3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of acrylamide. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not

the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of acrylamide are indicated in Table 3-4 and Figure 3-2. Because cancer effects could occur at lower exposure levels, Figure 3-2 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

3.2.1.1 Death

Human data are available from two cohort mortality studies of occupational exposure to acrylamide, one by Collins et al. (1989) with most recent follow up by Marsh et al. (2007) and one by Sobel et al. (1986) with follow up by Swaen et al. (2007). In these studies, no significant associations were found between occupational exposure to acrylamide and incidences of death from all causes. See Section 3.1.2.7 (Cancer) for more detailed information regarding these cohorts and assessments of death due to cancers.

Reliable information regarding death in animals following inhalation exposure to acrylamide is limited. In a study performed for the American Cyanamid Company (1953a), two dogs, seven rats, and seven guinea pigs (sex and strain unspecified) were exposed to acrylamide dust at 15.6 mg/m^3 for 6 hours/day, 5 days/week for up to 12 exposures in a 16-day period. Four of the seven rats died overnight following the first exposure period and two of the remaining rats died a few days later. One of the dogs died on study day 15; there were no deaths among the guinea pigs. The study authors stated that >90% of the particles were in the respirable range (0.3–1.2 μm).

Reliable inhalation mortality data for each species are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.2 Systemic Effects

No human or animal data were located regarding cardiovascular, gastrointestinal, musculoskeletal, hepatic, renal, endocrine, or dermal effects following inhalation exposure to acrylamide.

Respiratory Effects. Available information regarding acrylamide-associated respiratory effects is restricted to complaints of nose and throat irritation in a group of tunnel workers who had been occupationally exposed to acrylamide and N-methylolacrylamide in a chemical grouting agent for 2 months (Hagmar et al. 2001). Increasing incidences of complaints were associated with increasing levels of hemoglobin adducts of acrylamide.

Hematological Effects. No data were located regarding hematological effects in humans following inhalation of acrylamide. Available information in animals is limited to a study in which four rats were exposed to acrylamide as a "saturated" vapor for 6 hours/day, 5 days/week for 3 months at a mean analytical concentration of 1.65 ppm (4.8 mg/m^3); the exposures did not affect hematology results (American Cyanamid Company 1954).

Ocular Effects. Available information regarding acrylamide-associated ocular effects is restricted to complaints of eye irritation in a group of tunnel workers who had been occupationally exposed to acrylamide and N-methylolacrylamide in a chemical grouting agent for 2 months (Hagmar et al. 2001). Increasing incidences of complaints were associated with increasing levels of hemoglobin adducts of acrylamide.

Body Weight Effects. No data were located regarding body weight effects in humans following inhalation of acrylamide. Available information in animals is limited to a study in which four rats were exposed to acrylamide as a "saturated" vapor for 6 hours/day, 5 days/week for 3 months at a mean analytical concentration of 1.65 ppm (4.8 mg/m^3); the exposures did not affect body weights (American Cyanamid Company 1954).

Table 3-1 Levels of Significant Exposure to Acrylamide - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	LOAEL	Reference Chemical Form	Comments
ACUTE EXPOSURE									
Death									
1	Rat (NS)	4 d 6 hr/d				15.6	(death of 4/7 rats in 1 day and 2 others before day 8)	American Cyanamid Company 1953a	
Neurological									
2	Rat (NS)	4 d 6 hr/d				15.6	(loss of coordination and equilibrium on exposure day 4)	American Cyanamid Company 1953a	
INTERMEDIATE EXPOSURE									
Death									
3	Dog (NS)	16 d 5 d/wk 6 hr/d				15.6	(death of 1/2 dogs on day 15)	American Cyanamid Company 1953a	
Systemic									
4	Cat	3 mo 5 d/wk 6 hr/d	Hemato	4.8				American Cyanamid Company 1954 Acrylamide	
			Bd Wt	4.8					
Neurological									
5	Gn Pig (NS)	16 d 5 d/wk 6 hr/d		15.6				American Cyanamid Company 1953a	
6	Dog (NS)	16 d 5 d/wk 6 hr/d				15.6	(CNS effects including loss of equilibrium and coordination)	American Cyanamid Company 1953a	

Table 3-1 Levels of Significant Exposure to Acrylamide - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
7	Cat	3 mo 5 d/wk 6 hr/d		4.8			American Cyanamid Company 1954 Acrylamide	

a The number corresponds to entries in Figure 3-1.

Bd Wt = body weight; CNS = central nervous system; d = day(s); Gn pig = guinea pig; Hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; wk = week(s)

Figure 3-1 Levels of Significant Exposure to Acrylamide - Inhalation

Acute (≤14 days)

c-Cat -Humans f-Ferret n-Mink
d-Dog k-Monkey j-Pigeon o-Other
r-Rat m-Mouse e-Gerbil
p-Pig h-Rabbit s-Hamster
q-Cow a-Sheep g-Guinea Pig

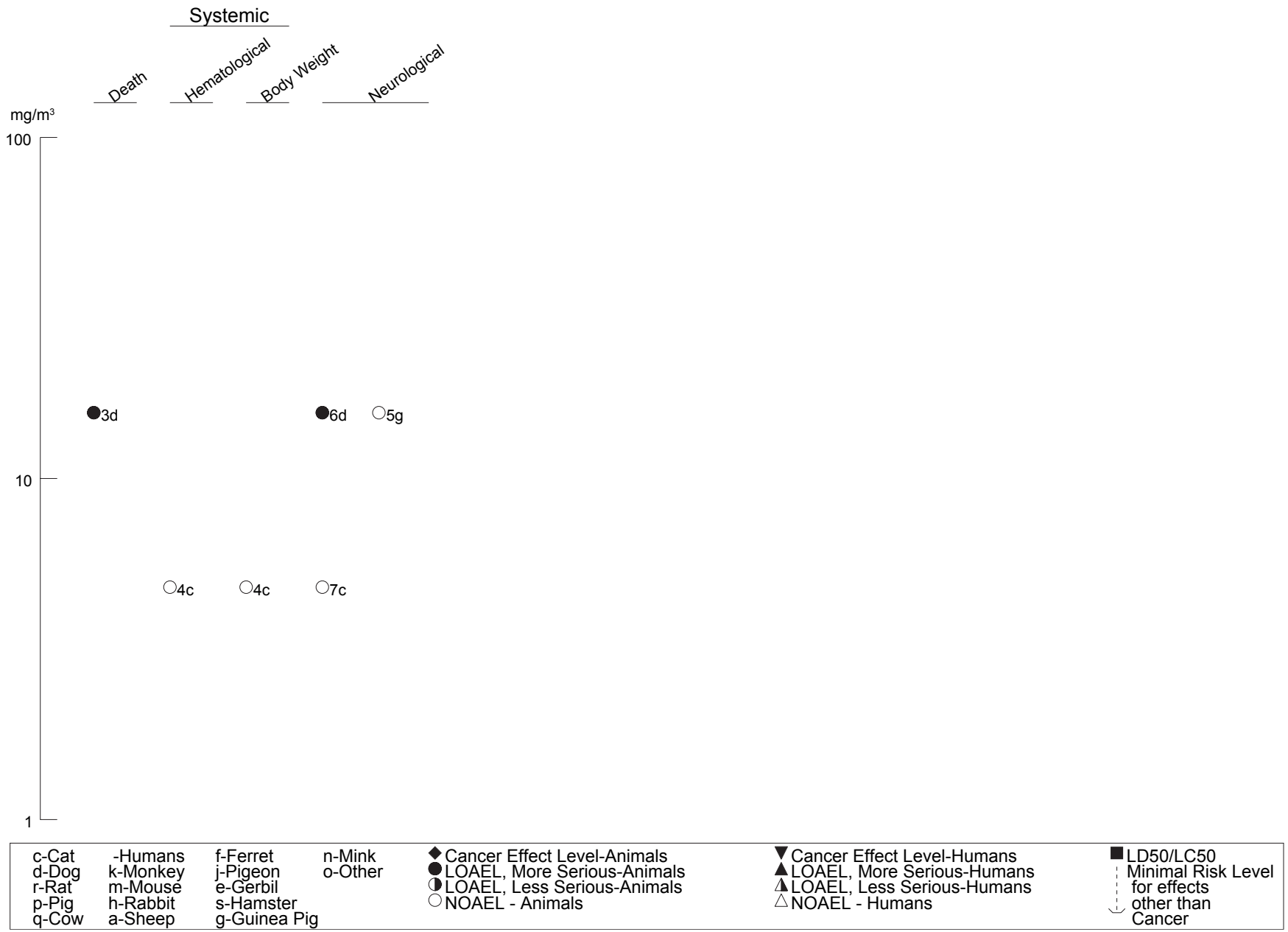
◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◭ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

Figure 3-1 Levels of Significant Exposure to Acrylamide - Inhalation *(Continued)*

Intermediate (15-364 days)



3.2.1.3 Immunological and Lymphoreticular Effects

No data were located regarding immunological or lymphoreticular effects in humans or animals following inhalation exposure to acrylamide.

3.2.1.4 Neurological Effects

Information in humans is available from numerous case reports in which acrylamide exposure has been associated with signs of impaired neurological performance in central and peripheral nervous systems that include impaired motor function and muscle weakness (Auld and Bedwell 1967; Davenport et al. 1976; Dumitru 1989; Fullerton 1969; Garland and Patterson 1967; Gjerløff et al. 2001; Igisu et al. 1975; Kesson et al. 1977; Mapp et al. 1977; Mulloy 1996; Takahashi et al. 1971). Human data are also available from cross-sectional studies that included self-reported symptoms and neurological evaluations of acrylamide-exposed workers with potential for inhalation and dermal (and possibly oral) exposure (Bachmann et al. 1992; Calleman et al. 1994; Hagmar et al. 2001; He et al. 1989; Myers and Macun 1991). Although the case reports and cross-sectional studies provide supportive evidence of acrylamide-induced neurotoxicity, they lack information regarding relative contributions of natural exposure routes (inhalation, oral, dermal), exposure-response relationships, and other confounding exposures. They are therefore unsuitable for meaningful quantitative risk analysis.

He et al. (1989) evaluated health end points in workers employed for 1–18 months at a factory in China that began producing acrylamide and polyacrylamide in 1984. A referent group consisted of unexposed workers from the same town. Concentrations of acrylamide in the workplace air (determined by gas chromatography) reached 5.56–9.02 mg/m^3 between March and June 1985 during polymerization when there was an exceptional increase in production, and decreased to an average of 0.0324 mg/m^3 (range not specified) after July 1985. The workers were evaluated in October 1985. The study authors reported that heavy skin contamination by aqueous acrylamide monomer was common among the workers. An acrylamide level of 410 mg/L was measured in the water in which three of the workers washed their hands. Personal interviews were conducted to obtain information on demographic factors, occupational history, symptoms, past illnesses, and family history. Physical and neurological examinations, visual acuity and visual field testing, skin temperature measurements, electrocardiography, and electroencephalography were performed. Sixty-nine of the exposed workers and 48 of the referent workers were subjected to electroneuromyographic examinations.

As shown in Table 3-2, significantly greater percentages of the acrylamide-exposed group reported skin peeling from the hands, anorexia, numbness and coldness in hands and feet, lassitude, sleepiness, muscle weakness, clumsiness of the hands, unsteady gait, difficulty in grasping, and stumbling and falling. The authors stated that initial symptoms of skin peeling were the result of dermal exposure to aqueous acrylamide and that other symptoms appeared following 3–10 months of occupational exposure. Greater percentages of acrylamide-exposed workers exhibited erythema of the hands, sensory impairments (vibration, pain, and touch sensation), diminished reflexes, and intention tremor (Table 3-2). Electrical activity, monitored in both the abductor pollicis brevis and abductor digiti minimi muscles of the hand, revealed electromyographic abnormalities in the acrylamide-exposed workers that included denervation potentials (3/69 exposed workers), prolonged duration of motor units (40/69), increased polyphasic potentials (29/69), and discrete pattern of recruitment (9/69). These abnormalities were not seen in referent workers, with the exception of prolonged duration of motor units (4/48 referents). Significantly increased mean duration and mean amplitude of motor unit potentials and significantly decreased mean amplitude of sensory unit potentials were seen in the acrylamide-exposed group compared to the referent group. Assessment of visual acuity and visual field, nerve conduction velocity, electrocardiography, and electroencephalography revealed no significant exposure-related effects.

Calleman et al. (1994) performed a cross-sectional analysis of hemoglobin adduct formation and neurological effects in a group of 41 factory workers who were exposed to acrylamide (and acrylonitrile, from which acrylamide is formed) for 1 month to 11.5 years (mean 3 years) during the production of acrylamide and polyacrylamide at a factory in China. As determined by station sampling and gas chromatography, mean acrylamide air concentrations were 1.07 and 3.27 mg/m^3 in the synthesis and polymerization rooms, respectively, during the summer of 1991. Mean exposure concentrations during the time of collection of biomarker data (September 1991) were lower, averaging 0.61 and 0.58 mg/m^3 in synthesis and polymerization rooms, respectively. Information regarding demographic factors, smoking and drinking habits, height and weight, occupational history, past illnesses, current symptoms, and reproductive history were collected by questionnaire. Neurological examinations were performed approximately 1 hour after a work shift. Vibration sensitivity thresholds were measured in fingers and toes. Physical and neurological examinations and electroneuromyographic (ENMG) testing were performed. For each test, a nonexposed referent group was included. Quantitative assessment of contributions of dermal and inhalation exposure were not made, although in the synthesis area of the factory where neurological symptoms were most severe, dermal contact was considered to have been the major exposure route.

Table 3-2. Self-Reported Neurological Symptoms and Observed Clinical Signs Among Acrylamide Workers and Nonexposed Workers

	Acrylamide group (n=71)		Reference group (n=51)	
Symptom	Number	Percent	Number	Percent
Skin peeling from the hands	38	53.5[a]	2	3.9
Numbness in the hands and feet	15	21.1[b]	2	3.9
Lassitude	14	19.7[b]	1	1.9
Sleepiness	12	16.9[b]	0	0
Muscle weakness	11	15.4[b]	0	0
Clumsiness of the hands	8	11.2[a]	0	0
Anorexia	8	11.2[a]	1	1.9
Unsteady gait	6	8.4[a]	0	0
Coldness of the hands and feet	6	8.4[a]	0	0
Difficulty in grasping	5	7.0[a]	0	0
Stumbling and falling	5	7.0[a]	0	0
Sweating	27	38.0	14	27.4
Dizziness	7	9.8	2	3.9
Cramping pain	6	8.4	5	9.8
Sign				
Erythema of hands	16	22.5[b]	0	0
Skin peeling from the hands	16	22.5[b]	1	1.9
Sensory impairments				
Vibration sensation	12	16.9[b]	0	0
Pain sensation	7	9.8[a]	0	0
Touch sensation	6	8.4[a]	0	0
Position sensation	1	1.4	0	0
Muscle atrophy in hands	4	5.6	0	0
Diminished reflexes				
Biceps	12	16.9[b]	0	0
Triceps	10	14.0	4	7.8
Knee	11	15.4[a]	1	1.9
Ankle	8	11.2[a]	1	1.9
Loss of reflexes				
Biceps	3	4.2	0	0
Triceps	5	7.0	1	1.9
Knee	5	7.0[a]	0	0
Ankle	17	23.9[b]	0	0
Intention tremor	13	18.3[a]	2	3.9
Positive Romberg's sign	15	21.1	3	5.8

[a]$p<0.05$
[b]$p<0.01$ (χ^2 test)

Source: He et al. 1989

As shown in Table 3-3, a variety of symptoms and signs of adverse health effects were noted in acrylamide-exposed workers (Calleman et al. 1994). Other significant ($p<0.01$) effects in the exposed workers included increased (magnitude ≥60%) vibration threshold, decreased (10–20%) conduction velocity in the peroneal and sural nerves, and increased (25–36%) latency in median, ulnar, and peroneal nerves. Neurotoxicity index scores, a quantitative expression of the severity of peripheral neuropathy, decreased with physical distance from the synthesis room where the monomer itself was handled. This relationship was not reflected by results of hand or foot vibration sensitivity measurements.

Hagmar et al. (2001) performed a health examination on a group of 210 tunnel construction workers who had been occupationally exposed for 2 months to a chemical grouting agent containing acrylamide and N-methylolacrylamide. Workers were expected to have experienced dermal exposure as well as inhalation exposure. Venous blood samples were drawn and questionnaires and physical examinations were administered 1–5 weeks after exposure was stopped. Quantitative exposure data were limited to two personal air samples showing concentrations of 0.27 and 0.34 mg/m^3 for the sum of acrylamide and N-methylolacrylamide; further analysis suggested that the air contained a 50:50 mixture of these compounds. The health examination included an extensive questionnaire and a physical examination that included unspecified tests of peripheral nerve function. Blood samples for the analysis of adducts of acrylamide with N-terminal valines in hemoglobin were drawn within a month after construction work was completed. A group of 50 subjects who claimed recently developed or deteriorated peripheral nervous function at the initial physical examination was subjected to more detailed neurophysiologic examinations and 6-month follow-up clinical (n=29) and neurophysiological (n=26) examinations. Those with remaining symptoms were examined for up to 18 months postexposure.

Hemoglobin adduct levels for 18 nonsmoking unexposed referents varied between 0.02 and 0.07 nmol/g globin. Adduct levels in 47 of the 210 tunnel workers did not exceed the highest level of the referents. The remaining workers were divided into three categories according to adduct levels as follows: 89 with 0.08–0.29 nmol/g globin, 36 with 0.3–1.0 nmol/g globin, and 38 with 1.0–17.7 nmol/g globin. The study authors noted a significant ($p<0.05$) association between self-reported exposure categories and adduct levels. Significant positive correlations ($p<0.05$) between prevalence of self-reported peripheral nervous symptoms, irritant symptoms, and symptoms of general discomfort with adduct levels were found. For example, in the groups with adduct levels <0.08 nmol/g globin, 0.08–0.29 nmol/g globin, 0.3–1.0 nmol/g globin, and >1.0 nmol/g globin, incidences of reported numbness or tingling in the feet or legs were 2/47 (4%), 10/89 (11%), 9/36 (25%), and 14/38 (37%), respectively. Irritant symptoms and symptoms of

Table 3-3. Prevalence of Symptoms and Signs of Adverse Health Effects in Acrylamide-Exposed Workers and Controls

Symptom or sign	Exposed (percent)	Controls (n=10)
Numbness of extremities	29/41 (71)[a]	0
Fatigue	29/41 (71)[a]	0
Sweating of hands and feet	28/41 (71)[a]	0
Skin peeling	24/41 (59)[a]	0
Menstruation disorders	4/7 (57)	NA
Loss of pain sensation	22/41 (54)[a]	0
Loss of touch sensation	19/41 (46)[b]	0
Dizziness	18/41 (44)[b]	0
Anorexia	17/41 (41)[b]	0
Loss of vibration sensation	17/41 (41)[b]	0
Nausea	16/41 (39)[b]	0
Loss of ankle reflexes	12/41 (29)	0
Headache	11/41 (27)	0
Unsteady gait	9/41 (22)	0
Loss of knee jerk	8/41 (20)	0
Unsteady Romberg sign	8/41 (20)	0
Loss of triceps reflexes	4/41 (10)	0
Loss of biceps reflexes	4/41 (10)	0

[a]$p<0.01$ (χ^2 test)
[b]$p<0.05$

Source: Calleman et al. 1994

general discomfort typically disappeared following the end of a workday, whereas peripheral nervous symptoms persisted. Follow-up examinations revealed that 58% of the subjects with early signs of impaired peripheral nervous function improved, while only 4% showed signs of deterioration.

Myers and Macun (1991) investigated peripheral neuropathy in a cohort of 66 workers in a South African factory that produced polyacrylamide. The investigation followed clinical diagnosis of peripheral neuropathy in five workers at the factory. The workforce was divided into a number of exposure categories, based on environmental sampling and discussions with workers. Exposure levels for the various tasks ranged from 0.07 to 2.5 times the National Institute of Occupational Safety and Health (NIOSH) recommended exposure limit (REL) of 0.3 mg/m^3. Workers were then classified as being exposed to airborne acrylamide when exposure levels exceeded the REL (n=22), and unexposed when exposure levels were below the REL (n=41). Workers completed a questionnaire that was designed to capture social, medical, and occupational history. A standard blind neurological examination was also performed.

The exposed group showed higher prevalences of abnormalities for all symptoms (weakness, sensation, balance, fatigue, visual, loss of weight, urogenital, and fingertip skin), most signs (fingertip effects, light touch, tactile discrimination, pain), and reflexes, coordination, motor weakness, gait, and Rombergism. Statistically significant differences between exposed and unexposed groups for individual effects were seen only for abnormal sensation symptoms and signs in fingertip skin (including color, peeling, and sweating). The overall prevalence of acrylamide-related abnormalities among the exposed was 66.7%, which was statistically significantly higher ($p<0.05$) than that of the unexposed group (prevalence of 14.3%). The authors stated that most workers observed to have abnormalities (number not reported) were employed in areas where exposures were highest (1.6–2.5 times the REL).

Bachmann et al. (1992) performed a follow-up investigation in July 1990 at the same South African factory that had been examined in 1986 by Myers and Macun (1991). The study design was similar to that of Myers and Macun (1991), but included measurements of vibration sensation threshold. Among 82 workers employed at follow-up, increased prevalences of symptoms of tingling and numbness in hands and feet, weakness and pain in arms and legs, peeling hand skin, and sweating hands were reported by exposed workers, compared with those classified as being unexposed. The symptoms of numbness, limb pain, and peeling and sweating of hands were statistically significantly increased in exposed workers. Results of clinical examinations provided supporting evidence for the reported increased symptoms of peeling and sweating of the hands. No gross neurological abnormalities were found. Mean vibration

sensation thresholds were similar among unexposed and exposed groups, even when adjusting for age, and no association was found between vibration thresholds and any symptoms.

Information regarding neurological effects in animals exposed to acrylamide by the inhalation route is limited to a single study report in which seven rats, seven guinea pigs, and two dogs were exposed to dust of acrylamide at an analytical concentration of 15.6 mg/m^3 for 6 hours/day, 5 days/week for up to 12 exposures in a 16-day period (American Cyanamid Company 1953a). Reported signs of neurological effects in the three rats that survived to exposure termination on day 4 included loss of equilibrium and coordination. There was no mention of neurological signs in the exposed dogs, although one of the dogs lost weight and died on day 15. No toxic signs were seen in the guinea pigs.

3.2.1.5 Reproductive Effects

No data were located regarding reproductive effects in humans or animals following inhalation exposure to acrylamide.

3.2.1.6 Developmental Effects

No data were located regarding developmental effects in humans or animals following inhalation exposure to acrylamide.

3.2.1.7 Cancer

Human data are available from two cohort mortality studies of occupational exposure to acrylamide, one by Collins et al. (1989) with most recent follow up by Marsh et al. (2007) and one by Sobel et al. (1986) with follow up by Swaen et al. (2007). Exposure to acrylamide was considered to have occurred primarily via inhalation and dermal exposure.

