dependant tumorigenic effect. ." Environ Health Persp. 68: 53-60.

Carlson, G and P Weaver (1985). "Distribution and binding of 14C acrylamide to macromolecules in SENCAR and BALB/c mice following oraland topical administration." Toxicol Appl Pharmacol 79: 307-313.

Cavanagh, J.B. (1964). "The significance of the dying-back process in experimental and human neurological disease." Int Rev Exp Pathol 3: 219-267.

Cavanagh, JB (1979). "The dying back process." Arch Pathol Lab Med 103: 659-664.

Cavanagh, JB (1982). "The pathokinetics of acrylamide intoxication: a reassessment of the problem." Neuropathol Appl Neurobiol 8: 315-336.

Chapin, RE, PA Fail, JD George, et al. (1995). "The reproductive and neural toxicities of acrylamide and three analogues in Swiss mice, evaluated using the continuous breeding protocol." Fundam Appl Toxicol 27(1): 9-24.

Chiak, R. and M Vontorkova (1988). "Cytogenetic effects of acrylamide in the bone marrow of mice." Mutation Research 209: 91-94.

Chihak, R and M Vontorkova (1990). "Activity of acrylamide in single-, double-, and triple-dose mouse bone marrow micronucleus assays." Mutation Research 234: 125-127.

Collins, J, G Swaen and et al (1989). "Mortality patterns among workers exposed to acrylamide." J Occup Med 31(7): 614-617.

Crofton, KM, S Padilla, HA Tilson, et al. (1996). "The impact of dose rate on the neurotoxicity of acrylamide: the interaction of administered dose, target tissue concentrations, tissue damage, and functional effects." Toxicology and Applied Pharmacology 139(1): 163-176.

Das, M, H Mukhtar and P Seth (1982). "Effect of acrylamide on brain and hepatic mixed-function oxidases." Toxicol Appl Pharmacol 66: 420-426.

DeGrandchamp, RL, Lowndes, HE. (1990a). "Early degeneration and sprouting at the rat neuromuscular junction following acrylamide administration." Neuropath Appl Neurobiol 16: 239-254.

DeGrandchamp, RL, Reuhl, KR, Lowndes, HE. (1990b). "Synaptic terminal degeneration and remodeling at the rat neuromuscular junction resulting from a single exposure to acrylamide." Tox Appl Pharmacol 105: 422-433.

Deng, H, F He, S Zhang, et al. (1993). "Quantitative measurements of vibration threshold in healthy adults and acrylamide workers." Int. Arch. Occup. Environ. Health 65: 53-56.

DeRojas, TC, Goldstein, BD. (1987). "Primary afferent terminal function following acrylamide: alterations in the dorsal root potential and reflex." Tox Appl Pharmacol 88: 175-182.

Diembeck, W, H-J Dusing and M Akhiani (1998). Dermal Absorption and Penetration of Acrylamide ([C14]-Acrylamide as Tracer) in different Cosmetic Formulations and polyacrylamide-solution after Topical Application to Excised Pig Skin. Beiersdorf**:** 9.

Dixit, R, M Das, P Seth, et al. (1986). "Interaction of Acrylamide with Bovine Serum Albumin." Environmental Research 40: 365-371.

Dixit, R, M Das, PK Seth, et al. (1984). "Interaction of acrylamide with glutathione in rat erythrocytes." Toxicol Lett 23(3): 291-8.

Dixit, R, R Husain, H Mukhtar, et al. (1981a). "Effect of acrylamide on biogenic amine levels, monoamine oxidase, and cathepsin D activity of rat brain." Environ Res 26: 168-173.

Dixit, R, R Husain, H Mukhtar, et al. (1981b). "Acrylamide Induced Inhibition of Hepatic Glutathione-S-Transferase Activity in Rats." Toxicology Letters 7: 207-210.

Dixit, R, H Mukhtar, PK Seth, et al. (1980). "Binding of acrylamide with glutathione-S-transferases." Chem Biol Interact 32(3): 353-9.