Collins et al. (1989) conducted a cohort mortality study of all male workers (8,854, of which 2,293 were exposed to acrylamide) who had been hired between January 1, 1925 and January 31, 1973 at four American Cyanamid factories, three in the United States (Fortier, Louisiana [1295 workers]; Warners, New Jersey [7,153 workers]; and Kalamazoo, Michigan [60 workers]) and one in the Netherlands (Botlek [346 workers]). Estimations of acrylamide exposure were based on available monitoring data and worker knowledge of past jobs and processes. Mortality rates among the factory workers were compared with the expected number of deaths among men of the United States from 1925 to 1980 or the Netherlands

from 1950 to 1982 to derive standardized mortality ratios (SMRs) as a measure of relative risk for each cohort. No statistically significantly elevated all cause or cause-specific SMRs were found among acrylamide-exposed workers (including cancer of the digestive or respiratory systems, bone, skin, reproductive organs, bladder, kidney, eye, central nervous system, thyroid, or lymphatic system). Trend tests showed no increased risk of mortality due to cancer at several sites (digestive tract, respiratory system, prostate, central nervous system, or lymphopoietic system) with increasing level of exposure to acrylamide.

The most recent update report of the cohort of Collins et al. (1989) includes study periods of 1925–2002 for the 8,508 workers in the three facilities in the United States and 1965–2004 for the 344 workers at the Botlek plant in the Netherlands (the original cohort of 346 people included 2 females who were excluded in the follow up) (Marsh et al. 2007). Among the workers at the three facilities in the United States (during which 4,650 deaths occurred among the 8,508 workers in the period of 1925–2002), excess and deficit overall mortality risks were observed for cancer sites implicated in oral studies in experimental animals: brain and other central nervous system (SMR 0.67, 95% confidence interval [CI] 0.40–1.05), thyroid gland (SMR 1.38, 95% CI 0.28–4.02), and testis and other male genital organs (SMR 0.64, 95% CI 0.08–2.30); and for sites selected in the original report (Collins et al. 1989) of this cohort: respiratory system cancer (SMR 1.17, 95% CI 1.06–1.27), esophagus (SMR 1.20, 95% CI 0.86–1.63), rectum (SMR 1.25, 95% CI 0.84–1.78), pancreas (SMR 0.94, 95% CI 0.70–1.22), and kidney (SMR 1.01, 95% CI 0.66–1.46). None of the mortality excesses were statistically significant, except for respiratory system cancer, which Collins et al. (1989) attributed to muriatic acid (hydrochloric acid) exposure. No significantly elevated SMRs were found for rectal, pancreatic, or kidney cancers in exploratory exposure-response analyses conducted according to the following exposure parameters and categories: duration of employment (<1, 1–, and 15+ years), time since first employment (<20, 20–, and 30+ years), duration of exposure (unexposed, 0.001–, 5–, and 20+ years), cumulative exposure (<0.001, 0.001–, 0.03–, and 0.30+ mg/m^3-years), and estimated mean exposure concentrations (unexposed, 0.001–, 0.02–, and 0.3+ mg/m^3).

Sobel et al. (1986) conducted a mortality study on a cohort of 371 workers assigned to acrylamide and polymerization operations at a Dow Chemical facility in the United States between 1955 and 1979. Analysis and review of air monitoring data and job classifications resulted in estimates of personal 8-hour time-weighted average acrylamide concentrations of 0.1–1.0 mg/m^3 before 1957, 0.1–0.6 mg/m^3 from 1957 to 1970, and 0.1 mg/m^3 thereafter. SMRs, calculated for categories in which at least two deaths were observed, were based on mortality of white males in the United States. No significantly increased incidences of cancer-related deaths were observed within the cohort.

Followup to the Sobel et al. (1986) study cohort was expanded to include employees hired through 2001 (Swaen et al. 2007). Exposure to acrylamide was retrospectively assessed based on personal samples from the 1970s onwards and area samples from the entire study period. Fewer acrylamide workers died (n=141) than expected (n=172.1). No cause-specific SMR for any of the investigated types of cancer was exposure related. The authors reported more total pancreatic cancer deaths (n=5) than expected (n=2.3) (SMR 222.2, 95% CI 72.1–518.5); however, three of the five were in the low-dose group, with no apparent dose-response relationship with acrylamide exposure.

Meta-analyses of the most recent results from the two major cohort studies that assessed cancer risk and occupational exposure to acrylamide (Marsh et al. 2007; Swaen et al. 2007) were performed by Pelucchi et al. (2011b). The results indicate a lack of increased risk of cancers of the digestive tract, pancreas, lung, or kidney among these cohorts.

No data were located regarding cancer in animals following inhalation exposure to acrylamide. EPA (IRIS 2012) calculated an inhalation unit risk of $1x10^{-4}$ per $\mu g/m^3$ for acrylamide based on results of a 2-year cancer bioassay in orally-exposed male and female F344 rats (Johnson et al. 1986) and route-to-route extrapolation (see Section 3.2.2.7 for details of the oral study). The air concentrations associated with risk of $1x10^{-4}$, $1x10^{-5}$, $1x10^{-6}$, and $1x10^{-7}$ are 1, 0.1, 0.01, and 0.001 mg/m^3, respectively. These risk levels are presented in Figure 3-1.

3.2.2 Oral Exposure

3.2.2.1 Death

There are no reports of human deaths associated with oral exposure to acrylamide.

Acrylamide has been demonstrated to be lethal to laboratory animals following a single oral dose. Reported oral LD_{50} values in rats range from 150 to 413 mg/kg (American Cyanamid Company 1973, 1977; Dow Chemical Company 1957; Fullerton and Barnes 1966; McCollister et al. 1964; Tilson and Cabe 1979; Union Carbide Corporation 1947). Reported LD_{50} values in mice, guinea pigs, and rabbits range from 107 to 195 mg/kg (American Cyanamid Company 1951; Dow Chemical Company 1957; Hashimoto et al. 1981; McCollister et al. 1964).

Repeated oral exposure to acrylamide has also been associated with death in laboratory animals. In one rat study, a single 100 mg/kg dose was not lethal, but two 100 mg/kg doses administered 24 hours apart resulted in mortalities (Fullerton and Barnes 1966). Sublet et al. (1989) reported death in a group of male Long-Evans hooded rats administered acrylamide by daily gavage for 5 days at a dose of 75 mg/kg/day. Longer repeated-dose exposure periods to lower daily doses are lethal as well. For example, a dose level of 50 mg/kg was lethal to rats receiving 12 daily gavage doses of 50 mg/kg in a 15-day period; the deaths occurred within a few days following the cessation of dosing (Fullerton and Barnes 1966). All mice (4/sex) given acrylamide in the drinking water at a concentration resulting in an estimated dose of 150 mg/kg/day were sacrificed moribund on the 10^{th} day of treatment (NTP 2011b). No deaths occurred in groups given acrylamide in the drinking water for 14 days at exposure levels resulting in estimated doses ranging from 2 to 76 mg/kg/day or in other mice receiving acrylamide from the food for 14 days at estimated doses up to 75 mg/kg/day (NTP 2011b). Similar treatment of rats to acrylamide in the drinking water or food resulted in the death of one high-dose (77 mg/kg/day) male from the drinking water study; there were no deaths in the female rats exposed via the drinking water or food (doses up to 70 and 63 mg/kg/day) or the male rats exposed via the food (doses up to 52 mg/kg/day). An acrylamide gavage dose of 30 mg/kg/day resulted in the death of 4/10 male and 2/10 female rats during the third week of daily dosing (Schulze and Boysen 1991). No deaths occurred in rats or mice receiving acrylamide from the drinking water for 13 weeks at estimated doses as high as 22–26 mg/kg/day (rats) and 70–83 mg/kg/day (mice) (NTP 2011b). Thirteen weeks of exposure to acrylamide in the food resulted in the death of one male mouse each at estimated dose levels of 32 and 59 mg/kg/day; there were no deaths in female mice at estimated doses as high as 64 mg/kg/day or among similarly-treated male and female rats at estimated doses as high as 14 and 18 mg/kg/day, respectively (NTP 2011b). During the last 4 months of a 2-year study in which male and female rats received acrylamide from the drinking water at an estimated dose level of 2 mg/kg/day, decreased survival of both sexes was noted; the increased mortality was statistically significant by study termination (Johnson et al. 1984, 1986). In other studies of rats and mice administered acrylamide in the drinking water for 2 years, significantly decreased survival was noted at estimated doses of ≥0.9 mg/kg/day (female rats), ≥4.6 mg/kg/day (female mice), and 9 mg/kg/day (male rats) (NTP 2011b).

Reliable acute oral LD_{50} values for death and other mortality data for each species are recorded in Table 3-4 and plotted in Figure 3-2.

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Wistar)	Once (GW)				294 M (LD50)	American Cyanamid Company 1973	
2	Rat (Sprague-Dawley)	Once (GW)				413 M (LD50)	American Cyanamid Company 1977	
3	Rat (NS)	Once (GW)				180 (LD50)	Dow Chemical Company 1957; McCollister et al. 1964	
4	Rat (albino)	Once (GW)				203 F (LD50)	Fullerton and Barnes 1966 Acrylamide	
5	Rat (albino)	2 d 1 x/d (GW)				100 (most rats died within 3 days following 2 days of dosing)	Fullerton and Barnes 1966 Acrylamide	
6	Rat (Fischer- 344)	14 d (W)				76.6 M (1/4 died)	NTP 2011	
7	Rat (Fischer- 344)	Once (GW)				175 M (LD50)	Tilson and Cabe 1979 Acrylamide	
8	Rat (albino)	Once (GW)				316 M (LD50)	Union Carbide Corporation 1947	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
9	Mouse (albino)	Once (G)				195 M (LD50)	American Cyanamid Company 1951	
10	Mouse	Once (NS)				107 M (LD50)	Hashimoto et al. 1981 Acrylamide	
11	Mouse (B6C3F1)	14 d (W)				150 (moribund sacrifice of 4/4 males and 4/4 females on treatment day 10)	NTP 2011 Acrylamide	
12	Gn Pig (NS)	Once (GW)				180 (LD50)	Dow Chemical Company 1957; McCollister et al. 1964	
13	Rabbit (NS)	Once (GW)				150 (LD50)	Dow Chemical Company 1957; McCollister et al. 1964	
Systemic								
14	Rat (Fischer- 344)	13 d (W)	Bd Wt	5 M	20 M (8% decreased mean body weight)		Burek et al. 1980 Acrylamide	
15	Rat (Fischer- 344)	14 d (W)	Bd Wt	37.4 M 39.4 F	70 F (15% lower terminal body weight)	76.6 M (44% lower terminal body weight)	NTP 2011	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
16	Rat (Fischer- 344)	14 d (F)	Bd Wt	22.4 M 63.4 F		51.7 M (28% lower terminal body weight)	NTP 2011 Acrylamide	
17	Rat (Long- Evans)	5 d 1 x/d (GW)	Bd Wt	5 M	15 M (significantly depressed body weight gain during 5 days of acrylamide administration)		Tyl et al. 2000b Acrylamide	
18	Rat (Fischer- 344)	Gd 6-17 (GW)	Bd Wt	20 F			Walden et al. 1981 Acrylamide	Maternal body weight
19	Mouse (B6C3F1)	14 d (W)	Bd Wt	66.7 M 75.8 F		150 (marked weight loss and moribund sacrifice at treatment day 10)	NTP 2011 Acrylamide	
20	Mouse (B6C3F1)	14 d (F)	Bd Wt	72.8 M 75.7 F			NTP 2011 Acrylamide	
21	Mouse ddY	Once (NS)	Bd Wt	100 M	150 M (significantly depressed body weight)		Sakamoto et al. 1988 Acrylamide	
22	Gn Pig (NS)	Once (GW)	Bd Wt		126 (very slight initial body weight loss)		Dow Chemical Company 1957; McCollister et al. 1964	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
23	Rabbit (NS)	Once (GW)	Bd Wt		63 (slight initial weight loss)		Dow Chemical Company 1957; McCollister et al. 1964	
Neurological								
24	Rat (Fischer- 344)	7 d (W)		20 M			Burek et al. 1980 Acrylamide	
25	Rat (Wistar)	Up to 21 d 1 x/d (G)				25 M (convulsions and ataxia as early as treatment day 14)	Dixit et al. 1981 Acrylamide	
26	Rat (albino)	Once (GW)				203 F (fine tremors)	Fullerton and Barnes 1966 Acrylamide	
27	Rat (albino)	Once (GW)				100 (fine tremors)	Fullerton and Barnes 1966 Acrylamide	
28	Rat (albino)	2 d 1 x/d (GW)				100 (generalized weakness)	Fullerton and Barnes 1966 Acrylamide	
29	Rat (NS)	Once (GW)				126 F (lethargy)	McCollister et al. 1964 Acrylamide	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
30	Rat (Fischer- 344)	14 d (W)		37.4 M 39.4 F		76.6 M (hind-leg paralysis in 4/4 males) 70 F (hind-leg paralysis in 4/4 females)	NTP 2011	
31	Rat (Fischer- 344)	14 d (F)		22.4 M 29.4 F		51.7 M (hind-leg paralysis in 4/4 males) 63.4 F (hind-leg paralysis in 4/4 females)	NTP 2011 Acrylamide	
32	Rat (Fischer- 344)	Once (GW)		100 M		200 M (decreases in hindlimb grip strength and locomotory performance)	Tilson and Cabe 1979 Acrylamide	
33	Rat (Long- Evans)	5 d 1 x/d (GW)		30 M		45 M (clinical signs of neurotoxicity)	Tyl et al. 2000b Acrylamide	
34	Rat (Fischer- 344)	Gd 6-17 (GW)		20 F			Walden et al. 1981 Acrylamide	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form	Comments
35	Mouse (BALB/c)	12 d (W)				25.8 F	(decreased rotarod performance, increased hindlimb splay as early as days 6-8)	Gilbert and Maurissen 1982 Acrylamide	
36	Mouse (B6C3F1)	14 d (W)		66.7 M 75.8 F		150	(hind-leg paralysis in 1/4 males and 1/4 females prior to moribund sacrifice)	NTP 2011 Acrylamide	
37	Mouse (B6C3F1)	14 d (F)		72.8 M 75.7 F				NTP 2011 Acrylamide	
38	Dog (Mongrel)	Once (C)				100	(severe neurological impairment of the limbs)	American Cyanamid Company 1953c	
39	Rabbit (NS)	Once (GW)				126	(tremors)	McCollister et al. 1964 Acrylamide	
Reproductive									
40	Rat (Fischer- 344)	14 d (W)		37.4 M		76.6 M	(seminiferous tubule degeneration in 4/4 males)	NTP 2011	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)			
41	Rat (Fischer- 344)	14 d (F)		22.4 M		51.7 M	(seminiferous tubule degeneration in 2/4 males)	NTP 2011 Acrylamide	
42	Rat (Long- Evans)	5 d 1 x/d (GW)		5 M[b]		15 M	(depressed fertility, increased preimplantation loss)	Sublet et al. 1989 Acrylamide	
43	Rat (Long- Evans)	5 d 1 x/d (GW)		30 M		45 M	(significantly increased postimplantation losses)	Tyl et al. 2000b Acrylamide	A significant trend for increased postimplantation loss was observed at doses from 15 to 60 mg/kg/day
44	Rat (Fischer- 344)	5 d 1 x/d (GW)				30 M	(significantly elevated pre- and post-implantation losses)	Working et al. 1987 Acrylamide	
45	Mouse (B6C3F1)	14 d (W)		66.7 M				NTP 2011 Acrylamide	
46	Mouse (B6C3F1)	14 d (F)		72.8 M				NTP 2011 Acrylamide	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
47	Mouse ddY	Once (NS)				100 M (histologic abnormalities in spermatids)	Sakamoto et al. 1988 Acrylamide	
INTERMEDIATE EXPOSURE								
Death								
48	Rat (albino)	21 d (F)				47 M (4/10 deaths during 21 days of exposure)	American Cyanamid Company 1953b	
49	Rat (Fischer- 344)	28 d (W)				25 M (death of 8/10 male rats during exposure week 4)	American Cyanamid Company 1991	
						24 F (death of 5/10 female rats during exposure week 4)		
50	Rat (albino)	12 doses in 15 d (GW)				50 (death within a few days posttreatment)	Fullerton and Barnes 1966 Acrylamide	
51	Rat (Sprague-Dawley)	5 wk 7 d/wk 1 x/d (GW)				30 (death of 4/10 males and 2/10 females during treatment week 3)	Schulze and Boysen 1991 Acrylamide	
Systemic								
52	Rat (albino)	21 d (F)	Bd Wt			47 M (severely depressed body weight gain)	American Cyanamid Company 1953b	Magnitude not specified

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
53	Rat (Sprague-Dawley)	2 wk premating and Gd 0-19 (F)	Bd Wt	3.82 F			American Cyanamid Company 1979 Acrylamide	
54	Rat (Fischer- 344)	28 d (W)	Endocr	12 M	19 M (decreased serum testosterone, 73% less than controls)		American Cyanamid Company 1991	
			Bd Wt	12 M 9 F		19 (emaciation)		
55	Rat (Fischer- 344)	up to 93 d (W)	Hemato	1 F	5 F (decreases in packed cell volume, erythrocytes, hemoglobin)		Burek et al. 1980 Acrylamide	
56	Rat (Fischer- 344)	16 d (dams) Gd 6-21 38 d (pups) Gd 6-Ppd 22 (GW)	Bd Wt	5 F			Ferguson et al. 2010	
57	Rat (Sprague-Dawley)	Gd 6-20 1 x/d (GW)	Bd Wt	2.5 F	7.5 F (12% decreased maternal weight gain)		Field et al. 1990 Acrylamide	Weight gain minus gravid uterine weight

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
58	Rat (Wistar)	21 d lactation period 1 x/d (G)	Bd Wt			25 F (net maternal weight loss during 21-day lactation treatment period)	Friedman et al. 1999 Acrylamide	
59	Rat (Fischer- 344)	13 wk (W)	Hemato	8.6 M 12.3 F	22.3 M (congestion and pigment in spleen, erythroid cell hyperplasia in bone marrow)		NTP 2011	
					26.3 F (congestion and pigment in spleen, erythroid cell hyperplasia in bone marrow)			
			Bd Wt	8.6 M 6 F	12.3 F (10% lower mean terminal body weight)	22.3 M (29% lower mean terminal body weight)		
60	Rat (Fischer- 344)	13 wk (F)	Bd Wt	5.5 M 6.6 F	14.2 M (15% lower mean terminal body weight)		NTP 2011	
					17.9 F (14% lower mean terminal body weight)			
61	Rat (CD)	6 wk Gd 6-Ld 21 (W)	Bd Wt	7.89 F	14.56 F (8% depressed mean body weight)		Ogawa et al. 2011	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
62	Rat (Sprague-Dawley)	5 wk 7 d/wk 1x/d (GW)	Bd Wt			30 (decreased mean body weight, 27% lower than controls)	Schulze and Boysen 1991 Acrylamide	
63	Rat (Sprague-Dawley)	4 wk 5 d/wk 1 x/d (G)	Bd Wt	15 M	30 M (14% lower mean body weight than controls)		Shi et al. 2011	
64	Rat (Fischer- 344)	12 wk Ld 1-21 (dams) 9 wk postweaning (pups) (W)	Bd Wt	4.4 M 4.9 F			Takami et al. 2011	
65	Rat (Wistar)	90 d (W)	Bd Wt	23.7 M			Tanii and Hashimoto 1983 Acrylamide	
66	Rat (Sprague-Dawley)	8 wk 1x/d (GW)	Bd Wt		5 M (>10% depressed mean body weight during most of the 8-week treatment period)		Wang et al. 2010	
67	Rat (Sprague-Dawley)	Gd 6- Ld 10 1 x/d (GW)	Bd Wt	5 F		10 F (approximately 33% depressed maternal body weight gain during 10 days of postpartum exposure)	Wise et al. 1995 Acrylamide	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
68	Mouse (CD-1)	Gd 6-20 1 x/d (GW)	Bd Wt	45 F			Field et al. 1990 Acrylamide	
69	Mouse (ICR)	Up to 38 d (W)	Bd Wt			90.8 M (body weight loss)	Ko et al. 1999 Acrylamide	
70	Mouse (B6C3F1)	13 wk (F)	Bd Wt	32.1 M 13.9 F	59.4 M (12% lower mean terminal body weight)	64 F (22% lower mean terminal body weight)	NTP 2011	
71	Mouse (B6C3F1)	13 wk (W)	Bd Wt	32.8 M 31.4 F	70 M (13% lower mean terminal body weight) 83.1 F (12% lower mean terminal body weight)		NTP 2011	
72	Dog	8 wk (F)	Bd Wt			7 (up to 12% weight loss)	Satchell and McLeod 1981 Acrylamide	
73	Cat	Up to 16 wk (F)	Bd Wt		15 (body weight loss, magnitude unspecified)		Post and McLeod 1977a Acrylamide	
Neurological								
74	Monkey	44-61 d 5 d/wk 1 x/d				10 F (clinical signs of peripheral neuropathy)	Dow Chemical Company 1981; Maurissen et al. 1983 Acrylamide	Histopathological evaluation of nerve tissue not performed