Dixit, R, H Mukhtar, PK Seth, et al. (1981c). "Conjugation of acrylamide with glutathione catalysed by glutathione-S- transferases of rat liver and brain." Biochem Pharmacol 30(13): 1739-44.

Dixit, R, PK Seth and H Mukjtar (1982). "Metabolism of acrylamide into urinary mercapturic acid and cysteine conjugates in rats." Drug Metab Dispos 10(2): 196-7.

Edwards, PM, Parker, VH (1977). "A simple, sensitive and objective method for early assessment of acrylamide neuropathy in rats." Tox Appl Pharmacol 40: 589-591.

Ehling, U and A Neuheuser-Klaus (1992). "Reevaluation of the induction of specific-locus mutations in spermatogonia of the mouse by acrylamide." Mutation Research 283: 185-191.

Eskin, T, L Lapham, J Maurissen, et al. (1985). "Acrylamide effects on the Macaque visual system. II. Retinogeniculate morphology." Invest Ophthaknik Vis Sci 26(3): 317-329.

Field, EA, CJ Price, RB Sleet, et al. (1990). "Developmental toxicity evaluation of acrylamide in rats and mice." Fundam Appl Toxicol 14: 502-12.

Frantz, S, M Dryzga, N Freshour, et al. (1995). "In vivo/in vitro determination of cutaneous penetration of residual monomer from polyacrylamides." Toxicol. Sci. 5(1-S): A39.

Friedman, M, L Dulak and M Stedham (1995). "A lifetime oncogenicity study in rats with acrylamide." Fundam Appl Toxicol 27: 95-105.

Fullerton, P and J Barnes (1966). "Peripheral neuropathy in rats produced by acrylamide." Br J Ind Med 23: 210-221.

Generoso, W, G Sega, A Lockhart, et al. (1996). "Dominant lethal mutations, heritable translocations, and unscheduled DNA synthesis induced in male mousegerm cells by glycidamide, a metabolite of acrylamide." Mutat Res 371: 175-183.

Ghetti, B, Wisneiwski, HM, Cook, RD, Schaumburg, HH. (1973). "Changes in the CNS after acute and chronic acrylamide intoxication." Am J Pathol 70: 78A.

Gilbert, S and J Maurissen (1982). "Assessment of the effects of acrylamide, methylmercury, and 2,5-hexanedione on motor functions in mice." J Toxicol Environ Health 10: 31-41.

Gold, BG, Austin, DR, Griffin, JW (1991). "Regulation of aberrant neurofilament phosphorylation in neuronal perikarya. II. Correlation with continued axonal elongation following axotomy." J Neuropath Exp Neurol 50: 627-648.

Gold, BG, Price, DL, Griffin, JW, Rosenfeld, J, Hoffman, PN, Sternberger, NH, Sternberger, LA (1988). "Neurofilament antigens in acrylamide neuropathy." J Neuropath Exp Neurol 47: 145-157.

Goldstein, BD (1985). "Acrylamide neurotoxicity: altered spinal monosynaptic response to quipazine, a sertonin agonist in cats." Tox Appl Pharmacol 78: 436-444.

Goldstein, BD, Lowndes, HE (1979). "Spinal cord defect in the peripheral neuropathy resulting from acrylamide." NeuroToxicology 1: 75-87.

Goldstein, BD, Lowndes, HE (1981). "Group Ia primary afferent terminal defect in cats with acrylamide neuropathy." NeuroToxicology 2: 297-312.

Goodman, D, J Ward, R Squire, et al. (1979). "Neoplastic and nonneoplastic lesions in aging F344 rats." Toxicol Appl Pharmacol 48: 237-248.

Griffin, JW, Price, DL, Drachman, DB (1977). "Impaired axonal regeneration in acrylamide intoxication." J Neurobiol 8: 355-370.

Gutierrez-Espeleta, G., L. Hughes and W Pierorsch (1992). "Acrylamide dermal exposure produces genetic damage in male mouse germ cells." Fundam. Appl. Toxicol. 18: 189-192.

Hagmar, L, M Tornqvist, C Norander, et al. (2001). "Health effects of occupational exposure to acrylamide using hemoglobin adducts as biomarkers of internal dose." Scand. J. Work Environ. Health 27(4): 219-226.

Haseman, JK, J Arnold and SL Eustis (1990). Tumor incidences in the Fischer 344 rat: NTP historical data. Pathology of the Fischer Rat. G. Boorman and e. al. New York, Academic Press**:** 555-564.

Haseman, JK, JR Hailey and RW Morris (1998). "Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: a National Toxicology Program update." Toxicol Pathol 26: 428-441.

Hashimoto, K and WN Aldridge (1970). "Biochemical studies on acrylamide, a neurotoxic agent." Biochem. Pharmacol. 19(9): 2591-604.

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

Hashimoto, K and H Tanii (1985). "Mutagenicity of Acrylamide and its Analogues in Salmonella typhimurium." Mutation Research 158(3): 129-133.

He, F, S Zhang, H Wang, et al. (1989). "Neurological and electroneuromyographic assessment of the adverse effects of acrylamide on occupationally exposed workers." Scand. J. Work Environ. Health 15: 125-129.

Hersch, M, J McLeod, P Satchell, et al. (1989). "Breathing pattern, lung inflation reflex and airway tone in acrylamide neuropathy." Resp. Physiol 76: 257-276.

Hopkins, A (1970). "The effect of acrylamide on the peripheral nervous system of the babbon." J Neurol Neurosurg Psychiat 33: 805-816.

Hopkins, A, Gilliatt, RW (1971). "Motor and sensory nerve conduction velocity in the baboon: normal values and changes during acrylamide neuropathy." J Neurol Neurosurg Psychiat 34: 415-426.

Husain, R, R Dixit, M Das, et al. (1987). "Neurotoxicity of acrylamide in developing rat brain: changes in the levels of brain biogenic amines and activities of monoamine oxidase and acetylcholine esterase." Indust Health 25: 19-28.

Ikeda, G, E Miller, P Sapienza, et al. (1987). "Comparative tissue distribution and excretion of fl-"C]acrylamide in beagle dogs and miniature pigs." Food Chem. Toxic. 25(11): 871-875.

Jennekens, FGI, Veldman, H, Schotman, P, Gispen, WH (1979). "Sequence of motor nerve terminal involvement in acrylamide neuropathy." Acata Neuropath 46: 57-63.

Johnson, KA, SJ Gorzinski, KM Bodner, et al. (1986). "Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats." Toxicol Appl Pharmacol 85(2): 154-168.

Kirman, CR, Gargas, ML, Deskin, R, Tonner-Navarro, LE, Andersen, ME (2002). "Using Monte Carlo sensitivity analyses to prioritize data neeeds in developing a physiologically based pharmacokinetic (PBPK) model for acrylamide." The Toxicologist 66(1-S): 155.

Kligerman, AD, AL Atwater, MF Bryant, et al. (1991). "Cytogenetic studies of ethyl acrylate using C57BL/6 mice." Mutagenesis 6(2): 137-41.

Knaap, A, P Kramers, C Voogd, et al. (1988). "Mutagenic activity of acrylamide in eukaryotic systems but not in bacteria." Mutagenesis 3: 263-268.

Lehning, EJ, Balaban, CD, Ross, JF, LoPachin, RM (2002a). "Acrylamide neuropathy. II. Spatiotemporal characteristics of nerve cell damage in rat brainstem and spinal cord." NeuroToxicology 23: 415-429.

Lehning, EJ, Balaban, CD, Ross, JF, LoPachin, RM (2002b). "Acrylamide neuropathy. III. Spatiotemporal characteristics of nerve cell damage in rat forebrain." NeuroToxicology 24(In Press).