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
75	Monkey	6-10 wk 5 d/wk 1 x/d				10 (degenerative changes in visual nerve fibers)	Eskin et al. 1985 Acrylamide	
76	Monkey	NS 1 x/d				30 (peripheral neuropathy)	Leswing and Ribelin 1969 Acrylamide	
77	Monkey (NS)	up to 363 d 5 d/wk (F)		3 F		10 F (clinical signs of peripheral neuropathy)	McCollister et al. 1964 Acrylamide	Only 1 animal per dose group; no clear signs of toxicity at 3 mg/kg/day
78	Monkey	33-47 d 5 d/wk 1 x/d				10 F (ataxia, adverse visual effects)	Merigan et al. 1985 Acrylamide	
79	Rat (albino)	21 d (F)				47 M (paralysis of hind limbs)	American Cyanamid Company 1953b	
80	Rat (albino)	NS 5 d/wk (G)				25 M (clinical signs of peripheral neuropathy)	American Cyanamid Company 1959	
81	Rat (Sprague-Dawley)	2 wk premating and Gd 0-19 (F)		3.82 F			American Cyanamid Company 1979 Acrylamide	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
82	Rat (Fischer- 344)	28 d (W)		12 M 9 F		19 (clinical signs of peripheral neuropathy)	American Cyanamid Company 1991	Histopathological evaluation of nerve tissue not performed
83	Rat (Fischer- 344)	up to 93 d (W)		0.2[c] M	1 M (reversible ultrastructural degeneration in sciatic nerve fibers)	20 (progressive signs of peripheral neuropathy)	Burek et al. 1980 Acrylamide	
84	Rat (Wistar)	Up to 21 d 1 x/d (G)				25 M (complete hindlimb paralysis by treatment day 21)	Dixit et al. 1981 Acrylamide	
85	Rat (Wistar)	21 d lactation period 1 x/d (G)				25 F (progressive clinical signs of neuropathy beginning as early as treatment day 4)	Friedman et al. 1999 Acrylamide	electron microscopic examinations were not performed on sciatic nerve
86	Rat (albino)	12 doses in 15 d (GW)				50 (severe weakness)	Fullerton and Barnes 1966 Acrylamide	
87	Rat (albino)	variable (F)				6 (slight leg weakness after 40 weeks of exposure)	Fullerton and Barnes 1966 Acrylamide	
88	Rat (albino)	38 d (F)				25 (severe leg weakness)	Fullerton and Barnes 1966 Acrylamide	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
89	Rat (albino)	Variable 5 d/wk 1 x/d (GW)				25 (severe leg weakness by 28 days of treatment)	Fullerton and Barnes 1966 Acrylamide	
90	Rat (albino)	55 doses 5 d/wk 1 x/d (GW)		10 F			Fullerton and Barnes 1966 Acrylamide	Histopathological evaluation of nerve tissue not performed
91	Rat (albino)	Variable 1 d/wk 1 x/d (GW)				100 (signs of severe neuropathy by the third dose)	Fullerton and Barnes 1966 Acrylamide	Younger rats appeared to be less severely affected.
92	Rat (Fischer- 344)	21 d (W)		3 M 10 F		10 M (clinical and histopathologic evidence of peripheral neuropathy) 30 F (clinical and histopathologic evidence of peripheral neuropathy)	Gorzinski et al. 1979	
93	Rat (Fischer- 344)	3 mo 6 mo (W)		0.5 M	2 M (electron microscopic degenerative effects in sciatic nerve fibers)		Johnson et al. 1984, 1986 Acrylamide	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
94	Rat (Fischer- 344)	13 wk (W)		8.6 M 6 F		22.3 M (hind-leg paralysis in 8/8 males) 12.3 F (hind-leg paralysis in 4/8 females)	NTP 2011	
95	Rat (Fischer- 344)	13 wk (F)		5.5 M 6.6 F		14.2 M (hind-leg paralysis) 17.9 F (hind-leg paralysis)	NTP 2011	
96	Rat (CD)	6 wk Gd 6-Ld 21 (W)		3.72 F	7.89 F (slightly abnormal gait)	14.56 F (severely abnormal gait)	Ogawa et al. 2011	
97	Rat (Sprague-Dawley)	5 wk 7 d/wk 1x/d (GW)				10 (degenerative effects in nerve fibers)	Schulze and Boysen 1991 Acrylamide	
98	Rat (Sprague-Dawley)	4 wk 5 d/wk 1 x/d (G)		5 M		15 M (neurotoxicity evidenced by clinical signs and biochemical and histologic lesions in cerebellum)	Shi et al. 2011	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
99	Rat (Sprague-Dawley)	Gd 6-Ppd 21 (W)		3.72 F	7.89 F (abnormal gait)		Takahashi et al. 2009 Acrylamide	
100	Rat (Fischer- 344)	12 wk Ld 1-21 (dams) 9 wk postweaning (pups) (W)		4.4 M 4.9 F			Takami et al. 2011	
101	Rat (Wistar)	90 d (W)		8.8 M		14.5 M (decreased rotarod performance)	Tanii and Hashimoto 1983 Acrylamide	Electron microscopic evaluations of nerve tissues not performed
102	Rat (Fischer- 344)	4 wk 5 d/wk (GW)				10 M (hindlimb dysfunction)	Tilson and Cabe 1979 Acrylamide	
103	Rat (Fischer- 344)	16 wk (W)		5 F		5 M (slight axonal fragmentation in peripheral nerves of 6/6 males assessed)	Tyl et al. 2000a Acrylamide	Assessment included clinical signs, histopathological evaluations of peripheral nerves
104	Rat (Sprague-Dawley)	Gd 6- Ld 10 1 x/d (GW)		10 F		15 F (hindlimb splay in 100% of dams during the first few days of postpartum exposure)	Wise et al. 1995 Acrylamide	Histopathology of peripheral nerve tissue was not performed

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
105	Rat (Sprague-Dawley)	4 wk 5 d/wk 1 x/d (G)				15 M (hind-leg splay)	Yuxin et al. 2011	
106	Rat (Long- Evans)	10 wk (W)				7.9 M (increased incidences of hindlimb splay) 14.6 F (increased incidences of foot splay)	Zenick et al. 1986 Acrylamide	NOAEL for neurological effects not established due to lack of histopathologic assessment of peripheral nerve tissue
107	Mouse (CD-1)	Gd 6-20 1 x/d (GW)		15 F		45 F (up to 48% incidence of hindlimb splay)	Field et al. 1990 Acrylamide	
108	Mouse	8 wk 2 x/wk (NS)				36 M (clinical signs of peripheral neuropathy, testicular atrophy)	Hashimoto et al. 1981 Acrylamide	
109	Mouse (ICR)	Up to 38 d (W)				90.8 M (clinically and histopathologically confirmed peripheral neuropathy)	Ko et al. 1999 Acrylamide	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
110	Mouse (ICR)	NS (W)				91.8 M (clinical signs of peripheral neuropathy; ultrastructural degeneration in cutaneous nerve terminals)	Ko et al. 2000; Ko et al. 2002 Acrylamide	
111	Mouse (B6C3F1)	13 wk (F)		32.1 M 13.9 F		59.4 M (hind-leg paralysis in 8/8 males) 64 F (hind-leg paralysis in 8/8 females)	NTP 2011	
112	Mouse (B6C3F1)	13 wk (W)		32.8 M 31.4 F		70 M (hind-leg paralysis in 8/8 males) 83.1 F (hind-leg paralysis in 8/8 females)	NTP 2011	
113	Dog (Mongrel)	19 wk 6 d/wk 1 x/day (C)		1		8 (loss of coordination in the rear extremities)	American Cyanamid Company 1953c	
114	Dog (Mongrel)	29 d 7 d/wk 1 x/day (C)				10 F (incoordination and weakness of the hind legs)	American Cyanamid Company 1953c	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form	Comments
115	Dog	6-7 wk (C)				5.7	(clinical evidence of neuropathy)	Hersch et al. 1989a Acrylamide	
116	Dog	8 wk (F)				7	(clinical signs of peripheral neuropathy)	Satchell and McLeod 1981 Acrylamide	
117	Cat	NS 1 x/d				20	(peripheral neuropathy)	Leswing and Ribelin 1969 Acrylamide	
118	Cat (NS)	up to 367 d 5 d/wk (F)		1		3	(twitching motion in hindquarters at 26 days, slightly unsteady gait at 47 days, definite weakness in the hindquarters at 68 days)	McCollister et al. 1964 Acrylamide	Histopathological evaluation of nerve tissue not performed
119	Cat	Up to 16 wk (F)				15	(initial weakness of hindlimbs and subsequent paralysis of fore- and hind-limbs)	Post and McLeod 1977a Acrylamide	
120	Baboon	up to 137 d 1 x/d (F)				10	(peripheral neuropathy)	Hopkins 1970 Acrylamide	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
121	Rat (Fischer- 344)	28 d (W)		12 M		19 M (atrophy of the testes and/or seminal vesicles)	American Cyanamid Company 1991	
122	Rat (Fischer- 344)	13 wk (W)		2.1 M 12.3 F		4.5 M (germinal epithelium degeneration in testes of 5/8 males) 26.3 F (8/8 females in anestrus)	NTP 2011	
123	Rat (Fischer- 344)	13 wk (F)		1.4 M 17.9 F		2.8 M (germinal epithelium degeneration in testes)	NTP 2011	
124	Rat (Long- Evans)	80 d (W)		1.5 M		2.8 M (male-mediated increased postimplantation loss)	Smith et al. 1986 Acrylamide	
125	Rat (Fischer- 344)	12 wk Ld 1-21 (dams) 9 wk postweaning (pups) (W)		2.1 M		4.4 M (degenerative effects in seminiferous epithelium of testis and epididymis)	Takami et al. 2011	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
126	Rat (Fischer- 344)	16 wk (W)		2		5 M (dominant lethal mutation effects) 5 (decreases in implantations and number of live pups)	Tyl et al. 2000a Acrylamide	
127	Rat (Sprague-Dawley)	8 wk 1x/d (GW)			5 M (decreased epididymal sperm concentration)		Wang et al. 2010	
128	Rat (Sprague-Dawley)	4 wk 5 d/wk 1 x/d (G)		5 M		15 M (decreased sperm count, increased sperm abnormality)	Yuxin et al. 2011	
129	Rat (Long- Evans)	10 wk (W)				7.9 M (male-mediated reproductive effects including decreased percentage impregnation of nonexposed females and increased postimplantation loss)	Zenick et al. 1986 Acrylamide	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
130	Mouse (CD-1)	16-22 wk (W)		3.1 M		7.5 M (increased early resorptions, total postimplantation loss, decreased numbers of live fetuses, decreased numbers of live pups; apparently male mediated)	Chapin et al. 1995 Acrylamide	
131	Mouse NMRI	2 mo (W)				5 M (decreased sperm motility, increased percentage of immotile sperm)	Kermani-Alghoraishi et al. 2010	
132	Mouse (B6C3F1)	13 wk (F)		32.1 M 35.1 F		59.4 M (germinal epithelium degeneration in testes) 64 F (8/8 females in anestrus)	NTP 2011	
133	Mouse (B6C3F1)	13 wk (W)		32.8 M 31.4 F		70 M (germinal epithelium degeneration in testes of 6/8 males) 83.1 F (6/8 females in anestrus)	NTP 2011	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
134	Mouse ddY	4 wk (W)		9 M 18.7 F		13.3 M (decreased number of fetuses/dam)	Sakamoto and Hashimoto 1986 Acrylamide	
Developmental								
135	Rat (Sprague-Dawley)	2 wk premating and Gd 0-19 (F)		3.82			American Cyanamid Company 1979 Acrylamide	Assessment included mating and pregnancy indices, litter data, and offspring growth and survival
136	Rat (Fischer- 344)	16 d (dams) Gd 6-21 38 d (pups) Gd 6-Ppd 22 (GW)			5 (30-49% decreased open field activity)		Ferguson et al. 2010	
137	Rat (Sprague-Dawley)	Gd 6-20 1 x/d (GW)		15			Field et al. 1990 Acrylamide	Assessment included external, visceral, and skeletal examinations of offspring
138	Rat (Fischer- 344)	15 wk Gd 1-21 Ppd 1-84 (GW)		1.3	6 (effects on measures of cognitive motivation)		Garey and Paule 2007 Acrylamide	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
139	Rat (Fischer- 344)	Gd 6 through 8 mo of age (GW)		1	5 (decreased performance in an incremental repeated acquisition task, a measure of learning ability)		Garey and Paule 2010	
140	Rat (Fischer- 344)	5 wk Gd 7-Ppd 22 (GW)		5	10 (deficient negative geotaxis and rotarod performance)		Garey et al. 2005	
141	Rat (Wistar)	Throughout lactation via mothers or 5 d 1 x/d (G)				25 M (neurochemical changes in brain regions)	Husain et al. 1987 Acrylamide	
142	Rat (CD)	6 wk Gd 6-Ld 21 (W)				3.72 (biochemical indicators of compensatory regulation to repair acrylamide-impaired neurogenesis in the pup brain)	Ogawa et al. 2011	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
143	Rat (Sprague-Dawley)	Gd 6-Ppd 21 (W)		7.89		14.56 (>40% decreased mean pup body weight)	Takahashi et al. 2009 Acrylamide	
144	Rat (Sprague-Dawley)	Gd 6- Ld 10 1 x/d (GW)			5 F (significantly decreased pup body weight during the preweaning period, as much as 9% lower than controls)		Wise et al. 1995 Acrylamide	
					15 (increased overall horizontal activity and decreased auditory startle response)			
145	Rat (Long- Evans)	10 wk (W)		5.1 F	8.8 F (female-mediated decreased pup body weight)		Zenick et al. 1986 Acrylamide	
146	Mouse (CD-1)	Gd 6-20 1 x/d (GW)		15	45 (decreased mean fetal body weight; 15% lower than controls)		Field et al. 1990 Acrylamide	Assessment included external, visceral, and skeletal examinations of offspring
CHRONIC EXPOSURE								
Death								
147	Rat (Fischer- 344)	2 yr (W)				2 (significantly decreased survival after 24 months of treatment)	Johnson et al. 1984, 1986 Acrylamide	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
148	Rat (Fischer- 344)	2 yr (W)				4.02 F (significantly decreased survival to terminal sacrifice)	NTP 2011	
149	Mouse (B6C3F1)	2 yr (W)				8.93 M (decreased survival) 4.65 F (decreased survival)	NTP 2011	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
150	Rat (Fischer- 344)	2 yr (W)	Resp	2 M 3 F[d]			Friedman et al. 1995 Acrylamide	
			Cardio	2 M 3 F[d]				
			Gastro	2 M 3 F[d]				
			Hemato	2 M 3 F[d]				
			Musc/skel	2 M 3 F[d]				
			Hepatic	2 M 3 F[d]				
			Renal	2 M 3 F[d]				
			Endocr	2 M 3 F[d]				

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
151	Rat (Fischer- 344)	2 yr (W)	Resp	2			Johnson et al. 1984, 1986 Acrylamide	
			Cardio	2				
			Gastro	2				
			Hemato	2				
			Musc/skel	2				
			Hepatic	2				
			Renal	2				
			Endocr	2				

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
152	Rat (Fischer- 344)	2 yr (W)	Resp	2.71 M 4.02 F			NTP 2011	
			Gastro	2.71 M 4.02 F				
			Hemato	2.71 M 4.02 F				
			Endocr	1.84 F	4.02 F (cytoplasmic vacuolation in adrenal cortex)			
			Ocular	2.71 M 4.02 F				
			Bd Wt	1.32 M 1.84 F	2.71 M (14% lower mean body weight than controls at week 104) 4.02 F (15% lower mean body weight than controls at week 104)			

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
153	Mouse (B6C3F1)	2 yr (W)	Resp	4.11 M	8.93 M (alveolar epithelium hyperplasia)		NTP 2011	
			Gastro	4.11 M	8.93 M (hyperplasia in forestomach epithelium)			
			Hemato	4.11 M 2.23 F	8.93 M (hematopoietic cell proliferation in the spleen)			
					4.65 F (hematopoietic cell proliferation in the spleen)			
			Ocular	4.11 M	8.93 M (cataracts)			
				2.23 F	4.65 F (cataracts)			
Neurological								
154	Rat (Fischer- 344)	2 yr (W)		0.5[e] M	2 M (light microscopic evidence of peripheral nerve degeneration)		Friedman et al. 1995 Acrylamide	electron microscopy not conducted
155	Rat (Fischer- 344)	2 yr (W)		0.5		2 (light microscopic evidence of moderate to severe degeneration in sciatic nerve fibers)	Johnson et al. 1984, 1986 Acrylamide	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
156	Rat (Fischer- 344)	2 yr (W)		1.32 M 1.84 F		2.71 M (degenerative effects in retina and sciatic nerve) 4.02 F (degenerative effects in sciatic nerve)	NTP 2011	
157	Mouse (B6C3F1)	2 yr (W)		8.93 M 9.96 F			NTP 2011	
158	Cat (NS)	up to 367 d 5 d/wk (F)		1		3 (clinical signs of peripheral neuropathy)	McCollister et al. 1964 Acrylamide	
Reproductive								
159	Rat (Fischer- 344)	2 yr (W)		2			Friedman et al. 1995 Acrylamide	Gross and histopathological evaluations of reproductive organs and tissues
160	Rat (Fischer- 344)	2 yr (W)		2			Johnson et al. 1984, 1986 Acrylamide	Gross and histopathological evaluations of reproductive organs and tissues

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
161	Mouse (B6C3F1)	2 yr (W)		8.93 M 2.23 F	4.65 F (ovarian cysts)		NTP 2011	
Cancer								
162	Rat (Fischer- 344)	2 yr (W)				1 F (CEL: mammary gland fibroadenomas and adenomas or carcinomas combined)	Friedman et al. 1995 Acrylamide	Tunica vaginalis mesotheliomas and thyroid gland tumors observed at high dose (2 and 3 mg/kg/day in males and females, respectively)
163	Rat (Fischer- 344)	2 yr (W)				0.5 M (CEL: testicular sac mesotheliomas)	Johnson et al. 1984, 1986 Acrylamide	At 2 mg/kg/day, increased incidences of tumors at other sites in both males and females

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
164	Rat (Fischer- 344)	2 yr (W)				2.71 M (CEL: tumors in epididymis, heart, pancreas, thyroid gland) 4.02 F (CEL: tumors in clitoral gland, mammary gland, oral mucosa or tongue, skin, thyroid gland)	NTP 2011	

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
165	Mouse (B6C3F1)	2 yr (W)				4.11 M (CEL: forestomach squamous cell papilloma)	NTP 2011	
						1.04 M (CEL: Harderian gland adenoma)		
						8.93 M (CEL: alveolar/bronchiolar adenoma)		
						4.65 F (CEL: skin tumors)		
						9.96 F (CEL: benign ovarian granulose cell tumors)		
						2.23 F (CEL: tumors in lung, mammary gland)		
						1.1 F (CEL: Harderian gland adenoma)		

Table 3-4 Levels of Significant Exposure to Acrylamide - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments

a The number corresponds to entries in Figure 3-2.

b Study results used to derive an acute-duration oral minimal risk level (MRL) of 0.01 mg/kg/day for acrylamide, as described in detail in Appendix A. PBPK modeling and benchmark dose (BMD) analysis were performed using administered acrylamide doses and corresponding male-mediated unsuccessful impregnation incidence data to identify a human equivalent dose (HED) of 0.31 mg/kg/day, which was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans using PBPK modeling and 10 for human variability).

c Study results used to derive an intermediate-duration oral minimal risk level (MRL) of 0.001 mg/kg/day for acrylamide, as described in detail in Appendix A. The rat NOAEL of 0.2 mg/kg/day was subjected to PBPK modeling to identify an HED of 0.038 mg/kg/day, which was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans using PBPK modeling and 10 for human variability).

d Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

e Study results used to derive a chronic-duration oral minimal risk level (MRL) of 0.001 mg/kg/day for acrylamide, as described in detail in Appendix A. PBPK modeling and BMD analysis were performed using administered acrylamide doses and corresponding incidence data for degenerative sciatic nerve changes to identify a HED of 0.042 mg/kg/day, which was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans using PBPK modeling and 10 for human variability).

Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; (F) = feed; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestation day; Gn Pig = guinea pig; (GW) = gavage in water; Hemato = hematological; Ld = lactation day; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; Ppd = post-parturition day; Resp = respiratory; (W) = drinking water; wk = week(s); x = time(s); yr = year(s)

Figure 3-2 Levels of Significant Exposure to Acrylamide - Oral

Acute (≤14 days)

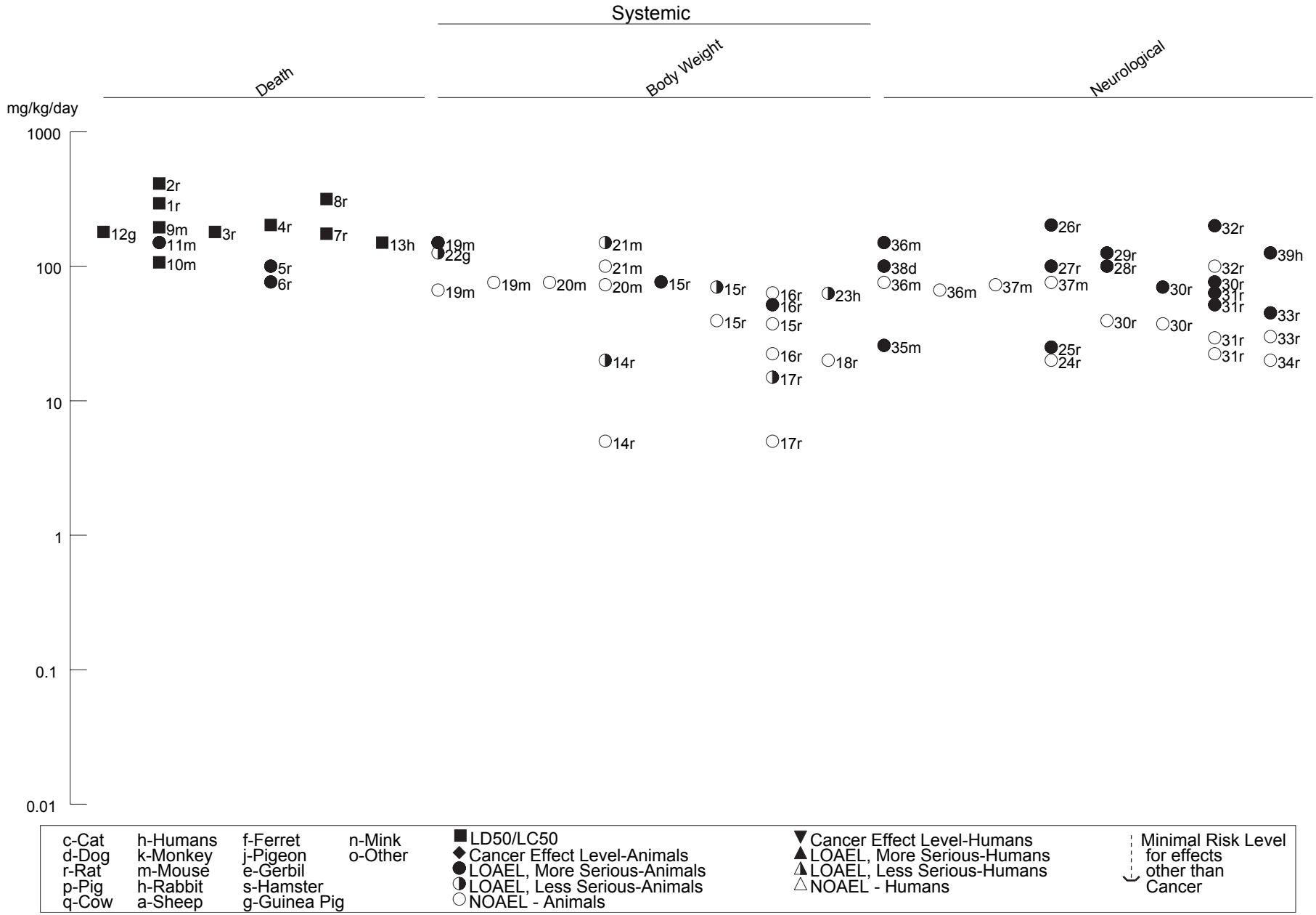


Figure 3-2 Levels of Significant Exposure to Acrylamide - Oral *(Continued)*

Acute (≤14 days)

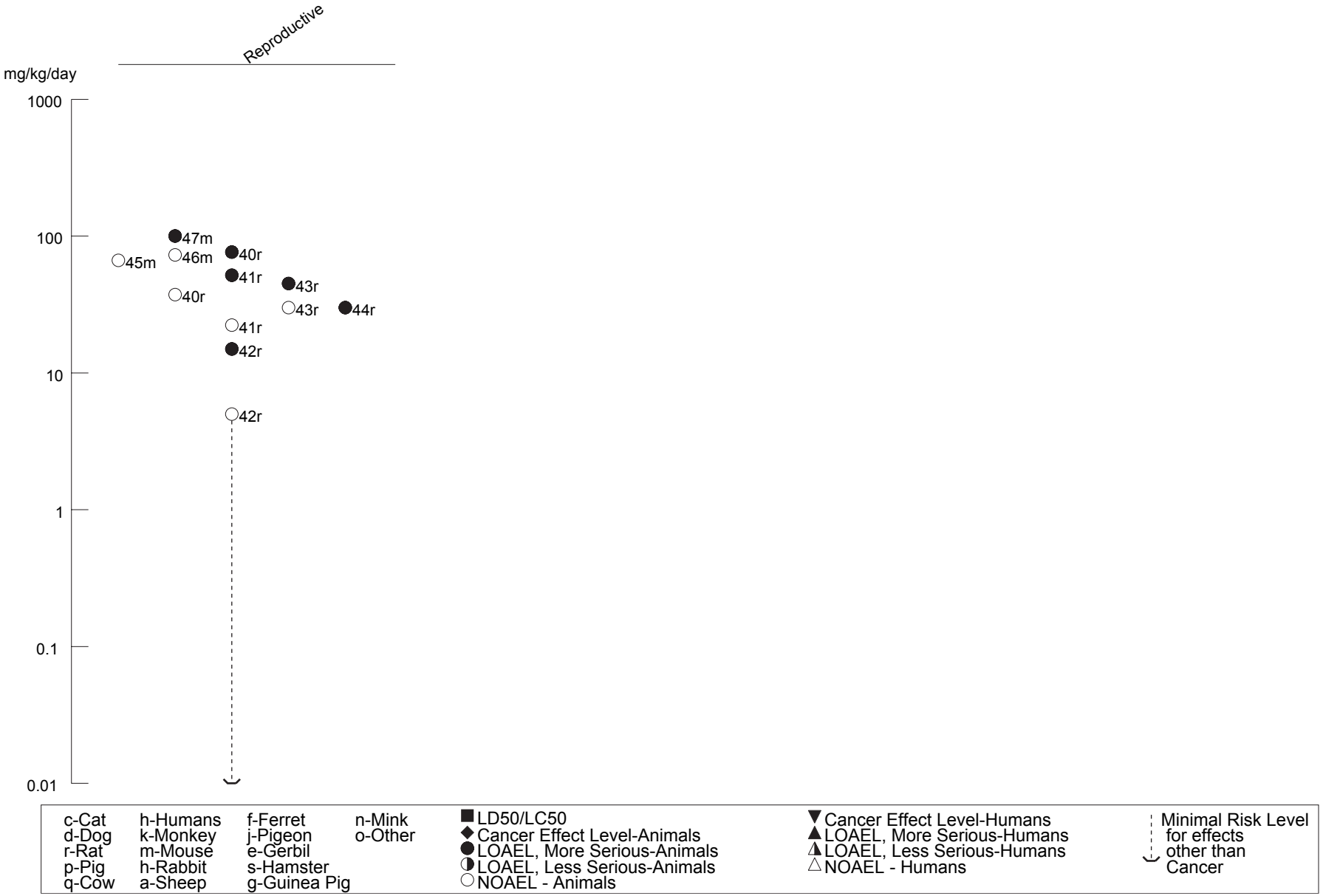


Figure 3-2 Levels of Significant Exposure to Acrylamide - Oral *(Continued)*

Intermediate (15-364 days)

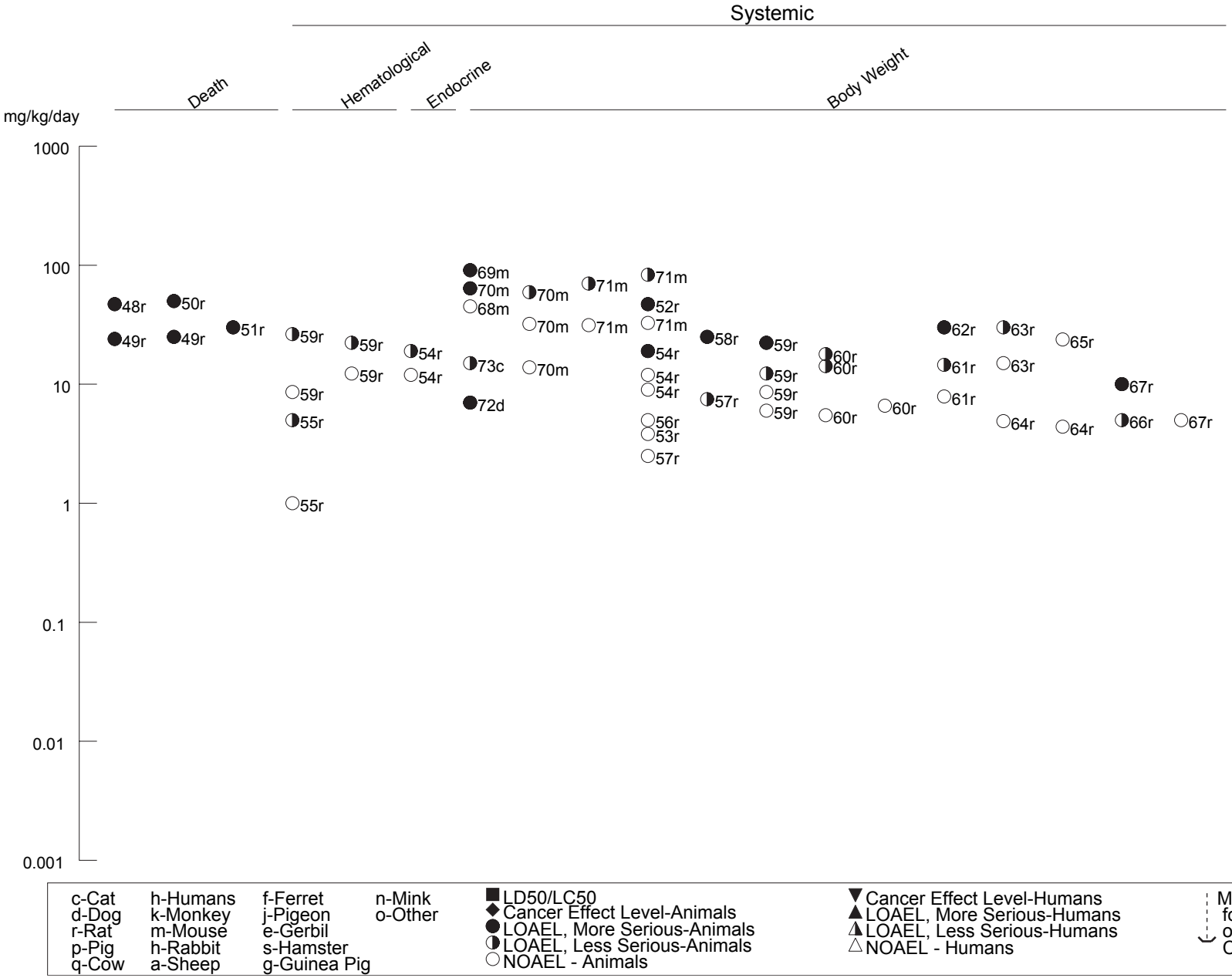


Figure 3-2 Levels of Significant Exposure to Acrylamide - Oral *(Continued)*

Intermediate (15-364 days)

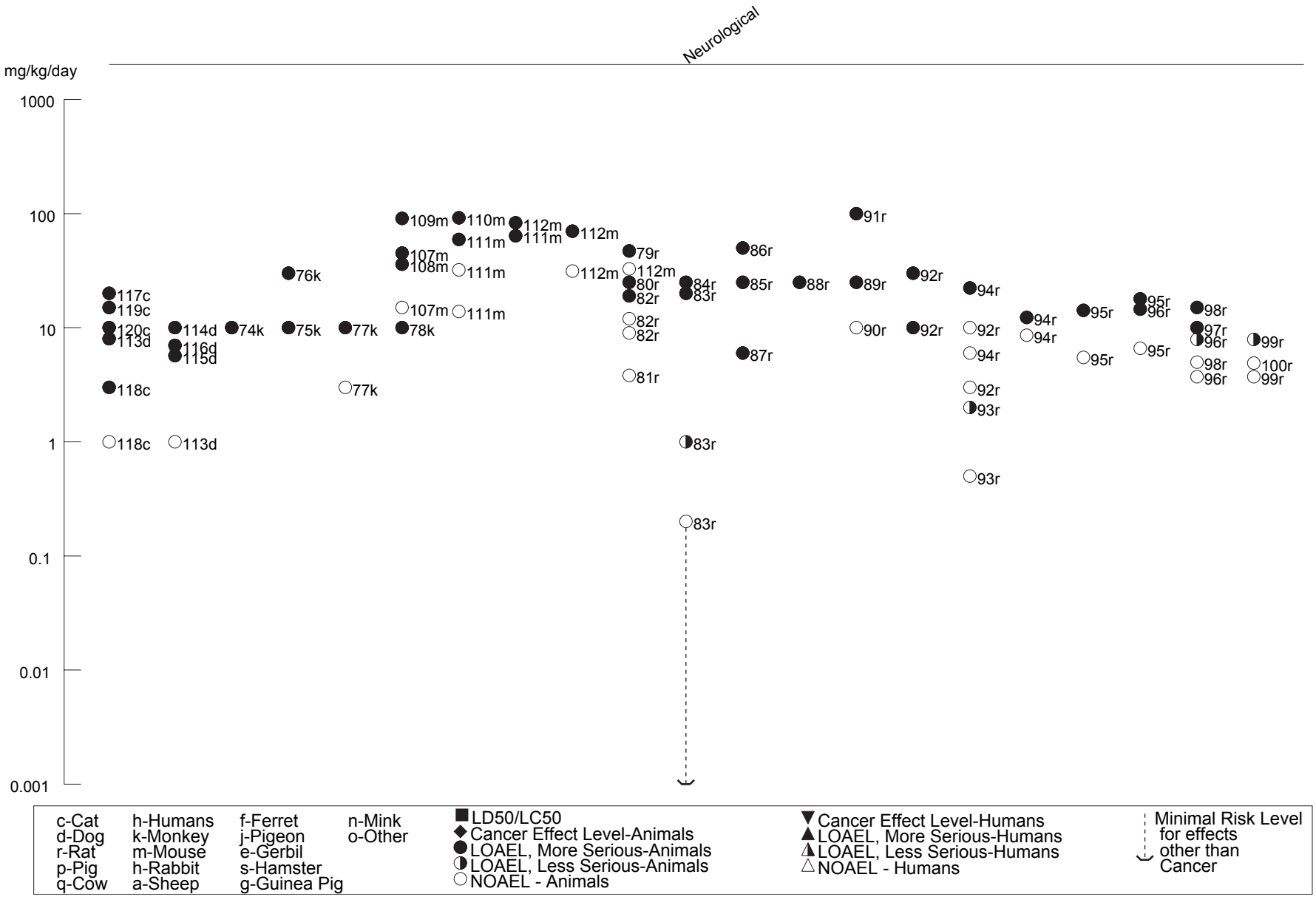


Figure 3-2 Levels of Significant Exposure to Acrylamide - Oral *(Continued)*

Intermediate (15-364 days)

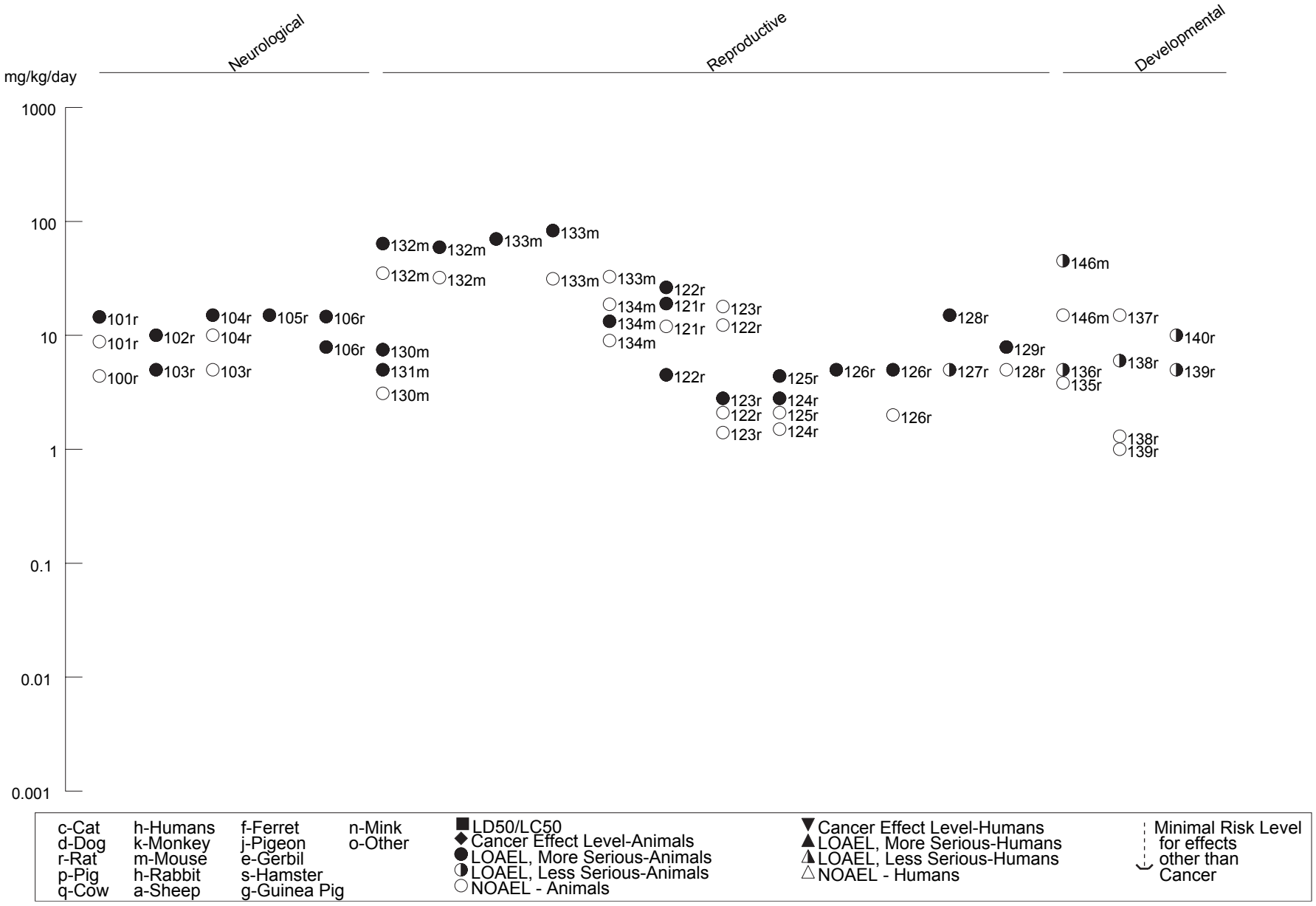
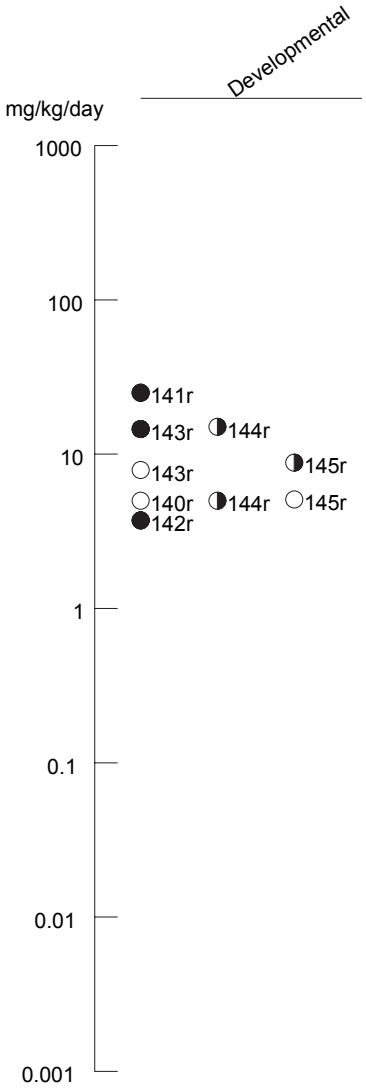


Figure 3-2 Levels of Significant Exposure to Acrylamide - Oral *(Continued)*

Intermediate (15-364 days)



c-Cat, d-Dog, r-Rat, p-Pig, q-Cow; h-Humans, k-Monkey, m-Mouse, h-Rabbit, a-Sheep; f-Ferret, j-Pigeon, e-Gerbil, s-Hamster, g-Guinea Pig; n-Mink, o-Other

■ LD50/LC50
◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◮ LOAEL, Less Serious-Humans
△ NOAEL - Humans

Minimal Risk Level for effects other than Cancer

Figure 3-2 Levels of Significant Exposure to Acrylamide - Oral *(Continued)*

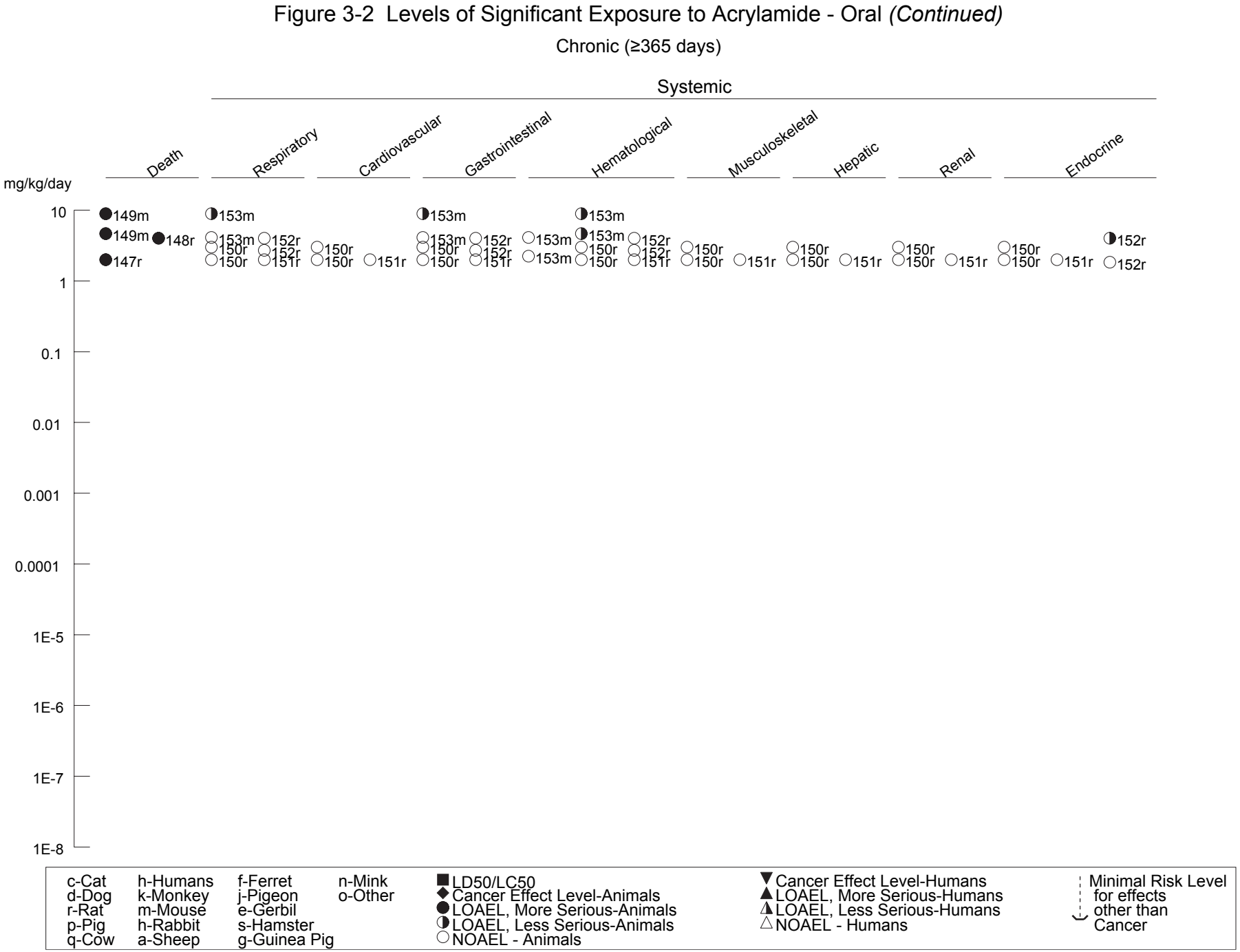
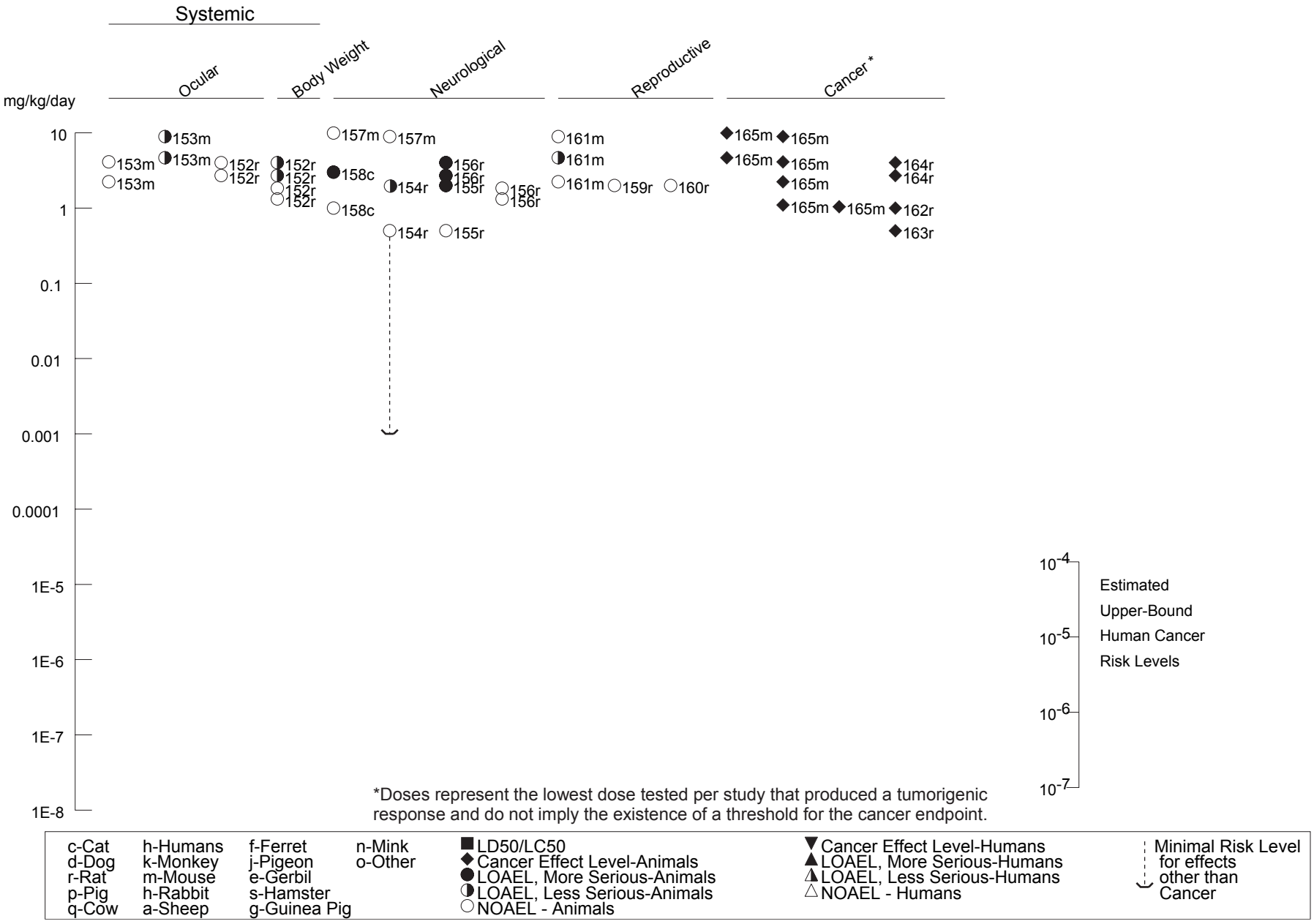


Figure 3-2 Levels of Significant Exposure to Acrylamide - Oral *(Continued)*

Chronic (≥365 days)



3.2.2.2 Systemic Effects

The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-4 and plotted in Figure 3-2.