Lehning, EJ, RM LoPachin, J. Mathew, et al. (1994). "Changes in Na-K ATPase and protein Kinase C activities in peripheral nerve of acrylamide-treated rats." J Toxicol Environ Health 42(3): 331-342.

Lehning, EJ, A Persaud, KR Dyer, et al. (1998). "Biochemical and morphologic characterization of acrylamide peripheral neuropathy." Toxicology and applied Pharmacology 151(2): 211-221.

Lehning, EL, Balaban, CD, Ross, JF, Reid, ML, LoPachin, RM (2002c). "Acrylamide neuropathy. I. Spatiotemprotal characteristics of nerve cell damage in rat cerebellum." NeuroToxicology 23: 397-414.

Leswing, RJ, Ribelin, WE (1969). "Physiologic and pathologic changes in acrylamide neuropathy." Arch Environ Health 18: 22-29.

LoPachin, R. M., J. F. Foss and Lehning E. J. (2002). "Nerve Terminals as the Primary Site of Acrylamdie Action: A Hypothesis." NeuroToxicology 23: 43-59.

LoPachin, RM, Lehning, EJ, Opanashuk, LA, Jortner, BS (2000). "Rate of neurotoxicant exposure determines morphologic manifestations of distal axonopathy." Tox Appl Pharmacol 167: 75-86.

LoPachin, RM, Ross, JF, Reid, ML, Dasgupta, S, Mansukhani, S, Lehning, EJ (2001). "Neurological evaluation of toxic axonopathies in rats: acrylamide and 2,5-hexanedione." NeuroToxicology 23: 95-110.

Lowndes, HE, Baker, T (1976). "Studies on drug-induced neuropathies. III. Motor nerve deficit in cats with experimental acrylamide neuropathy." Europ J Pharmacol 35: 177-184.

Lowndes, HE, Baker, T, Cho, E-S, Jortner, BS (1978a). "Position sensitivity of de-efferented muscle spindles in experimental acrylamide neuropathy." J Pharmacol Exp Ther 205: 40-48.

Lowndes, HE, Baker, T, Michelson, LP, Vincent-Ablazey, M. (1978b). "Attenuated dynamic respoonses of primary endings of muscle spindles: A basis for depressed tendon responses in acrylamide neuropathy." Ann Neurol 3: 433-437.

Maekawa, A, Y Kurokawa, M Takahashi, et al. (1983). "Spontaneous tumors in F344/DuCrj rats." Gann 74: 365-372.

Marchetti, F, X Lowe, J Bishop, et al. (1997). "Induction of chromosomal aberrations in mouse zygotes by acrylamide treatment of male germ cells andtheir correlation with dominant lethality and heritable translocations." Environ Mol Mutagen 30: 410-417.

Marlowe, C, MJ Clark, RW Mast, et al. (1986). "The Distribution of (14C)Acrylamide in Male and Pregnant Swiss-Webster Mice byWhole Body Autoradiography." Toxicol and Appl. Pharmacol 86: 457-465.

Marsh, G, L Lucas, A Youk, et al. (1999). "Mortality patterns among workers exposed to acrylamide: 1994 follow up." J Occup Environ Med 56: 181-190.

Marty, J-P (1998). In Vitro Percutaneous Absorption of Acrylamide Across Human Skin. Paris, France, Faculty of Pharmacy, Université de Paris Sud.

Maurissen, J, B Weiss and C Cox (1990). "Vibration sensitivity recovery after a second course of acrylamide intoxication." Fund Appl Toxicol 15(1): 93-98.

Maurissen, J, B Weiss and H Davis (1983). "Somatosensory thresholds in monkeys exposed to acrylamide." Toxicol Appl Pharmacol 71(2): 266-279.

McCollister, D, F Oyen and V Rowe (1964). "Toxicology of acrylamide." Toxicol. Appl. Pharmacol 6: 172-181.