Respiratory Effects. No data were located regarding respiratory effects in humans following oral exposure to acrylamide.

No clinical signs or gross or histopathological evidence of respiratory effects were observed in male or female F344 rats administered acrylamide in the drinking water for up to 93 days at concentrations resulting in doses up to 20 mg/kg/day (Burek et al. 1980) or for up to 2 years at concentrations resulting in doses up to 2 or 3 mg/kg/day (Friedman et al. 1995; Johnson et al. 1984, 1986). NTP (2011b) reported increased incidences of alveolar epithelium hyperplasia in male B6C3F1 mice receiving acrylamide from the drinking water for 2 years at an estimated dose of 9 mg/kg/day.

Cardiovascular Effects. No data were located regarding cardiovascular effects in humans following oral exposure to acrylamide.

No clinical signs or gross or histopathological evidence of cardiovascular effects were observed in male or female F344 rats administered acrylamide in the drinking water for up to 93 days at concentrations resulting in doses up to 20 mg/kg/day (Burek et al. 1980) or up to 2 years at concentrations resulting in doses up to 2 or 3 mg/kg/day (Friedman et al. 1995; Johnson et al. 1984, 1986).

Gastrointestinal Effects. No data were located regarding gastrointestinal effects in humans following oral exposure to acrylamide.

No clinical signs or gross or histopathological evidence of gastrointestinal effects were observed in male or female F344 rats administered acrylamide in the drinking water for up to 93 days at concentrations resulting in doses up to 20 mg/kg/day (Burek et al. 1980) or up to 2 years at concentrations resulting in doses up to 2 or 3 mg/kg/day (Friedman et al. 1995; Johnson et al. 1984, 1986). No clinical signs or gross or histopathological evidence of gastrointestinal effects were observed in male or female F344/N rats receiving acrylamide from the drinking water for 2 years at estimated doses as high as 2.71 and

4.02 mg/kg/day, respectively; among similarly-treated B6C3F1 mice, high-dose males (estimated dose of 9 mg/kg/day) exhibited increased incidence of hyperplasia in the forestomach epithelium (NTP 2011b).

Hematological Effects. No data were located regarding hematological effects in humans following oral exposure to acrylamide.

Burek et al. (1980) reported significant decreases in packed cell volume (PCV), erythrocyte counts, and hemoglobin in male and female F344 rats at day 76 during administration of acrylamide in their drinking water at a dose level of 20 mg/kg/day. These effects were also noted in females (but not males) dosed at 5 mg/kg/day for 93 days. Hematological evaluations at 3, 6, 12, 18, and 24 months on male and female F344 rats receiving acrylamide doses as high as 2 mg/kg/day from the drinking water for up to 2 years revealed no evidence of treatment-related effects (Johnson et al. 1984, 1986). However, NTP (2011b) reported congestion and pigmentation in the spleen and erythroid cell hyperplasia in the bone marrow of male and female F344/N rats receiving acrylamide from the drinking water for 13 weeks at estimated doses in the range of 22–26 mg/kg/day.

Musculoskeletal Effects. No data were located regarding musculoskeletal effects in humans following oral exposure to acrylamide.

Atrophy of skeletal muscles in the posterior area was noted in F344 rats receiving acrylamide at a dose level of 20 mg/kg/day from the drinking water for up to 93 days; however, this effect was considered the likely result of acrylamide-induced peripheral neuropathy (Burek et al. 1980).

Renal Effects. No data were located regarding renal effects in humans following oral exposure to acrylamide.

Urinalysis and gross and histopathological evaluations revealed no evidence of renal effects in male or female F344 rats administered acrylamide in the drinking water for up to 93 days at concentrations resulting in doses up to 20 mg/kg/day (Burek et al. 1980) or up to 2 years at concentrations resulting in doses up to 2 or 3 mg/kg/day (Friedman et al. 1995; Johnson et al. 1984, 1986). Repeated oral exposure to acrylamide has been associated with distention of the urinary bladders in some animal studies. For example, in 14-day repeated-dose studies, NTP (2011b) reported dilatation of the urinary bladder in most or all male and female F344/N rats receiving acrylamide from the drinking water or food at estimated doses in the range of 52–77 mg/kg/day; this effect was not reported in similarly-treated male and female

B56C3F1 mice at estimated doses as high as 73–150 mg/kg/day. Dilatation of the urinary bladder was observed in most F344/N rats and B6C3F1 mice receiving acrylamide from the drinking water or food for 13 weeks at estimated doses in the range of 14–26 mg/kg/day (rats) and 59–83 mg/kg/day (mice). Most of the animals that exhibited dilatation of the urinary bladder also showed hind-leg paralysis and degenerative histopathologic lesions in the sciatic nerve. It has been suggested that bladder distension may be the result of acrylamide-induced effects on nerve fibers that innervate the bladder (Fullerton and Barnes 1966; NTP 2011b).

Endocrine Effects. No data were located regarding endocrine effects in humans following oral exposure to acrylamide.

Significantly decreased mean serum testosterone levels (27 and 9% of the control mean) were noted in adult male F344 rats administered acrylamide in the drinking water for 28 days at concentrations resulting in estimated acrylamide doses of 19 and 25 mg/kg/day, respectively; there were no statistically significant effects on serum testosterone levels at 14-day interim assessment (American Cyanamid Company 1991). In the same study, serum levels of the thyroid hormones thyroxin (T_4) and triiodothyronine (T_3) and serum prolactin were assessed in both male and female F344 rats receiving acrylamide from the drinking water at estimated doses up to 24–25 mg/kg/day. High-dose males exhibited significantly increased mean T_4 concentration compared to controls at 14-day interim assessment and significantly decreased mean T_3 and T_4 concentrations at study termination. Terminal (28-day) mean T_3 level was significantly decreased in 19 mg/kg/day males as well. Mean T_3 and T_4 levels in treated female rats were not significantly different from controls at either time point. Serum prolactin levels were significantly decreased in 19 and 25 mg/kg/day males at the 14-day interim assessment, but not at terminal assessment. No significant effects on serum prolactin levels were seen in treated female rats. At terminal sacrifice, no significant treatment-related effects on thyroid weights were seen in males or females at any dose level.

Gross and histopathological assessments revealed no evidence of endocrine effects in male or female F344 rats administered acrylamide in the drinking water for up to 93 days at concentrations resulting in doses up to 20 mg/kg/day (Burek et al. 1980) or up to 2 years at concentrations resulting in doses up to 2 or 3 mg/kg/day (Friedman et al. 1995; Johnson et al. 1984, 1986). Cytoplasmic vacuolation was reported in the adrenal cortex of female F344/N rats receiving acrylamide from the drinking water for 2 years at an estimated dose of 4 mg/kg/day (NTP 2011b).

Dermal Effects. No data were located regarding dermal effects in humans or animals following oral exposure to acrylamide.

Ocular Effects. No data were located regarding ocular effects in humans following oral exposure to acrylamide.

Ophthalmological evaluations revealed no evidence of ocular effects in male or female F344 rats administered acrylamide in the drinking water for up to 93 days at concentrations resulting in doses up to 20 mg/kg/day (Burek et al. 1980) or up to 2 years at concentrations resulting in doses up to 2 or 3 mg/kg/day (Friedman et al. 1995; Johnson et al. 1984, 1986). NTP (2011b) reported significantly increased incidences of cataracts in male and female B6C3F1 mice receiving acrylamide from the drinking water for 2 years at estimated doses of 9 mg/kg/day (males) and 4.6 and 10 mg/kg/day (females).

Acrylamide-induced neurological effects on the visual system of orally-exposed primates are discussed in Section 3.2.2.4 (Neurological Effects).

Body Weight Effects. No data were located regarding body weight effects in humans following oral exposure to acrylamide.

Depressed body weight, including actual body weight loss, was consistently reported in laboratory animals following single or repeated oral exposure to acrylamide.

Slight initial weight loss (magnitude not specified) was reported following a single oral dose of 126 mg acrylamide/kg in rats and guinea pigs and 63 mg/kg in rabbits (Dow Chemical Company 1957). Significantly depressed body weight was observed for several days in mice dosed once at 150 mg/kg (lethal dose), but not at 100 mg/kg (sublethal dose) (Sakamoto et al. 1988).

Depressed body weight or actual body weight loss was observed in repeat-dosing oral studies of rats and mice (American Cyanamid Company 1953b, 1991; Burek et al. 1980; Ko et al. 1999; NTP 2011b; Ogawa et al. 2011; Schulze and Boysen 1991; Shi et al. 2011; Tyl et al. 2000b; Wang et al. 2010a). For example, at treatment day 13 of a 3-month study, significantly decreased mean body weights (8% lower than controls) were observed in male and female rats receiving acrylamide at 20 mg/kg/day from the drinking water (Burek et al. 1980). Significantly depressed body weight gain (approximately 40% less than controls) was noted in male Long-Evans rats receiving 15 mg/kg/day of acrylamide for 5 consecutive

days; there were no apparent acrylamide-induced effects at 5 mg/kg/day (Tyl et al. 2000b). Wang et al. (2010a) reported between 10 and 20% depressed body weight in male Sprague-Dawley weanling rats administered acrylamide by gavage at 5 or 10 mg/kg/day for up to 8 weeks. Male and female F344/N rats receiving acrylamide from the drinking water for 14 days at estimated doses in the range of 70–77 mg/kg/day exhibited 44% (males) and 15% (females) lower mean terminal body weights than their controls. Similar effects on terminal body weight were observed in rats receiving acrylamide from the drinking water for 13 weeks at estimated doses in the range of 14–18 mg/kg/day (NTP 2011b). Male and female B6C3F1 mice were less sensitive to acrylamide-induced body weight effects; the lowest estimated doses resulting in significantly depressed mean terminal body weight in the mice were 150 mg/kg/day for 14-day treatment and 70–83 mg/kg/day for 13-week treatment (NTP 2011b). In a 2-year oral study in F344/N rats (NTP 2011b), estimated doses of 2.7 mg/kg/day (males) and 4 mg/kg/day (females) resulted in 14–15% lower mean body weight at treatment week 104. Two years of drinking water exposure in B6C3F1 mice did not affect body weight at estimated doses as high as 9–10 mg/kg/day (NTP 2011b).

Dogs and cats are also susceptible to the body weight effects of orally-administered acrylamide. An 8-week dosing period to dogs at 7 mg/kg/day resulted in weight loss (Satchell and McLeod 1981). Cats dosed for 12–16 weeks at 15 mg/kg/day exhibited weight loss of unspecified magnitude (Post and McLeod 1977a).

Several groups of investigators assessed effects of maternal body weight on rat or mouse dams receiving oral acrylamide during gestation and/or lactation. A daily dose as low as 7.5 mg/kg/day during gestation days 6–20 resulted in approximately 12% depressed maternal weight gain in rats, but a NOAEL of 45 mg/kg/day was identified in similarly-treated mouse dams (Field et al. 1990). Net maternal weight loss was observed in rat dams administered acrylamide at 25 mg/kg/day during 21 days of lactation (Friedman et al. 1999). Wise et al. (1995) treated rat dams with 5 or 15 mg/kg/day from gestation day 6 through lactation day 10; no body weight effects were seen at the 5 mg/kg/day dose level, but approximately 33% depressed maternal weight gain was noted during the 10-day lactation period in the 15 mg/kg/day group.

3.2.2.3 Immunological and Lymphoreticular Effects

No data were located regarding immunological or lymphoreticular effects in humans or animals following oral exposure to acrylamide.

3.2.2.4 Neurological Effects

Neurological deficits are a hallmark of acrylamide toxicity. Most evidence in humans derives from occupational exposures that predominantly involved inhalation and dermal routes (see Section 3.2.1.4). Available information regarding the neurological effects of oral exposure in humans is limited to a case report of persistent peripheral neuropathy in a subject who intentionally ingested 18 g of acrylamide crystals (Donovan and Pearson 1987) and signs of central and peripheral neurological deficits in family members exposed (likely via oral and dermal routes) to acrylamide in well water at a concentration of 400 ppm (Igisu and Matsuoka 2002; Igisu et al. 1975). Epidemiologic studies designed to evaluate noncancer health effects in groups of orally-exposed subjects have not been conducted.

Neurological effects associated with oral exposure to acrylamide have been well characterized in laboratory animals and include clinical signs such as twitching, loss of balance, tremors, lethargy, and general weakness and more subtle indicators of functional deficits such as decreased rotarod performance and increased limb or foot splay. Evidence of degenerative lesions in peripheral nerve fibers, as observed by light and electron microscopy, have been detected at oral doses lower than those eliciting clinical signs and other overt indications of functional deficit.

Clinical signs have been elicited following single oral exposure of rats, rabbits, and dogs to doses in the range of 100–200 mg/kg (American Cyanamid Company 1953c; Fullerton and Barnes 1966; McCollister et al. 1964; Tilson and Cabe 1979). Five daily doses to rats at 45 mg/kg (Tyl et al. 2000b) or 75 mg/kg (Sublet et al. 1989) elicited typical clinical signs of peripheral neuropathy. Ko et al. (1999) noted the onset of altered gait as early as treatment day 5 in 3- and 8-week-old mice receiving acrylamide from the drinking water at approximately 90 mg/kg/day. Friedman et al. (1999) and Dixit et al. (1981) observed clinical signs of neurological effects as early as treatment days 4 and 14, respectively, in rats receiving acrylamide at 25 mg/kg/day. Gilbert and Maurissen (1982) reported decreased rotarod performance and increased hindlimb splay as early as days 6–8 of a 12-day exposure of mice receiving acrylamide from the drinking water at an estimated dose of 25.8 mg/kg/day. NTP (2011b) noted hind-leg paralysis in all male and female F344/N rats receiving acrylamide for 14 days from the drinking water at 70–77 mg/kg/day or from the food at 52–63 mg/kg/day (NTP 2011b). Among similarly-treated male and female B6C3F1 mice, hind-leg paralysis was observed in one of four males and one of four females receiving acrylamide from the drinking water at approximately 150 mg/kg/day; there were no indications of neurological effects in the male or female mice receiving acrylamide from the food at doses as high as 73–76 mg/kg/day (NTP 2011b).

Clinical signs and other indicators of acrylamide-induced functional neurological deficit, such as increased hindlimb splay and decreased rotarod performance, during intermediate-duration (15–364 days) exposure have been observed in several animal species. In rats and cats, dose levels in the range of 8–25 mg/kg/day elicited overt signs of neurological deficit as early as 1–8 weeks following the initiation of treatment (American Cyanamid Company 1991; Burek et al. 1980; Fullerton and Barnes 1966; Gorzinski et al. 1979; Leswing and Ribelin 1969; McCollister et al. 1964; Ogawa et al. 2011; Post and McLeod 1977a; Shi et al. 2011; Takahashi et al. 2009; Tanii and Hashimoto 1983; Tilson and Cabe 1979; Wise et al. 1995; Zenick et al. 1986). Slight leg weakness was reported after 40 weeks of oral dosing at 6 mg/kg/day in one rat study (Fullerton and Barnes 1966). In dogs, dose levels of 5.7–10 mg/kg/day elicited clinical signs within 3–4 weeks (American Cyanamid Company 1953c; Hersch et al. 1989a; Satchell and McLeod 1981). Hashimoto et al. (1981) noted typical signs of peripheral neuropathy and diminished rotarod performance in male mice treated at 36 mg/kg/day, 2 days/week for 8 weeks. Hind-leg paralysis and degenerative histopathologic lesions of sciatic nerve and spinal cord fibers were observed in male and female F344/N rats receiving acrylamide from the drinking water for 13 weeks at approximately 22–26 mg/kg/day; hind-leg paralysis was also noted in two of eight females receiving acrylamide at approximately 12 mg/kg/day (NTP 2011b). Most of the rats exhibiting hind-leg paralysis and degenerative lesions of sciatic nerve also exhibited atrophy of hind-leg skeletal muscle as well. Hind-leg paralysis, degenerative lesions of the sciatic nerve, and atrophy of hind-leg skeletal muscle were observed in most male and female F344/N rats receiving acrylamide from the food at approximately 14–18 mg/kg/day for 13 weeks (NTP 2011b). Similar effects were noted in male and female B6C3F1 mice receiving acrylamide for 13 weeks at approximately 70–83 mg/kg/day from the drinking water and 59–64 mg/kg/day from the food (NTP 2011b). Histopathological evidence of degenerative effects was noted in fibers from cervical and lumbar spinal cord, gasserian and dorsal root ganglia, and sciatic, tibial, and sural nerves of male and female Sprague-Dawley rats that had received acrylamide by gavage for 5 weeks at doses of 10 and 30 mg/kg/day (Schulze and Boysen 1991). The most severe changes were observed in the large fibers of the tibial and sural nerves of high-dose rats. In a 2-generation study of F-344 rats, Tyl et al. (2000a) reported degenerative effects in peripheral nerve fibers of F_0 (but not female or F_1 male or female) rats that had received acrylamide from the drinking water at 5 mg/kg/day for 16 weeks. Shi et al. (2011) reported treatment-related biochemical and histopathologic changes in the cerebellum of rats administered acrylamide by gavage at doses of 15 or 30 mg/kg/day for 4 weeks. In primates (monkeys, baboons), clinical signs were elicited by doses of 10–30 mg/kg/day for periods of 42–61 days (Eskin et al. 1985; Hopkins 1970; Leswing and Ribelin 1969; Maurissen et al. 1983; Merigan et al. 1985). Higher

levels of oral dosing typically result in earlier onset of clinical signs and more severe effects with continued dosing.

Histopathological assessment of acrylamide-induced neurological effects includes studies in which light and electron microscope evaluations were performed on peripheral nerve fibers of rats administered acrylamide in the drinking water (Burek et al. 1980; Johnson et al. 1984, 1985, 1986). In the study of Burek et al. (1980), the rats received estimated doses of 0.05–20 mg/kg/day for up to 93 days. This study identified a NOAEL of 0.2 mg/kg/day and a LOAEL of 1 mg/kg/day for increased incidences of ultrastructural degeneration (axolemma invaginations with cell organelles and/or dense bodies) in the sciatic nerve; higher doses elicited more pronounced degenerative effects that could also be detected by light microscopy. Clinical signs of peripheral neuropathy were elicited at the 20 mg/kg/day dose level. The study of Johnson et al. (1984, 1985, 1986) employed estimated dose levels of 0.01, 0.1, 0.5, and 2.0 mg/kg/day for up to 2 years. Interim evaluations of peripheral nerve fibers were performed at 3, 6, 12, and 18 months; however electron microscope evaluations at interims >12 months were inconclusive due to high incidences of degenerative results attributed in part to aging. The studies of Johnson et al. (1984, 1985, 1986) identified a NOAEL of 0.5 mg/kg/day and a LOAEL of 2 mg/kg/day for increased incidences of ultrastructural degeneration (axolemma invaginations) in the sciatic nerve at 3, 6, and 12 months and for light microscopy evidence of degenerative effects in peripheral nerve fibers at 12–24 months; there were no clinical signs of neurological effects at any dose level. Friedman et al. (1995) duplicated the study design of Johnson et al. (1986), but excluded electron microscopy in histopathological evaluations of peripheral nerve fibers. The study of Friedman et al. (1995) also identified a NOAEL of 0.5 mg/kg/day and a LOAEL of 2 mg/kg/day for peripheral nerve degeneration as revealed by light microscopy in the absence of clinical signs of neurological effects.

NTP (2011b) observed degenerative effects in sciatic nerve preparations from male and female F344/N rats receiving acrylamide for 2 years from the drinking water at approximately 2.7 mg/kg/day (males) and 4 mg/kg/day (females); some of the males exhibited degenerative effects in the retina as well. There were no indications of degenerative lesions in nerve preparations from male or female B6C3F1 mice receiving acrylamide from the drinking water for 2 years at approximate doses as high as 9–10 mg/kg/day (NTP 2011b). The NTP (2011b) studies did not include electron microscopic evaluation of nerve preparations.

Information regarding the neurodevelopmental toxicity of acrylamide is discussed in Section 3.2.2.6.

The highest NOAEL values and all LOAEL values from each reliable study for neurological effects in each species and duration category are recorded in Table 3-4 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

No studies were located regarding acrylamide-induced reproductive effects in humans.

Animal studies designed to assess the reproductive toxicity of orally-administered acrylamide revealed pre- and postimplantation losses and decreased numbers of live fetuses in rats and mice at repeated doses in the range of 3–60 mg/kg/day (Chapin et al. 1995; Sakamoto and Hashimoto 1986; Smith et al. 1986; Sublet et al. 1989; Tyl et al. 2000a, 2000b; Working et al. 1987; Zenick et al. 1986).

Results of dominant lethality testing (Chapin et al. 1995; Smith et al. 1986; Tyl et al. 2000a, 2000b) and crossover trials (Chapin et al. 1995; Sakamoto and Hashimoto 1986; Zenick et al. 1986) indicate that acrylamide induces male-mediated reproductive effects at repeated oral doses in the range of 2.8–19 mg/kg/day. Sublet et al. (1989) reported statistically significant moderately decreased sperm mobility in Long-Evans rats administered acrylamide at a gavage dose of 45 mg/kg/day for 5 days, but suggested that this effect was not solely responsible for poorer reproductive performance. In apparent contrast, Tyl et al. (2000b) found no significant effects on sperm parameters in Long-Evans hooded rats following repeated oral dosing at levels as high as 60 mg/kg/day and suggested that indicators of acrylamide-induced reproductive toxicity such as pre-and post-implantation loss and decreased numbers of live fetuses may be at least partly the result of impaired mating performance due to acrylamide neurotoxicity. Adverse effects on sperm parameters were observed in Sprague-Dawley rats administered acrylamide by oral gavage once/day, 5 days/week for 4 weeks (Yuxin et al. 2011) and in NMRI mice receiving acrylamide from the drinking water for 2 months at an estimated dose of 5 mg/kg/day (Kermani-Alghoraishi et al. 2010). In a study of F344 rat pups whose mothers were exposed to acrylamide in the drinking water during 3 weeks of lactation followed by 9 weeks of exposure of the pups directly via their drinking water, degenerative effects on seminiferous epithelium of testis and epididymis were observed at an estimated pup dose of 4.4 mg/kg/day; the NOAEL was 2.2 mg/kg/day (Takami et al. 2011). In a study of male Sprague-Dawley weanling rats administered acrylamide by gavage at 0, 5, or 10 mg/kg/day for 8 weeks, mean epididymal sperm concentrations in the 5 and 10 mg/kg/day dose groups were approximately 24 and 40% lower, respectively, than that of controls (Wang et al. 2010a). The study authors also reported significantly increased concentrations of Leydig cells and serum testosterone at 5 mg/kg/day and approximately 2-fold increases in these concentrations at 10 mg/kg/day.

Histologic indicators of degenerative effects were reported in spermatids of ddY mice administered acrylamide by daily gavage for 5 days at dose levels of 100 or 150 mg/kg/day (Sakamoto et al. 1988). Other investigators reported evidence of acrylamide-induced testicular atrophy in F344 rats receiving acrylamide in the drinking water for 28 or 90 days at concentrations resulting in estimated acrylamide doses of 19 or 5 mg/kg/day, respectively (American Cyanamid Company 1991; Burek et al. 1980). NTP (2011b) noted degeneration in the seminiferous tubules of male F344/N rats receiving acrylamide from the drinking water for up to 14 days at an approximate dose of 77 mg/kg/day; the NOAEL for this effect was 37 mg/kg/day. In other males similarly exposed (via the food), degeneration in the seminiferous tubules was observed at an approximate dose of 52 mg/kg/day; the NOAEL for this effect was 22 mg/kg/day (NTP 2011b). In similar 14-day studies of male B6C3F1 mice (NTP 2011b), no histopathologic evidence of reproductive toxicity was observed at acrylamide doses from the drinking water or food as high as 67 and 73 mg/kg/day, respectively. Results of 13-week oral studies include findings of degeneration of testicular germinal epithelium of male F344/N rats and B6C3F1 mice at doses in the range of 2.8–4.5 and 59–70 mg/kg/day, respectively, and anestrus in female F344/N rats and B6C3F1 mice at doses of 26 and 64–83 mg/kg/day, respectively (NTP 2011b). Atrophy of the testes and/or seminal vesicles was reported in F344 rats receiving acrylamide at 19 or 25 mg/kg/day from the drinking water for 28 days; this effect was not seen at 12 mg/kg/day (American Cyanamid Company 1991).

Gross and histopathologic examinations of reproductive organs and tissues from male rats receiving acrylamide from the drinking water for up to 2 years at estimated doses as high as 2 mg/kg/day revealed no signs of acrylamide-induced effects (Friedman et al. 1995; Johnson et al. 1984, 1986). However, NTP (2011b) observed increased numbers of ovarian cysts in female B6C3F1 mice receiving acrylamide from the drinking water for 2 years at an estimated dose of 4.6 mg/kg/day.

Prebreeding exposure of female mice to acrylamide at 18.7 mg/kg/day (Sakamoto and Hashimoto 1986) or female Long-Evans rats to doses up to 14.6 mg/kg/day (Zenick et al. 1986) did not adversely affect reproductive performance variables such as fertility or implantation when the animals were bred with nonexposed males. Gross and histopathologic examinations of reproductive organs and tissues from female rats receiving acrylamide from the drinking water for up to 2 years at estimated doses as high as 2–3 mg/kg/day revealed no signs of acrylamide-induced effects (Friedman et al. 1995; Johnson et al. 1984, 1986).

The highest NOAEL values and all LOAEL values from each reliable study for reproductive effects in each species and duration category are recorded in Table 3-4 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

No studies were located regarding acrylamide-induced developmental effects in humans.

Several reports are available in which developmental toxicity was assessed in the offspring of rat or mouse dams administered acrylamide via daily gavage during gestation and/or lactation (American Cyanamid Company 1979; Field et al. 1990; Friedman et al. 1999; Husain et al. 1987; Takahashi et al. 2009; Walden et al. 1981; Wise et al. 1995).

Body weight decreases and decreased auditory startle response were noted in offspring of female Sprague-Dawley rats exposed to 5 and 15 mg/kg-day, respectively, on gestation days 6–10 (Wise et al. 1995). No exposure-related fetal malformations or variations (gross, visceral, or skeletal) were found in offspring of Sprague-Dawley rats administered acrylamide at doses of 2.5, 7.5, or 15 mg/kg/day on gestation days 6–20 or in CD-1 mice at doses of 3, 15, or 45 mg/kg/day on gestation days 6–17 (Field et al. 1990). The highest dose in each species was maternally toxic, as evidenced by depressed maternal weight gain in the rats and mice and increased hindlimb splay in the mice. There were no indications of treatment-related developmental effects in the offspring of Sprague-Dawley rat dams administered acrylamide at doses up to nearly 4 mg/kg/day for 2 weeks premating and throughout gestation (American Cyanamid Company 1979). Decreased mean fetal body weight (approximately 15% lower than controls) was noted in the offspring of CD-1 mouse dams administered acrylamide by gavage at 45 mg/kg/day during gestation days 6–20; a NOAEL for this effect was 15 mg/kg/day (Field et al. 1990). Significantly depressed mean body weights were reported in offspring of Sprague-Dawley rat dams administered acrylamide in the drinking water at 100 ppm (mean acrylamide intake 14.56 mg/kg/day) from gestation day 6 through postnatal day 21; the male and female pups exhibited approximately 42 and 46% lower mean body weights, respectively, than unexposed control pups (Takahashi et al. 2009). At lower mean maternal exposure levels (25 and 50 ppm; mean doses of 3.72 and 7.89 mg/kg/day, respectively), pup body weights did not differ significantly from those of controls. There were no signs of acrylamide-induced neurotoxicity or testicular toxicity in pups of any exposure group. The 100 ppm dams exhibited increasing severity of gait abnormalities from postnatal day 2 onward; abnormal gait was observed in 50 ppm dams from postnatal day 18 onward.

Decreased performance in an operant test of cognitive motivation was reported in adolescent F344 rats of the 5 mg/kg/day dose group exposed via their gavaged mothers during gestation, followed by direct gavage treatment through weaning at postnatal day 22 and subsequent exposure to acrylamide via the drinking water until the pups were 12 weeks of age (Garey and Paule 2007). In a similarly-designed study in which the pups were exposed to acrylamide until they were 8 months of age, decreased performance in an incremental repeated acquisition task (a measure of learning ability) was reported in the 5 mg/kg/day dose group (Garey and Paule 2010).

Delayed pinnae detachment (a developmental landmark) and deficient negative geotaxis and rotarod performance were reported in F344 rat pups that had been exposed via their mothers (10 mg acrylamide/kg/day by gavage) during gestation followed by gavage of the pups at the same dose until postnatal day 22; these effects were not seen at doses ≤5 mg/kg/day (Garey et al. 2005). In a similarly-designed study that included 5 mg/kg/day as the highest dose tested, there were no effects on pup developmental landmarks or most behavioral tests; however, the high-dose pups exhibited 30–49% less open field activity than controls (Ferguson et al. 2010).

Periodic significantly decreased brain levels of selected catecholamines (noradrenaline, dopamine, 5-hydroxytryptamine) were noted in pups of rat dams administered acrylamide at 25 mg/kg/day during lactation only (Husain et al. 1987). Similar effects on brain catecholamines were observed in rat pups 12–21 days of age at the beginning of a 5-day period in which they were administered acrylamide by gavage at 25 mg/kg/day; these effects were not seen in rat pups that were 60 days of age at the initiation of dosing (Husain et al. 1987). Significant decreases in whole brain levels of noradrenaline, dopamine, and 5-hydroxytryptamine were observed in pups of rat dams administered acrylamide at 25 mg/kg/day during lactation. Walden et al. (1981) found significant alterations in intestine enzyme levels in young Sprague-Dawley rat pups whose mothers had received acrylamide via gavage on gestation days 6–17 at 20 mg/kg/day; the toxicological significance of these findings is uncertain. Friedman et al. (1999) reported increased mortality and reduced body weights in pups of rat dams dosed at 25 mg/kg/day during lactation; however, serious maternal toxicity was noted as well. Ogawa et al. (2011) reported dose-dependent increases in indicators of a compensatory regulatory mechanism for correcting impaired neurogenesis in the brain of rat pups whose mothers had received acrylamide from the drinking water between gestation day 6 and lactation day 21 at estimated doses ≥3.72 mg/kg/day. The pups from the high-dose dams (14.56 mg/kg/day estimated dose) exhibited nearly 50% depressed mean body weight as well.

The highest NOAEL values and all LOAEL values from each reliable study for developmental effects in each species and duration category are recorded in Table 3-4 and plotted in Figure 3-2.

3.2.2.7 Cancer

Available epidemiology studies on increased risk of cancer from acrylamide in food include case-control studies (Lin et al. 2010; Michels et al. 2006; Mucci et al. 2003, 2004, 2005; Pelucchi et al. 2006, 2007, 2011a; Wilson et al. 2009a) prospective cohort studies (Hogervorst et al. 2007, 2008a, 2008b, 2009a, 2009b; Larsson et al. 2009a, 2009b, 2009c, 2009d, 2009e; Mucci et al. 2006; Schouten et al. 2009; Wilson et al. 2009b, 2010). These studies provide mixed results regarding possible associations between dietary acrylamide intake and selected cancer types. See Section 3.2.1.7 (Cancer) for information regarding cancer among acrylamide workers primarily exposed via inhalation and dermal routes.

Case-control Studies. No statistically significant associations were found between self-reported consumption of foods with high (300–1,200 μg/kg) or moderate (30–299 μg/kg) acrylamide concentrations and increased risk of large bowel, kidney, or bladder cancer in a population-based case-control study (692 controls and 875, 391, and 186 large bowel, kidney, and bladder cancer cases, respectively) (Augustsson et al. 1999; Mucci et al. 2003). Information on intake of various foods and nutrients was assessed by questionnaire. Cancer cases were born in Sweden between 1918 and 1942 and resided in Stockholm for at least 1 month between November 1992 and December 1994. Controls were selected from a Swedish national population registry and matched to cases by age and gender.

Mucci et al. (2005) assessed acrylamide intake of >43,000 women, including 667 breast cancer cases, who were enrolled in the Swedish Women's Lifestyle and Health Cohort. The estimated average daily acrylamide intake among the participants was 25.9 μg/day and was based on results of food frequency questionnaires (FFQs) and the Swedish National Food Administration database of information on acrylamide content of selected food items. After ranking the women into quintiles of estimated acrylamide intake (means of 12, 20, 25, 31, and 44 μg/day), there was no significant increased risk of breast cancer in the higher quintiles compared to the lowest quintile.

A Swedish nationwide, population-based case-control study reported a significant association between dietary acrylamide intake and risk of esophageal cancer (Lin et al. 2010). The study included 189 cases of exophageal adenocarcinoma, 262 cases of gastroexophageal junctional adenocarcinoma, 167 cases of esophageal squamous cell carcinoma, and 820 control participants. Participation rates ranged from 73 to

88%. Dietary acrylamide intake was assessed by questionnaire and categorized into quartiles. For the highest quartile, the adjusted risk of all esophageal tumors combined was increased (odds ratio [OR] 1.23; 95% CI 1.02–1.75) and was higher among overweight or obese patients (OR 1.88; 95% CI 1.06-3.34). The association appeared to be strongest for esophageal squamous cell carcinoma, particularly among nonsmokers in the highest quartile of acrylamide exposure (OR 2.82; 95% CI 1.16–6.87).

Within an integrated network of Italian and Swiss hospital-based case-control studies to investigate the relation between dietary acrylamide intake and cancers at several sites, no significant associations were found between estimated dietary acrylamide intake and cancers of the oral cavity and pharynx, esophagus, large bowel, larynx, breast, ovary, or prostate (Pelucchi et al. 2006). Dietary acrylamide intake was estimated based on results of FFQs and average content of acrylamide in foods from resources of the World Health Organization and Swiss Federal Office of Public Health. In case-control study that included four areas of Italy, no significant association was found between total dietary acrylamide intake and renal cell cancer (Pelucchi et al. 2007). In this study, statistically significantly elevated ORs (1.49, 95% CI 1.18–1.87; 1.70, 95% CI 1.25–2.30) were noted for weekly white bread portions of 7–<21 and ≥21, respectively; however, the study authors indicated that the relationship between white bread consumption and renal cell cancer might be explained by a high glycemic content and consequent effect on levels of insulin-like growth factors. Another case-control study performed by Pelucchi and coworkers in northern Italy (Pelucchi et al. 2011a) found no significant association between dietary acrylamide and pancreatic cancer (OR 1.01; 95% CI 0.92–1.10, for a 10 µg/day increase in acrylamide intake).

Wilson et al. (2009a) conducted a case-control study to assess possible associations between acrylamide and prostate cancer risk using two measures of acrylamide exposure: intake from FFQs and acrylamide-hemoglobin adduct levels in blood samples. Dietary data were available for 1,499 prostate cancer cases and 1,118 controls from a Cancer of the Prostate in Sweden (CAPS) population-based case-control study. Acrylamide-hemoglobin adduct levels were measured in blood samples from a subset of 170 prostate cancer cases and 161 controls. Controls were randomly selected from the Swedish Population Registry and were frequency matched to cases by 5-year age groups and region of residence. No significant association was found between acrylamide exposure (as measured by FFQ or acrylamide-hemoglobin adduct levels) and risk of prostate cancer.

Michels et al. (2006) conducted a case-control study to evaluate whether diet during preschool age affected a woman's risk of breast cancer later in life. Cases and controls were selected from participants

in two prospective cohort studies, the Nurses' Health Study and the Nurses' Health Study II. Information concerning childhood diet of the nurses at ages 3–5 years was obtained from FFQs filled out by the mothers of the participants. The median year of birth of the mothers was 1914 for case mothers and 1913 for control mothers. The results indicated an increased risk of breast cancer among woman who had frequently consumed French fries at preschool age. For one additional serving of French fries per week, the OR for breast cancer adjusted for adult life breast cancer risk factors was 1.27 (95% CI=1.12–1.44). Consumption of whole milk was associated with a slightly decreased risk of breast cancer (covariate-adjusted OR for every additional glass of milk per day=0.90; 95% CI=0.82–0.99). Intake of none of the nutrients calculated was related to the breast cancer risk in this study. The authors noted that they did not observe a similar association of breast cancer with frequent consumption of hot dogs or ground beef, suggesting that French fry consumption was not a marker of "fast food" habits. The study results suggest a possible association between diet before puberty and the subsequent risk of breast cancer, but the conclusions and the study are of limited use. No information is available on cooking methods or acrylamide content in the foods being evaluated, and the ability of mothers to accurately recall preschool diets of their daughters is questionable.

No significant associations were found between acrylamide-hemoglobin or glycidamide-hemoglobin adduct levels and total breast cancer in a Danish nested case-control study that examined breast cancer and acrylamide exposure using acrylamide-hemoglobin and glycidamide-hemoglobin adduct levels in red blood cells as presumed biomarkers for oral exposure to acrylamide (Olesen et al. 2008). After adjusting for confounding factors including smoking behavior, the study authors noted that a 10-fold increase in acrylamide-hemoglobin adduct levels was associated with a 1.9 (95% CI 0.9–4.0) times higher risk of breast cancer and a 5-fold increase (which corresponds to the range in acrylamide-hemoglobin adduct levels among nonsmokers) was associated with a 1.6 (95% CI 0.9–2.6) times higher risk. A significant positive association was observed between acrylamide-hemoglobin adduct level and ER+ breast cancer; a 10-fold increase in adduct level was associated with a 4.9 (95% CI 1.2–20) times increased risk in smokers and 2.7 (95% CI 1.1–6.6) times increased risk after adjustment for smoking. However, this study is limited by the relatively small number of subjects (374 cases and 374 controls) and uncertainty regarding extrapolation of acrylamide exposure as assessed by a few months of acrylamide-hemoglobin adduct measurements to a lifetime of exposure.

Mucci et al. (2004) analyzed data from a large population-based Swedish case-control study of renal cell cancer. FFQs were used to collect information on intake of 11 food items with elevated acrylamide levels as ascertained through extensive food databases in Sweden and the United States and quartiles of daily

food and acrylamide intake were created. This study found no evidence that food items with elevated acrylamide were associated with a higher risk of renal cell cancer risk.

Prospective Cohort Studies. Recent and ongoing prospective studies designed to evaluate possible associations between acrylamide in food and risk of cancers at various sites include cohorts from Sweden (Larsen et al. 2009a, 2009b, 2009c, 2009d; Mucci et al. 2006), the United States (Wilson et al. 2009b, 2010), and the Netherlands (Hogervorst et al. 2007, 2008a, 2008b). Most studies found no statistically significant associations between acrylamide in food and risks of cancers of the oro-hypopharynx, larynx, or thyroid gland (Schouten et al. 2009); esophagus, stomach, or pancreas (Hirvonen et al. 2010; Hogervorst et al. 2008b); colon or rectum (Hirvonen et al. 2010; Hogervorst et al. 2008b; Larsen et al. 2009c; Mucci et al. 2006); bladder or prostate (Hirvonen et al. 2010; Hogervorst et al. 2008a; Larsson et al. 2009e); lung (Hogervorst et al. 2009b); brain (Hogervorst et al. 2009a); breast (Hogervorst et al. 2007; Larsson et al. 2009d; Wilson et al. 2009b, 2010); endometrium (Hogervorst et al. 2007; Larsson et al. 2009a); ovarian epithelium (Larsson et al. 2009b), or lymphomas (Hirvonen et al. 2010).

However, Wilson et al. (2010) reported increased risk for endometrial cancer among "high" acrylamide consumers (relative risk [RR] for highest versus lowest quintile=1.41; 95% CI 1.01–1.97; p for trend=0.03) among women in the Nurses' Health Study. Wilson et al. (2010) also reported a slightly increased risk for ovarian serous tumors (RR 1.58; 95% CI 0.99–2.52; p for trend=0.04). Hirvonen et al. (2010) reported increased risk for lung cancer in the highest quintile (compared to the lowest quintile) of dietary acrylamide intake (RR 1.18; 95% CI 1.01–1.38; p for trend=0.11) within a cohort of 27,111 male smokers identified through the Finnish Cancer Registry without history of cancer prior to a 10.2-year follow-up period. Two prospective studies of a Dutch population reported increased risks of postmenopausal endometrial and ovarian cancer (Hogervorst et al. 2007) and renal cell cancer (Hogervorst et al. 2008a) with increasing dietary acrylamide in prospective studies of a Dutch population, but in these studies, estimations of dietary acrylamide levels in foods on the market at baseline in 1986 were based on food samples analyzed since 2001 and questionnaires did not include details regarding specifics of food preparation. Some of the tumor sites observed in animal studies (thyroid, testis, central nervous system) have not been evaluated in humans, and there are limitations in some of the study methods and cohort sizes in the prospective studies.

Pelucchi et al. (2011b) performed meta-analyses for various cancer end points from a number of prospective cohort and case-control studies that assessed dietary intake of acrylamide. The meta-analyses results indicated a lack of increased risk for cancers of the esophagus, colorectum, colon, rectum, breast,

endometrium, ovary, prostate, bladder, and kidney. The relative risk of 1.12 (95%CI 0.80–1.57; p for heterogeneity=0.055) was considered to indicate that a possible association between dietary acrylamide intake and kidney cancer should not be excluded.

Information regarding the carcinogenicity of acrylamide in orally-exposed animals is available from two similarly-designed 2-year bioassays (Friedman et al. 1995; Johnson et al. 1984, 1985, 1986) in which groups of male and female F344 rats were administered acrylamide in the drinking water at concentrations calculated to provide doses up to 2 mg/kg/day (3 mg/kg/day for high-dose female rats of the Friedman et al. 1995 study). Selected tumor types with significantly increased incidences in acrylamide-treated rats from both studies are summarized in Table 3-5. Significantly increased incidences of mesotheliomas of the tunica vaginalis testis were observed by Johnson et al. (1984, 1986) in male rats at the two highest dose levels (0.5 and 2 mg/kg/day). Additional findings included significantly increased incidences of thyroid (follicular cell) adenomas (no carcinomas), mesotheliomas of the tunica vaginalis testis, and benign adrenal pheochromocytoma in high-dose males and mammary gland benign tumors (adenoma, fibroadenomas, or fibroma), central nervous system tumors of glial origin, thyroid (follicular cell) adenomas or adenocarcinomas, squamous papillomas of the oral cavity, uterine adenocarcinomas, benign clitoral gland adenomas, and pituitary gland adenomas in high-dose females. The study of Friedman et al. (1995) noted increased incidences of tunica vaginalis mesothelioma and thyroid gland (follicular cell) adenoma (and adenoma or carcinoma) in high-dose males and thyroid gland follicular cell neoplasms (adenomas and carcinomas combined) in high-dose females and mammary gland tumors (fibroadenomas or combined fibroadenomas and carcinomas) in low- and high-dose (1 and 3 mg/kg/day) females. The findings of statistically significant increased incidences of adrenal pheochromocytomas in male rats, oral cavity tumors in female rats, central nervous system tumors of glial origin, and clitoral or uterine tumors in female rats in the earlier bioassay (Johnson et al. 1986) were not replicated in the second bioassay (Friedman et al. 1995); however, Rice (2005) reported that the study of Friedman et al. (1995) did not include examination of all the brains or spinal cords in the treatment groups and that seven reported cases of a morphologically distinctive category of primary brain tumor described as “malignant reticulosis” were excluded from the analysis.