Miller, MJ, DE Carter and IG Sipes (1982). "Pharmacokinetics of acrylamide in Fisher-334 Rats." Toxicology and Applied Pharmacology 63: 36-44.

Moore, M, A Amtower, C Doerr, et al. (1987). "Mutagenicity and clastogenicity of acrylamide in L5178Y mouse lymphoma cells." Environ Mutagen 9: 261-267.

Morgan-Hughes, JA, Sinclair, S, Durston, JHJ (1974). "The pattern of peripheral nerve regeneration induced by crush in rats with severe acrylamide neuropathy." Brain Res 97: 215-250.

Moser, VC, Anthon, DC, Sette, WF, MacPhail, RC (1992). "Comparison of subchronic neurotoxicity of 2-hydroxyethyl acrylate and acrylamide in rats." Fundam Appl Toxicol 118: 343-352.

Mukhtar, H, R Dixit and P Seth (1981). "Reduction in cutaneous and hepatic glutathione contents, glutathione-S-transferases and aryl hydrocarbon hydroxylase activities following topical application of acrylamide to mouse." Tox. Lett. 9: 153-156.

Myall, OT, SL Allen and WG McLean (1990). "The effect of acrylamide on the induction of ornithine decarboxylase in the dorsal root ganglion of the rat." Brain Res 523(2): 295-7.

Myers, JE and I Macun (1991). "Acrylamide neuropathy in a South African factory: an epidemiologic investigation." American Journal of Industrial Medicine 19(4): 487-493.

Park, Jougjoa, Lisa M Kamendulis, Marvin A Friedman, et al. (2002). "Acrylamide-Induced Cellur Transformation." Toxicological Sciences 65: 177-183.

Paulet, G. V. (1975). "De la toxicite de quelques esters acryliques et methacryliques de l'acrylamide et des polyacrylamides." Arch Mal Prof 36: 58-60.

Paulsson, B., J. Grawe and M. Tornqvist (2002a). "Hemoglobin adducts and micronucleus frequencies in mouse and rat after acrylamide or N-methylolacry treatment." Mutation Research 516: 101-111.

Paulsson, B., N. Kotova, J. Grawe, et al. (2002b). "Induction of micronuclei in mouse and rat by glycidamide, the genotoxic metabolite of acrylamide." Mutation Research In press.

Post, E and J McLeod (1977). "Acrylamide autonomic neuropathy in the cat. 1. Neurophysiological and histological studies." J Neurol Sci 33: 353-374.

Prineas, J (1969). "The pathogenesis of dying-back polyneuropathies. Part II. An ultrastructural study of experimental acrylamide intoxication in the cat." J Neuropath Exp Neurol 28: 598-621.

Ramsey, J, JD Young and SJ Gorzinski (1984). Acrylamide: Toxicodynamics in the Rat. Midland, MI, Dow Chemical Company.

Robinson, M, RJ Bull, GL Knutsen, et al. (1986). "A combined carcinogen bioassay utilizing both the lung adenoma and skin papilloma protocols." Environ Health Perspect 68: 141-5.

Russell, L., P. Hunsicker, N. Cacheiro, et al. (1991). "Induction of specific-locus mutations in male germ cells of the mouse by acrylamide monomer." Mutation Research 262: 101-107.

Russo, A., G. Gabbani and B. Simoncini (1994). "Weak genotoxicity of acrylamide on the premiotic and somatic cells of the mouse." Mutat Res 309: 263-272.

Sakamoto, J and K Hashimoto (1986). "Reproductive toxicity of acrylamide and related compounds in mice-- effects on fertility and sperm morphology." Arch Toxicol 59(4): 201-205.

Satchell, P and J McLeod (1981). "Megaoesophagus due to acrylamide neuropathy." J. Neurol. Neurosurg Psych 44: 906-913.

Schaumburg, HH, Wisniewski, HM, Spencer, PS (1974). "Ultrastructural studies of the dying-back process. I. Peripheral nerve terminal and axon degeneration in systemic acrylamide intoxication." J Neuropath Exp Neurol 33: 260-284.