Table 3-6 (rats) and Table 3-7 (mice) summarize relevant tumor incidence data obtained in more recent cancer bioassays of male and female F344/N rats and B6C3F1 mice administered acrylamide in the drinking water for up to 2 years (NTP 2011b). Treatment-related increased incidences of cancers of the epididymis, heart, pancreas, and thyroid gland were noted in the high-dose male rats (2.71 mg/kg/day) and increased incidences of cancers of the clitoral gland, mammary gland, oral mucosa or tongue, skin,

Table 3-5. Incidence of Tumors with Statistically Significant Increases in 2-Year Bioassays with F344 Rats Exposed to Acrylamide in Drinking Water

	Dose (mg/kg/day)							
Reference/tumor type	0	0	0.01	0.1	0.5	1.0	2.0	3.0
Johnson et al. 1986; males								
Follicular cell adenoma	1/60	–	0/58	2/59	1/59	–	7/59[a]	–
Tunica vaginalis mesothelioma	3/60	–	0/60	7/60	11/60[a]	–	10/60[a]	–
Adrenal pheochromocytoma	3/60	–	7/59	7/60	5/60	–	10/60[a]	–
Johnson et al. 1986; females								
Follicular cell adenoma/ carcinoma	1/58	–	0/59	1/59	1/58	–	5/60[b]	–
Mammary adenocarcinoma	2/60	–	1/60	1/60	2/58	–	6/61	–
Mammary benign	10/60	–	11/60	9/60	19/58	–	23/61[a]	–
Mammary benign + malignant[c]	12/60	–	12/60	10/60	21/58	–	29/61[a]	–
Central nervous system tumors of glial origin	1/60	–	2/59	1/60	1/60	–	9/61[a]	–
Oral cavity malignant + benign	0/60	–	3/60	2/60	3/60	–	8/60[a]	–
Uterus adenocarcinoma	1/60	–	2/60	1/60	0/59	–	5/60[b]	–
Clitoral adenoma, benign	0/2	–	1/3	3/4	2/4	–	5/5[b]	–
Pituitary gland adenoma	25/59	–	30/60	32/60	27/60	–	32/60[b]	–
Friedman et al. 1995; males[d]								
Follicular cell adenoma/carcinoma		2/102[f]	–	12/203	5/101	–	17/75[a]	–
Tunica vaginalis mesothelioma[e]	4/102	4/102	–	9/204	8/102	–	13/75[a]	–
Friedman et al. 1995; females[d]								
Follicular cell adenoma/ carcinoma	1/50 7/46	1/50 4/50	– –	– –	– –	10/100 21/94[a]	– –	23/100[a] 30/95[a]
Mammary benign + malignant								

[a]Statistically significantly ($p<0.05$) different from control, Fisher's Exact test.
[b]Statistically significantly ($p<0.05$) different from control, after Mantel-Haenszel mortality adjustment.
[c]Incidences of benign and adenocarcinoma were added herein, based on an assumption that rats assessed with adenocarcinoma were not also assessed with benign mammary gland tumors.
[d]Two control groups were included in the study design to assess variability in background tumor responses.
[e]Incidences reported herein are those originally reported by Friedman et al. (1995) and not those reported in the reevaluation study by Latropoulos et al. (1998).
[f]The data reported in Table 4 in Friedman et al. (1995) noted one follicular cell adenoma in the second control group; however, the raw data obtained in the Tegeris Laboratories (1989) report (and used in the time-to-tumor analysis) listed no follicular cell adenomas in this group. The corrected number for adenomas (0) and the total number (2) of combined adenomas and carcinomas in the second control group are used in the tables of this assessment.

Sources: Friedman et al. 1995; Johnson et al. 1986

Table 3-6. Incidence of Tumors with Statistically Significant Increases in a 2-Year Bioassay with F344 Rats Exposed to Acrylamide in Drinking Water

	Concentration of acrylamide in the drinking water (mM)				
Tumor type	0	0.0875	0.175	0.35	0.70
Males (dose in mg/kg/day)	**0**	**0.33**	**0.66**	**1.32**	**2.71**
Epididymis					
Malignant mesothelioma	2/48[a]	2/48	1/48	5/48	8/48[b]
Heart					
Malignant schwannoma	1/48	2/48	3/48	4/48	6/48[b]
Pancreatic islets					
Adenoma	1/46	2/48	4/48	1/48	6/48[b]
Thyroid gland (follicular cell)					
Carcinoma	1/47	2/48	3/47	6/48	6/48[b]
Adenoma or carcinoma	1/47	3/48	4/47	6/48	9/48[c]
Females (dose in mg/kg/day)	**0**	**0.44**	**0.88**	**1.84**	**4.02**
Clitoral gland					
Carcinoma	1/48	6/48	12/47[c]	3/48	8/47[c]
Mammary gland					
Fibroadenoma	16/48	18/48	24/46[b]	22/47[b]	31/48[c]
Oral mucosa or tongue					
Squamous cell papilloma or carcinoma	0/48	2/48	1/48	3/48	5/48[b]
Skin					
Subcutaneous fibroma, fibrosarcoma, or sarcoma	1/48	0/48	0/48	1/48	5/48[b]
Thyroid gland (follicular cell)					
Adenoma or carcinoma	0/48	0/48	2/48	3/48	4/47[b]

[a]Number of animals with neoplasm per number of animals examined microscopically.
[b]Statistically significantly ($p \leq 0.05$) different from control with adjustment for intercurrent mortality in Poly-3 test.
[c]Statistically significantly ($p < 0.01$) different from control with adjustment for intercurrent mortality in Poly-3 test.

Source: NTP 2011b

Table 3-7. Incidence of Tumors with Statistically Significant Increases in a 2-Year Bioassay with B6C3F1 Mice Exposed to Acrylamide in Drinking Water

	Concentration of acrylamide in the drinking water (mM)				
Gender (dose)/tumor type	0	0.0875	0.175	0.35	0.70
Males (dose in mg/kg/day)	**0**	**1.04**	**2.20**	**4.11**	**8.93**
Harderian gland					
Adenoma	2/46[a]	13/46[b]	27/47[b]	36/47[b]	39/47[b]
Lung					
Alveolar/bronchiolar adenoma	5/47	6/46	13/47[c]	10/45	19/48[b]
Forestomach (squamous cell)					
Papilloma	0/46	2/45	2/46	6/47[c]	6/44[b]
Papilloma or carcinoma	0/46	2/45	2/46	7/47[b]	8/44[b]
Females (dose in mg/kg/day)	**0**	**1.10**	**2.23**	**4.65**	**9.96**
Harderian gland					
Adenoma	0/45	8/44[b]	20/48[b]	32/47[b]	31/43[b]
Lung					
Alveolar/bronchiolar adenoma	1/47	4/47	6/48	11/45[b]	19/45[b]
Mammary gland					
Adenocanthoma or adenocarcinoma	0/47	4/46	7/48[b]	4/45[c]	17/42[b]
Ovary					
Benign granulose cell tumor	0/46	1/45	0/48	1/45	5/42[c]
Skin					
All tumor morphologies	1/48	0/46	4/48	11/45[b]	9/43[b]
Forestomach (squamous cell)					
Papilloma	4/46	0/46	2/48	5/45	8/42[c]

[a]Number of animals with neoplasm per number of animals examined microscopically.
[b]Statistically significantly ($p<0.01$) different from control with adjustment for intercurrent mortality in Poly-3 test.
[c]Statistically significantly ($p<0.05$) different from control with adjustment for intercurrent mortality in Poly-3 test.

Source: NTP 2011b

and thyroid gland were observed in the high-dose female rats (4.02 mg/kg/day). Significantly increased incidences of Harderian gland adenoma and Harderian gland adenoma or carcinoma (combined) were observed in all acrylamide-treated groups of male and female B6C3F1 mice beginning at doses of 1.04–1.1 mg/kg/day (NTP 2011b). Significantly increased incidences of cancers at other sites included squamous cell papilloma and squamous cell papilloma or carcinoma (combined) of the forestomach in males at doses ≥4.11 mg/kg/day, alveolar/bronchiolar adenoma or carcinoma (combined) in males at doses ≥2.20 mg/kg/day, alveolar/bronchiolar adenoma and adenoma or carcinoma (combined) in females at doses ≥4.65 mg/kg/day, skin tumors (including sarcoma, fibrosarcoma, fibrous histocytoma, myxosarcoma, and neurofibrosarcoma) in females at doses ≥4.65 mg/kg/day, mammary gland adenocanthoma or adenocarcinoma (combined) in females at doses ≥2.23 mg/kg/day, and benign granulose tumor of the ovary in high-dose females (9.96 mg/kg/day) (NTP 2011b).

The cancer effect levels (CELs) from the rat cancer bioassays of Johnson et al. (1984, 1986) and Friedman et al. (1995) and the rat and mouse cancer bioassays of NTP (2011b) are recorded in Table 3-4 and plotted in Figure 3-2.

The potential for acrylamide to initiate skin tumors was examined in two studies of female mice administered acrylamide at oral dose levels of 0, 12.5, 25, or 50 mg/kg/day, 6 times during a 2-week period, followed 2 weeks later by dermal applications of 12-O-tetradecanoylphorbol-13-acetate (TPA, a tumor promoter) to the shaved back 3 times/week for 20 weeks (Bull et al. 1984a, 1984b). Incidences of skin papillomas and skin carcinomas were significantly elevated in the mice receiving acrylamide at oral dose levels of 25 and/or 50 mg/kg/day, indicating that acrylamide initiated skin tumors under the conditions of the studies.

EPA, IARC, and the Department of Health and Human Services have concluded that acrylamide is likely to be carcinogenic to humans. This conclusion is based on lack of adequate human data and sufficient evidence of carcinogenicity in the animal studies summarized above. The Department of Health and Human Services (NTP 2011a) assigned the cancer descriptor "*reasonably anticipated to be a human carcinogen*". IARC (1994, 2011) assigned acrylamide to Group 2A (probably carcinogenic to humans).
EPA, IARC, and the Department of Health and Human Services have concluded that acrylamide is likely to be carcinogenic to humans. This conclusion is based on lack of adequate human data and sufficient evidence of carcinogenicity in the animal studies summarized above. The Department of Health and Human Services (NTP 2011a) assigned the cancer descriptor "*reasonably anticipated to be a human carcinogen*". IARC (1994, 2011) assigned acrylamide to Group 2A (probably carcinogenic to humans).

EPA characterized acrylamide as "likely to be carcinogenic to humans" (EPA 2010; IRIS 2012) and derived an oral slope factor of 0.5 per mg/kg/day) for acrylamide based on the summed risks for increased incidence of thyroid tumors and tunica vaginalis mesotheliomas in male F344 rats exposed to acrylamide in the drinking water for 2 years (Johnson et al. 1986). For cancer risks of $1x10^{-4}$, $1x10^{-5}$, $1x10^{-6}$, and $1x10^{-7}$, the corresponding dose levels are $2x10^{-5}$, $2x10^{-6}$, $2x10^{-7}$, and $2x10^{-8}$ mg/kg/day, respectively. These risk levels are presented in Figure 3-2. The human oral slope factor was derived using the rat $BMDL_{10}$ of 0.15 mg/kg/day determined from BMD analysis of the summed tumor incidence data from Johnson et al. (1986) as the point of departure, which was converted to a human equivalent dose (HED_{BMDL10}) of 0.194 mg/kg/day using glycidamide area under the curve (AUC) as the dose metric. EPA noted that the slope factor for acrylamide should not be used with exposures exceeding the HED_{BMDL10} and that age-dependent adjustment factors should be applied to the slope factor when assessing cancer risks to individuals <16 years of age (EPA 2010; IRIS 2012).

3.2.3 Dermal Exposure

3.2.3.1 Death

There are no reports of human deaths associated with dermal exposure to acrylamide.

Acrylamide has been demonstrated to be lethal to laboratory animals following a single dermal dose. Reported dermal LD_{50} values are 252 mg/kg in rats (American Cyanamid Company 1973) and 941 mg/kg in rabbits (American Cyanamid Company 1977). Dow Chemical Company (1957) reported that one of two rabbits administered a single 24-hour dermal dose of 1,000 mg/kg died 2 days following dosing.

Reliable acute dermal LD_{50} values for death and other mortality data for each species are recorded in Table 3-8.

3.2.3.2 Systemic Effects

No human or animal data were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, or endocrine effects following dermal exposure to acrylamide.

Table 3-8 Levels of Significant Exposure to Acrylamide - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL			Reference Chemical Form	Comments
				Less Serious		Serious		
ACUTE EXPOSURE								
Death								
Rat (albino)	Once					252 M mg/kg (LD50)	American Cyanamid Company 1973	
Mouse	5 d 1 x/d					200 M mg/kg/day (4/8 died during 30 days following cessation of dosing)	Gutierrez-Espeleta et al. 1992 Acrylamide	
Rabbit (New Zealand)	Once					941 M mg/kg (LD50)	American Cyanamid Company 1977	
Rabbit (NS)	Once					1000 mg/kg (death of 1/2 rabbits)	Dow Chemical Company 1957; McCollister et al. 1964	
Systemic								
Mouse	5 d 1 x/d	Dermal	125 M mg/kg/day				Gutierrez-Espeleta et al. 1992 Acrylamide	Histopathological evaluations of peripheral nerve fibers not performed
Rabbit (albino)	Once 18 h	Dermal		1120 M mg/kg	(slight dermal irritation in 1/3 rabbits)		American Cyanamid Company 1951	

Table 3-8 Levels of Significant Exposure to Acrylamide - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL			Reference Chemical Form	Comments
				Less Serious		Serious		
Neurological								
Rat (albino)	Once				200 M mg/kg	(unspecified CNS symptoms)	American Cyanamid Company 1973	
Reproductive								
Mouse	5 d 1 x/d		25 M mg/kg		50 M mg/kg	(significantly increased number of dead implants)	Gutierrez-Espeleta et al. 1992 Acrylamide	
INTERMEDIATE EXPOSURE								
Neurological								
Rabbit (New Zealand)	5 wk 3 x/d				50 mg/kg/day	(clinical signs of peripheral neuropathy)	Rohm and Haas Company 1975 Acrylamide	

d = day(s); hr = hour(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; wk = week(s); x = time(s)

The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-8.

Dermal Effects. Peeling of the skin was a common complaint among workers in factories with measurable acrylamide levels in the air and probable dermal exposure as well (Bachmann et al. 1992; Calleman et al. 1994; He et al. 1989; Myers and Macun 1991). Available information in animals is limited. Slight dermal irritation was reported in rats and rabbits following single dermal application of acrylamide in the range of 200–1,120 mg/kg (American Cyanamid Company 1951, 1973, 1977; Dow Chemical Company 1957). There was no evidence of dermal irritation in male mice receiving dermal applications of acrylamide for 5 days at doses ranging from 25 to 125 mg/kg/day (Gutierrez-Espeleta et al. 1992).

Ocular Effects. No data were located regarding ocular effects in humans following dermal exposure to acrylamide. Mild to moderate irritation was noted in the eyes of rabbits following ocular instillation of acrylamide (in water); irritation resolved during the ensuing 24 hours (American Cyanamid Company 1951; Dow Chemical Company 1957).

Body Weight Effects. No data were located regarding body weight effects in humans following dermal exposure to acrylamide. Available information in animals is restricted to a report of very slight initial weight loss (magnitude not specified) among a group of two rabbits administered a single 500 mg/kg dermal dose of acrylamide (Dow Chemical Company 1957) and another report of weight loss Table 3-8 in rabbits administered acrylamide dermally at 50 mg/kg/day for 5 weeks (Rohm and Haas Company 1975).

3.2.3.3 Immunological and Lymphoreticular Effects

No human or animal data were located regarding immunological or lymphoreticular effects following dermal exposure to acrylamide.

3.2.3.4 Neurological Effects

Available human data consist of occupational exposure scenarios with suspected inhalation and dermal exposure. See Section 3.2.1.4 for information regarding neurological effects in occupationally-exposed workers. Unspecified clinical signs of central nervous system effects were reported in rats that died following single dermal application of acrylamide at doses in the range of 200–800 mg/kg (American

Cyanamid Company 1973). Clinical signs that included shaking and loss of coordination were reported in rabbits following single dermal application at doses in the range of 784–1,568 mg/kg (American Cyanamid Company 1977). Clinical signs of hindlimb neuropathy became apparent during treatment week 4 in a study of rabbits administered acrylamide dermally for 5 weeks at 50 mg/kg/day (Rohm and Haas Company 1975).

The highest NOAEL values and all LOAEL values from each reliable study for neurological effects in each species and duration category are recorded in Table 3-8.

3.2.3.5 Reproductive Effects

No data were located regarding reproductive effects in humans following dermal exposure to acrylamide. Dermal applications of acrylamide to male mice for 5 days at doses ranging from 25 to 125 mg/kg/day followed by matings to unexposed female mice resulted in significantly increased numbers of dead implants (Gutierrez-Espeleta et al. 1992). At doses ≥50 mg/kg/day, significantly decreased numbers of living embryos were noted as well; this effect is indicative of dominant lethality.

The highest NOAEL values and all LOAEL values from each reliable study for reproductive effects in each species and duration category are recorded in Table 3-8.

3.2.3.6 Developmental Effects

No data were located regarding developmental effects in humans or animals following dermal exposure to acrylamide.

3.2.3.7 Cancer

No data were located regarding cancer associated with dermal exposure to acrylamide in humans.

The potential for acrylamide to initiate skin tumors was examined in a study of female mice administered acrylamide at dermal dose levels of 0, 12.5, 25, or 50 mg/kg/day, 6 times during a 2-week period, followed 2 weeks later by dermal applications of 12-O-tetradecanoylphorbol-13-acetate (TPA, a tumor promoter) to the shaved back 3 times/week for 20 weeks (Bull et al. 1984a). Incidences of skin papillomas and skin carcinomas were not significantly elevated at any acrylamide dose level. It was concluded that acrylamide did not act as a skin tumor initiator under the conditions of the study.

3.3 GENOTOXICITY

The genotoxicity of acrylamide has been studied both *in vivo* and *in vitro*. Studies are limited almost exclusively to laboratory rodents and nonmammalian species with the exception of a few *in vitro* assays of human cells. Results indicate that acrylamide is genotoxic and most potent in its ability to induce clastogenic effects (including heritable translocations in offspring of acrylamide-exposed male rodents mated with untreated females), deoxyribonucleic acid (DNA) damage, and gene mutations (including male-germ-cell-mediated dominant lethal mutations and heritable specific-locus mutations).

Table 3-9 summarizes the results of *in vivo* assays for acrylamide. Numerous assays have been performed in rodents. Dominant lethal mutations were induced consistently following administration of acrylamide via oral, dermal, or intraperitoneal injection in rodents (Adler et al. 2000; Chapin et al. 1995; Gutierrez-Espeleta et al. 1992; Sakamoto and Hashimoto 1986; Shelby et al. 1987; Smith et al. 1986; Sublet et al. 1989; Tyl et al. 2000a, 2000b; Working et al. 1987; Zenick et al. 1986). Exposure via intraperitoneal injection was also associated with specific locus mutations in offspring of male mice exposed to 50–125 mg/kg acrylamide before mating to untreated females (Ehling and Neuhäuser-Klaus 1992; Russell et al. 1991) and in offspring of pregnant female mice exposed to 50 or 75 mg/kg (Neuhäuser-Klaus and Schmahl 1989). Heritable or reciprocal translocations were noted in offspring of male mice exposed to 50–100 mg/kg acrylamide via intraperitoneal injection or dermal application before mating to untreated females (Adler 1990; Adler et al. 1994, 2004; Shelby et al. 1987). Intraperitoneal exposure to acrylamide also increased mutations at the tk and HPRT loci in lymphocytes of mice (von Tungeln et al. 2009) and at lac z loci in transgenic mice (Hoorn et al. 1993; Krebs and Favor 1997). Significantly increased mutation rates at the HPRT locus in splenic lymphocytes from Big Blue transgenic rats that had received acrylamide from the drinking water for 2 months at an estimated dose of 10 mg/kg/day (Mei et al. 2010) and in Big Blue transgenic mice that had received acrylamide from the drinking water for 3–4 weeks at estimated doses of 19–25 and 98–107 mg/kg/day (Manjanatha et al. 2006). Manjanatha et al. (2006) also noted a positive mutagenic response at the *cII* locus in liver cells at the high dose (98–107 mg/kg/day). Mei et al. (2010) also observed weakly positive mutagenic responses at the *cII* locus of bone marrow and thyroid cells from the acrylamide-treated rats and no significant effect on mutation rates at the *cII* locus of cells from testis, liver, or mammary gland. Significantly increased mutation rates were observed at the *cII* locus of testicular germ cells from Big Blue transgenic mice receiving acrylamide from the drinking water for 4 weeks at 19 or 98 mg/kg/day (Wang et al. 2010b).