Sega, G, E Generoso and P Brimer (1990). "Acylamide exposure induces a delayed unscheduled DNA synthesis in germ cells of male mice that iscorrelated with the temproal pattern of adduct formation in testis DNA." Environ Mol Mutagen 16: 137-142.

Sega, G, R Valdivia Alcota, C Tancongco, et al. (1989). "Acrylamide binding to the DNA and protoamine of spermiogenic stages in the mouse and its relationship to genetic damage." Mutation Research 216(4): 221-230.

Segerback, D, J Calleman, J Schroeder, et al. (1995). "Formation of N-7-(2-carbamoyl-2-hydroxyethylguanine in DNA of the mouse and rat following intraperitonealadministration of 14C acrylamide." Carcinogenesis 16: 1161-1165.

Shelby, M, K Cain, C Cornett, et al. (1987). "Acylamide: induction of heritable translocations in male mice." Environ Mutagen 9: 363-368.

Shelby, MD, KT Cain, LA Hughes, et al. (1986). "Dominant lethal effects of acrylamide in male mice." Mutation Research 173(1): 35-40.

Shell, L, Rozum, M, Jortner, BS, Ehrich, M. (1992). "Neurotoxicity of acrylamide and 2,5-hexanedione in rats evaluated using a functional observational battery and pathological examination." Neurotox Teratol 14: 273-283.

Sickles, D, ST Brady, A Testino, et al. (1996). "Acrylamide effects on kinesin related mitotic/meiotic motor proteins." J Neurosci Res 46: 7-17.

Sickles, D.W., J.D. Stone and M.A. Friedman (2002). "Fast axonal transport: A site of acrylamide neurotoxicity." Neurotoxicology 23: 223-251.

Smith, M., H. Zenick, R. Preston, et al. (1986). "Dominant lethal effects of subchronic acrylamide administration in the male Long-Evans rat." Mutation Research 173: 273-277.

Solleveld, H, J Haseman and E McConnell (1984). "Natural history of body weight gain, survival, and neoplasia in the F344 rats." J Natl Cancer Inst 72(4): 929-940.

Solomon, J, J Fedyk, F Mukai, et al. (1985). "Direct alkylation of 2'-deoxynucleosides and DNA following in vitro reaction with acrylamide." Cancer Res 45: 3465-3470.

Spencer, P.S. and H.H. Schaumberg (1976). Central-peripheral distal axonopathy - The pathology of dying-back polyneuropathies. Progress in Neuropathology. H. Zimmerman. New York, Grune and Stratton. 3**:** 253-276.

Spencer, PS, Schaumburg, HH (1977a). "Ultrastructural studies of the dying-back process IV. Differential vulnerability of PNS and CNS fibers in experimental central-peripoheral distal axonopathy." J Neuropath Exp Neurol 36: 300-320.

Spencer, PS, Schaumburg, HH (1977b). "Ultrastructural studies of the dying-pack process. III. The evolution of experimental peripheral giant axonal degeneration." J Neuropath Exp Neurol 36: 276-299.

Spencer, PS and HH Shaumburg (1974). "A review of acrylamide neurotoxicity. Part II Experimental animal neurotoxicity and pathologic mechanisms." Canad J Neurol Sci.: 152-69.

Spencer, PS, Thomas PK, (1974). "Ultrastructural studies of the dying-back process. II. The sequestration and removal by Schwann cells and oligodendrocytes of organelles from normal and diseased axons." J Neurocytol 3: 763-783.

Sterman, AB and N Sposito (1985). "2,5-Hexanedione and acrylamide produce reorganization of motoneuron perikarya." Neuropathol Appl Neurobiol 11(3): 201-12.

Sublet, VH, H Zenick and MK Smith (1989). "Factors associated with reduced fertility and implantation rates in females mated to acrylamide treated rats." Toxicology 55: 53-67.

Sumner, A, Asbury, AK (1974). "Acrylamide neuropathy: selective vulnerability of sencory fibers." Trans Ann Neurol Assoc 99: 78-83.

Sumner, A, Asbury, AK (1975). "Physiological studies of the dying-back phenomenon: muscle stretch afferents in acrylamide neuropathy." Brain 98: 91-100.

Sumner, SCJ, A Bahman, CC Williams, et al. (2001). Acrylamide, Metabolism, Distribution, and Hemoglobin Adducts in Male F344 Rats and B6C3F1 Mice Following Inhalation Exposure and Distribution and Hemoglobin Adducts Following Dermal Application to F344 Rats. Research Triangle Park, NC, CIIT.

Sumner, SCJ, JP MacNeela and TR Fennell (1992). "Characterization and quantitation of urinary metabolites of {1,2,3-13C}acrylamide in rats and mice using 13C nuclear magnetic resonance spectroscopy." Chem Res Toxicol 5: 81-9.

Sumner, SCJ, L Selvaraj, SK Nauhaus, et al. (1997). "Urinary Metabolites from F344 Rats and B6C3F1 Mice Coadministered Acrylamide and Acrylonitrile for 1 or 5 Days." Chemical Research In Toxicology 10(10): 1152-1160.

Suzuki, K, Pfaff, L (1973). "Acrylamide neuropathy in rats. An electron microscopic study of degeneration and regeneration." Acta Neuropathol 24: 197-203.

Tanii, H and K Hashimoto (1981). "Studies on in vitro metabolism of acrylamide and related compounds." Arch Toxicol 48: 157-166.

Tareke, E., P. Rydberg, P. Karlsson, et al. (2002). "Analysis of acrylamide, a carcinogen formed in heated foodstuffs." J. Agric. Food Chem 50: 4998-5006.

Tilson, H and P Cabe (1979). "The effects of acrylamide given acutely or in repeated doses on fore- and hind limb function in rats." Toxicol. Appl. Pharmacol. 47: 253-260.

Tsuda, H, C Chimizu, M Taketomi, et al. (1993). "Acrylamide; induction of DNA damage, chromosomal aberrations and cell transformation without gene mutations." Mutagenesis 8: 23-29.

Tsujihata, M, Engel, AG, Lambert, EH (1974). "Motor end-plate fine structure in acrylamide dying-back neuropathy: A sequential morphometric study." Neurology 24: 849-856.

Tyl, RW, MA Friedman, PE Losco, et al. (2000a). "Rat two-generation reproduction and dominant lethal study of acrylamide in drinking water." Reproductive Toxicology 14(5): 385-401.

Tyl, RW, MC Marr, CB Myers, et al. (2000b). "Relationship between acrylamide reproductive and neurotoxicity in male rats." Reproductive Toxicology 14(2): 147-57.

Warr, T., J. Parry, R. Callander, et al. (1990). "Methyl vinyl sulphone: a new class of Michael-type genotoxin." Mutat Res 245: 191-199.

Wise, L, L Gordon, K Soper, et al. (1995). "Developmental neurotoxicity evaluation of acrylamide in Sprague-Dawley rats." Neurotox. Teratol. 17(2): 189-198.

Working, PK, KS Bentley, ME Hurtt, et al. (1987). "Comparison of the dominant lethal effects of acrylonitrile and acrylamide in F344 rats." Mutagenesis 2: 215-20.

Xiao, Y. and A. Tates (1994). "Increased frequencies of micronuclei in early spermatids of rats following exposure of young primary spermatocytes to acrylamide." Mutation Research 309: 245-254.

Zeiger, E., B. Anderson, S. Haworth, et al. (1987). "Salmonella mutagenicity tests. III. Results from the testing of 255 chemicals." Environ Mutagen 9: 1-110.

Zenick, H, E Hope and MK Smith (1986). "Reproductive toxicity associated with acrylamide treatment in male and female rats." J Toxicol Environ Health 17: 457-472.