Table 3-9. Genotoxicity of Acrylamide *In Vivo*

Species (test system)	End point	Result	Test conditions	Reference
Mammalian gene mutation				
Mouse	Dominant lethal mutation	+	1x125 mg/kg, intraperitoneal injection of males before mating with untreated females	Adler et al. 2000
Mouse	Dominant lethal mutation	+	5x40 or 50 mg/kg, intraperitoneal injection of males before mating with untreated females	Shelby et al. 1987
Mouse	Dominant lethal mutation	+	6 weeks, 0.81, 3.19, or 7.22 mg/kg/day, drinking water of males before mating with untreated females	Chapin et al. 1995
Mouse	Dominant lethal mutation	+	4 weeks, 3.3–16.3 mg/kg/day estimated drinking water doses of males before mating with untreated females	Sakamoto and Hashimoto 1986
Rat	Dominant lethal mutation	+	64 days, 0.5 or 5.0 mg/kg/day drinking water of males before mating with untreated females	Tyl et al. 2000a, 2000b
Rat	Dominant lethal mutation	+	5 days, 5, 15, 30, 45, or 60 mg/kg drinking water of males before mating with untreated females	Sublet et al. 1989
Rat	Dominant lethal mutation	+	5 days, 30 mg/kg gavage of males before mating with untreated females	Working et al. 1987
Rat	Dominant lethal mutation	+	80 days, 1.5, 2.8, or 5.8 mg/kg/day, drinking water of males before mating with untreated females	Smith et al. 1986
Rat	Dominant lethal mutation	+	10 weeks, 4.65, 7.86, or 11.53 mg/kg/day estimated drinking water doses of males before mating with untreated females	Zenick et al. 1986
Mouse	Dominant lethal mutation	+	5 days, 25–125 mg/kg/day, dermal exposure of males before mating with untreated females	Gutierrez-Espeleta et al. 1992
Mouse (spleen lymphocytes, tk and HPRT loci)	Gene mutation	–	0, 0.14, or 0.70 mmol/kg, intraperitoneal injection on postnatal days 1, 8, or 15	Von Tungeln et al. 2009
Mouse (spleen lymphocytes, tk and HPRT loci)	Gene mutation	+	0, 0.14, or 0.70 mmol/kg, intraperitoneal injection on postnatal days 1–8	Von Tungeln et al. 2009

Table 3-9. Genotoxicity of Acrylamide *In Vivo*

Species (test system)	End point	Result	Test conditions	Reference
Muta® Mouse (lac z loci)	Gene mutation	+	5x50 mg/kg intraperitoneal injection	Hoorn et al. 1993
Muta® Mouse (lac z loci)	Gene mutation	–	1x50–100 mg/kg, intraperitoneal injection	Krebs and Favor 1997
Mouse (offspring coat color loci)	Gene mutation	+	1x50 or 75 mg/kg, intraperitoneal injection of pregnant females	Neuhäuser-Klaus and Schmahl 1989
Mouse (offspring coat color loci)	Gene mutation	+	3x50 or 75 mg/kg, intraperitoneal injection of pregnant females	Neuhäuser-Klaus and Schmahl 1989
Mouse (offspring coat color loci)	Gene mutation	+	5x50 mg/kg, intraperitoneal injection of males before mating with untreated females	Russell et al. 1991
Mouse (offspring coat color loci)	Gene mutation	+	1x100–125 mg/kg, intraperitoneal injection of males before mating with untreated females	Ehling and Neuhäuser-Klaus 1992
Mouse, Big Blue transgenic (splenic lymphocytes, HPRT locus)	Gene mutation	(+)	3–4 weeks, 0 or 19–25 mg/kg/day, drinking water	Manjanatha et al. 2006
Mouse, Big Blue transgenic (splenic lymphocytes, HPRT locus)	Gene mutation	+	3–4 weeks, 0 or 98–107 mg/kg/day, drinking water	Manjanatha et al. 2006
Mouse, Big Blue transgenic (liver cells, *cII* locus)	Gene mutation	+	3–4 weeks, 0 or 98–107 mg/kg/day, drinking water	Manjanatha et al. 2006
Mouse, male Big Blue transgenic (testicular germ cells, *cII* locus)	Gene mutation	+ both doses	4 weeks, 0, 19, or 98 mg/kg/day, drinking water	Wang et al. 2010b
Rat, male and female Big Blue transgenic (splenic lymphocytes, HPRT locus)	Gene mutation	(+) high dose	2 months, 0, 5, or 10 mg/kg/day, drinking water	Mei et al. 2010
Rat, male Big Blue transgenic (testis, liver; *cII* locus)	Gene mutation	–	2 months, 0, 5, or 10 mg/kg/day, drinking water	Mei et al. 2010
Rat, male Big Blue transgenic (bone marrow, thyroid; *cII* locus)	Gene mutation	(+)	2 months, 0, 5, or 10 mg/kg/day, drinking water	Mei et al. 2010
Rat, female Big Blue transgenic (mammary gland, liver; *cII* locus)	Gene mutation	–	2 months, 0, 5, or 10 mg/kg/day, drinking water	Mei et al. 2010

Table 3-9. Genotoxicity of Acrylamide *In Vivo*

Species (test system)	End point	Result	Test conditions	Reference
Rat, female Big Blue transgenic (bone marrow, thyroid; *cII* locus)	Gene mutation	(+)	2 months, 0, 5, or 10 mg/kg/day, drinking water	Mei et al. 2010
Rat, 3-week-old male *gpt* delta transgenic (*gpt* locus)	Gene mutation (liver)	–	4 weeks, 0, 3.01, 5.95, or 12.19 mg/kg/day, drinking water	Koyama et al. 2011a
Rat, 11-week-old male *gpt* delta transgenic (*gpt* locus)	Gene mutation (liver)	–	4 weeks, 0, 1.83, 3.54, or 7.05 mg/kg/day, drinking water	Koyama et al. 2011a
Rat, 3-week-old male *gpt* delta transgenic (*gpt* locus)	Gene mutation (testis)	+ high dose	4 weeks, 0, 3.01, 5.95, or 12.19 mg/kg/day, drinking water	Koyama et al. 2011a
Rat, 11-week-old male *gpt* delta transgenic (*gpt* locus)	Gene mutation (testis)	–	4 weeks, 0, 1.83, 3.54, or 7.05 mg/kg/day, drinking water	Koyama et al. 2011a
Chromosomal alterations in mammalian cells				
Mouse (bone marrow)	Chromosomal aberration	+	1x50–150 mg/kg, intraperitoneal injection	Adler et al. 1988
Mouse (bone marrow)	Chromosomal aberration	+	1x100 mg/kg, intraperitoneal injection	Čihák and Vontorková 1988
Mouse (bone marrow)	Chromosomal aberration	–	1x100–200 mg/kg, intraperitoneal injection	Shiraishi 1978
Mouse (bone marrow)	Chromosomal aberration	–	7–21 days, 78 mg/kg-day, diet	Shiraishi 1978
Rat (bone marrow)	Chromosomal aberration	–	1x100 mg/kg, intraperitoneal injection	Krishna and Theiss 1995
Mouse (spleen lymphocyte)	Chromosomal aberration	–	1x50–125 mg/kg, intraperitoneal injection	Backer et al. 1989
Mouse (splenocyte)	Chromosomal aberration	–	1x100 mg/kg, intraperitoneal injection	Kligerman et al. 1991
Mouse (spermatogonia)	Chromosomal aberration	–	1x50–150 mg/kg, intraperitoneal injection	Adler et al. 1988
Mouse (spermatogonia)	Chromosomal aberration	–	1x50–125 mg/kg, intraperitoneal injection	Backer et al. 1989
Mouse (spermatogonia)	Chromosomal aberration	–	5x50 mg/kg/day, intraperitoneal injection	Adler 1990
Mouse (spermatocyte)	Chromosomal aberration	+	1x100 mg/kg, intraperitoneal injection	Adler 1990
Mouse (first cleavage one-cell zygote)	Chromosomal aberration	+	1x75 or 125 mg/kg or 5x50 mg/kg/day, intraperitoneal injection of males before mating with untreated females	Pacchierotti et al. 1994

Table 3-9. Genotoxicity of Acrylamide *In Vivo*

Species (test system)	End point	Result	Test conditions	Reference
Mouse (first cleavage zygote)	Chromosomal aberration	+	5x50 mg/kg/day, intraperitoneal injection of males before mating with untreated females	Marchetti et al. 1997
Mouse (offspring spermatocyte)	Heritable translocation	+	5x40–50 mg/kg/day, intraperitoneal injection of males before mating with untreated females	Shelby et al. 1987
Mouse (offspring spermatid)	Heritable translocation	+	1x50–100 mg/kg, intraperitoneal injection of males before mating with untreated females	Adler et al. 1994
Mouse (offspring spermatocyte)	Heritable translocation	+	5x50 mg/kg/day, dermal exposure of males before mating with untreated females	Adler et al. 2004
Mouse (offspring spermatocyte)	Heritable translocation	+	5x50 mg/kg/day, intraperitoneal injection	Adler 1990
Mouse (bone marrow)	Polyploidy or aneuploid	+	1x100–200 mg/kg, intraperitoneal injection	Shiraishi 1978
Mouse (bone marrow)	Polyploidy or aneuploid	+	7–21 days, 78 mg/kg/day, diet	Shiraishi 1978
Mouse (bone marrow)	Spindle disturbance	–	1x120 mg/kg, intraperitoneal injection	Adler et al. 1993
Mouse (bone marrow)	Micronucleus	+	1x50–125 mg/kg, intraperitoneal injection	Adler et al. 1988
Mouse (bone marrow)	Micronucleus	+	1x100 mg/kg, intraperitoneal injection	Čihák and Vontorková 1988
Mouse (bone marrow)	Micronucleus	+	2 days, 25–100 mg/kg/day, intraperitoneal injection	Čihák and Vontorková 1988
Mouse (bone marrow)	Micronucleus	+	1x136 mg/kg, intraperitoneal injection	Knaap et al. 1988
Mouse (bone marrow)	Micronucleus	+	1, 2, or 3 days, 42.5–100 mg/kg/day, intraperitoneal injection	Čihák and Vontorková 1990
Sprague-Dawley rat (bone marrow)	Micronucleus	–	1x100 mg/kg, intraperitoneal injection	Paulsson et al. 2002
Rat (bone marrow)	Micronucleus	–	1x100 mg/kg, intraperitoneal injection	Krishna and Theiss 1995
Rat (bone marrow)	Micronucleus	+	1x0, 125, 150, or 175 mg/kg, gavage	Yener and Dikmenli 2009
Rat, 3-week-old male *gpt* delta transgenic (bone marrow)	Micronucleus	–	4 weeks, 0, 3.01, 5.95, or 12.19 mg/kg/day, drinking water	Koyama et al. 2011a

Table 3-9. Genotoxicity of Acrylamide *In Vivo*

Species (test system)	End point	Result	Test conditions	Reference
Rat, 11-week-old male *gpt* delta transgenic (bone marrow)	Micronucleus	–	4 weeks, 0, 1.83, 3.54, or 7.05 mg/kg/day, drinking water	Koyama et al. 2011a
Rat, 3-week-old male *gpt* delta transgenic (bone marrow)	Micronucleus	+ high dose	4 weeks, 0, 3.01, 5.95, or 12.19 mg/kg/day, drinking water	Koyama et al. 2011a
Rat, 11-week-old male *gpt* delta transgenic (bone marrow)	Micronucleus	–	4 weeks, 0, 1.83, 3.54, or 7.05 mg/kg/day, drinking water	Koyama et al. 2011a
Rat, Big Blue transgenic (reticulocyte)	Micronucleus	–	2 months, 0, 5, or 10 mg/kg/day, drinking water	Mei et al. 2010
Mouse, male Big Blue transgenic (reticulocyte)	Micronucleus	(+)	3–4 weeks, 0 or 98–107 mg/kg/day, drinking water	Manjanatha et al. 2006
Mouse (reticulocyte)	Micronucleus	+	1x50–100 mg/kg, intraperitoneal injection	Russo et al. 1994
Mouse (reticulocyte)	Micronucleus	+	1x25–100 mg/kg, intraperitoneal injection	Paulsson et al. 2002
Mouse (reticulocyte and nonchromatic erythrocyte)	Micronucleus	–	0, 0.14, or 0.70 mmol/kg, postnatal days 1, 8, or 15, intraperitoneal injection	Von Tungeln et al. 2009
Mouse (reticulocyte and nonchromatic erythrocyte)	Micronucleus	–	0, 0.14, or 0.70 mmol/kg, postnatal day 1–8, intraperitoneal injection	Von Tungeln et al. 2009
Mouse (reticulocyte)	Micronucleus	+	28 days, 0–24 mg/kg/day, gavage	Zeiger et al. 2009
Mouse (normochromatic erythrocyte)	Micronucleus	+	28 days, 0–24 mg/kg/day, gavage	Zeiger et al. 2009
Mouse (erythrocyte)	Micronucleus	+	5 days 0, 25, 50 mg/kg/day, intraperitoneal injection	Ghanayem et al. 2005b
Mouse (spleen lymphocyte)	Micronucleus	+	1x50–125 mg/kg, intraperitoneal injection	Backer et al. 1989
Mouse (splenocyte)	Micronucleus	+	1x100 mg/kg, intraperitoneal injection	Kligerman et al. 1991
Mouse (spermatid)	Micronucleus	+	1x10–100 mg/kg, intraperitoneal injection	Collins et al. 1992
Mouse (spermatid)	Micronucleus	+	1x50–100 mg/kg or 4x50 mg/kg/day, intraperitoneal injection	Russo et al. 1994
Lewis rat (spermatid)	Micronucleus	+	1x50–100 mg/kg or 4x50 mg/kg/day, intraperitoneal injection	Xiao and Tates 1994

Table 3-9. Genotoxicity of Acrylamide *In Vivo*

Species (test system)	End point	Result	Test conditions	Reference
Sprague-Dawley rat (spermatid)	Micronucleus	+	1x50–100 mg/kg or 4x50 mg/kg/day, intraperitoneal injection	Lähdetie et al. 1994
Mouse (germ cell)	Synaptonemal complex aberration	–	1x50–150 mg/kg intraperitoneal injection	Backer et al. 1989
Mouse (germ cell)	Synaptonemal complex asynapsis	+	1x50–150 mg/kg, intraperitoneal injection Asynapsis in meiotic prophase	Backer et al. 1989
Sister chromatid exchange				
Mouse (spleen lymphocytes)	Chromatid exchange	+	1x50–125 mg/kg, intraperitoneal injection	Backer et al. 1989
Mouse (splenocytes)	Chromatid exchange	+	1x100 mg/kg, intraperitoneal injection	Kligerman et al. 1991
DNA damage				
Mouse (spermatocytes and early spermatids)	DNA breakage	+	1x0–125 mg/kg, intraperitoneal injection	Sega and Generoso 1990
Mouse (bone marrow, spleen, liver, kidney, lungs, testes)	DNA breakage	+	1x0–125 mg/kg, intraperitoneal injection	Dobrzynska 2007
Mouse (leukocytes, liver, lung)	DNA breakage	+	5 days, 0, 25, or 50 mg/kg/day, intraperitoneal injection (wild type mice)	Ghanayem et al. 2005b
B6C3F1 mouse (blood leukocytes, liver cells, duodenal cells, testicular somatic cells)	DNA damage (Comet assay)	+	4 days, 0, 12.5, 25, 37.5, or 50 mg/kg/day, gavage	Recio et al. 2010
F344/N rat (blood leukocytes, thyroid cells, duodenal cells, testicular somatic cells)	DNA damage (Comet assay)	+	4 days, 0, 12.5, 25, 37.5, or 50 mg/kg/day, gavage	Recio et al. 2010
F344/N rat (liver cells, presumptive sperm cells)	DNA damage (Comet assay)	–	4 days, 0, 12.5, 25, 37.5, or 50 mg/kg/day, gavage	Recio et al. 2010
Rat, 3-week-old male *gpt* delta transgenic (liver cells)	DNA damage (Comet assay)	+ high dose	4 weeks, 0, 3.01, 5.95, or 12.19 mg/kg/day, drinking water	Koyama et al. 2011a
Rat, 11-week-old male *gpt* delta transgenic (liver cells)	DNA damage (Comet assay)	+ two high doses	4 weeks, 0, 1.83, 3.54, or 7.05 mg/kg/day, drinking water	Koyama et al. 2011a
Rat (hepatocyte)	Unscheduled DNA synthesis	–	1x100 mg/kg or 5x30 mg/kg/day, gavage	Butterworth et al. 1992

Table 3-9. Genotoxicity of Acrylamide *In Vivo*

Species (test system)	End point	Result	Test conditions	Reference
Rat (spermatocyte)	Unscheduled DNA synthesis	+	1x100 mg/kg or 5x30 mg/kg/day, gavage	Butterworth et al. 1992
Mouse (germ cell)	Unscheduled DNA synthesis	+	1x7.8–125 mg/kg, intraperitoneal injection	Sega et al. 1990
Mouse (testis)	DNA adduct (Alkylation)	+	1x46 mg/kg, intraperitoneal injection	Sega et al. 1990
Mouse (liver)	DNA adduct (Alkylation)	+	1x46 mg/kg, intraperitoneal injection	Sega et al. 1990
Sprague-Dawley rat (liver, lung, kidney, brain, testis)	DNA adduct (N7-GA-Gua)	+	1x46 mg/kg, intraperitoneal injection	Segerbäck et al. 1995
Mouse (liver, kidney, brain)	DNA adduct (N7-GA-Gua)	+	1x53 mg/kg, intraperitoneal injection	Segerbäck et al. 1995
Neonatal mouse (whole body)	DNA adduct (N7-GA-Gua, N3-GA-Ade)	+	1x50 mg/kg, intraperitoneal injection	Gamboa da Costa et al. 2003
Mouse (liver, lung, kidney)	DNA adduct (N7-GA-Gua, N3-GA-Ade)	+	1x50 mg/kg, intraperitoneal injection	Gamboa da Costa et al. 2003
Mouse (liver, lung)	DNA adduct (N7-GA-Gua, N3-GA-Ade)	+	1x1–10 mg/kg, intraperitoneal injection	Gamboa da Costa et al. 2003
Mouse (lung, liver, spleen, bone marrow)	DNA adduct (N7-GA-Gua, N3-GA-Ade)	+	0, 0.14, or 0.70 mmol/kg, intraperitoneal injection, postnatal day 1, 8, 15	Von Tungeln et al. 2009
Mouse (lung, liver, spleen)	DNA adduct (N7-GA-Gua, N3-GA-Ade)	+	0, 0.14, or 0.70 mmol/kg, postnatal days 1–8, intraperitoneal injection	Von Tungeln et al. 2009
Mouse (liver)	DNA adduct (GA-Gua)	+	28 days, 0–24 mg/kg/day, gavage	Zeiger et al. 2009
Mouse (liver, lung, kidney, leukocyte, testis)	DNA adduct (N7-GA-Gua, N3-GA-Ade)	+	1x50 mg/kg, intraperitoneal injection	Doerge et al. 2005a
Mouse (liver)	DNA adduct (N7-GA-Gua, N3-GA-Ade)	+	14 days, 1 mg/kg/day, drinking water	Doerge et al. 2005a
Rat (liver, brain, thyroid, leukocyte, mammary gland, testis)	DNA adduct (N7-GA-Gua, N3-GA-Ade)	+	1x50 mg/kg, intraperitoneal injection	Doerge et al. 2005a
Rat (liver)	DNA adduct (N7-GA-Gua)	+	14 days, 1 mg/kg/day, drinking water	Doerge et al. 2005a

Table 3-9. Genotoxicity of Acrylamide *In Vivo*

Species (test system)	End point	Result	Test conditions	Reference
Rat, 3-week-old male *gpt* delta transgenic (preparations from liver, testis, mammary, thyroid)	DNA adduct (N7-GA-Gua)	+[a]	4 weeks, 0, 3.01, 5.95, or 12.19 mg/kg/day, drinking water)	Koyama et al. 2011a
Rat, 11-week-old male *gpt* delta transgenic (preparations from liver, testis, mammary, thyroid)	DNA adduct (N7-GA-Gua)	+[b]	4 weeks, 0, 1.83, 3.54, or 7.05 mg/kg/day, drinking water	Koyama et al. 2011a
Nonmammalian gene mutation				
Drosophila melanogaster	Sex-linked recessive lethal	–	1x40–50 mM, abdominal injection	Knaap et al. 1988
D. melanogaster	Sex-linked recessive lethal	+	48 hours, 0.25–5 mM, larvae feeding	Tripathy et al. 1991
D. melanogaster	Somatic mutation and recombination	+	1–1.5 mM, larvae feeding until pupation	Knaap et al. 1988
D. melanogaster	Somatic mutation and recombination	+	10–30 mM, larvae feeding until pupation	Batiste-Alentorn et al. 1991
D. melanogaster	Somatic mutation and recombination	+	48 hours, 0.25–5 mM, larvae feeding	Tripathy et al. 1991

[a]The high-dose young rats exhibited a significantly higher concentration of N7-GA-Gua adducts in the liver than the older rats.
[b]All groups of acrylamide-dosed young rats exhibited significantly higher concentrations of N7-GA-Gua adducts in the testis than the older rats.

– = negative result; + = positive result; (+) = weakly positive result; DNA = deoxyribonucleic acid

Koyama et al. (2011a) assessed the effects of age on the rate of mutation at the *gpt* locus from liver and testicular cells of 3- and 11-week-old male *gpt* delta transgenic rats administered acrylamide in the drinking water for 4 weeks. The test was positive for gene mutation in testicular cells from the younger rats receiving acrylamide at the highest dose tested (approximately 12 mg/kg/day), but negative for gene mutation in liver cells from both age groups and negative for gene mutation in testicular cells from the older rats up to and including the highest dose tested (approximately 7 mg/kg/day).

Prominent clastogenic effects consistently associated with *in vivo* exposure include sister chromatid exchanges (Backer et al. 1989; Kligerman et al. 1991), micronucleus formation (Backer et al. 1989; Čihák and Vontorková 1988, 1990; Collins et al. 1992; Ghanayem et al. 2005b; Kligerman et al. 1991; Knaap et al. 1988; Koyama et al. 2011a; Lähdetie et al. 1994; Manjanatha et al. 2006; Paulsson et al. 2002; Russo et al. 1994; Yener and Dikmenli 2009; Xiao and Tates 1994; Zeiger et al. 2009), and aneuploidy or polyploidy (Shiraishi 1978). Acrylamide induced DNA damage in various rodent tissues (including testes and male germ cells) (Butterworth et al. 1992; Dobrzynska 2007; Ghanayem et al. 2005b; Koyama et al. 2011a; Recio et al. 2010; Sega and Generoso 1990; Sega et al. 1990). Acrylamide also induced DNA adduct formation (Doerge et al. 2005a; Gamboa da Costa et al. 2003; Koyama et al. 2011a; Sega et al. 1990; Segerbäck et al. 1995; Von Tungeln et al. 2009; Zeiger et al. 2009). Increases in chromosomal aberrations were observed in the first cleavage zygote of acrylamide-treated male mice mated with untreated females (Marchetti et al. 1997; Pacchierotti et al. 1994).

Assays for acrylamide-induced chromosomal aberrations in bone marrow, spleen, or spermatogonia of acrylamide-treated rodents produced both positive (Adler 1990; Adler et al. 1988; Čihák and Vontorková 1988) and negative (Adler 1990; Adler et al. 1988; Backer et al. 1989; Kligerman et al. 1991; Krishna and Theiss 1995; Shiraishi 1978) results. Acrylamide did not induce micronuclei in a small number of rat and mouse assays (Krishna and Theiss 1995; Paulsson et al. 2002; Von Tungeln et al. 2009). Results for synaptonemal complex aberrations in male mouse germ cells were negative or only weakly positive (Backer et al. 1989). Acrylamide did not induce spindle disturbances in the bone marrow of intraperitoneally-injected mice (Adler et al. 1993).

Somatic mutations and recombination (Batiste-Alentorn et al. 1991; Knaap et al. 1988; Tripathy et al. 1991) and sex-linked recessive lethal mutations (Tripathy et al. 1991) were induced in drosophila larval feeding assays. Negative results were obtained for sex-linked recessive lethal mutations in another assay that employed abdominal injection (Knaap et al. 1988).

Table 3-10 summarizes the results of *in vitro* assays. Acrylamide induced mutations at the tk and HPRT loci in several assays with mammalian cells including mouse lymphoma L5178Y cells (Barfknecht et al. 1988; Knaap et al. 1988; Mei et al. 2008b; Moore et al. 1987) and human promyelocytic leukemia HL-60 and NB4 cells (Ao et al. 2008). Koyama et al. (2011b) reported a weakly positive result for gene mutation in human lymphoblastoid cell lines (TK6, AHH-1, and h2E1v2) at acrylamide concentrations in the range of 3–15 mM and in the absence of exogenous activation; in assays of the TK6 cell line with exogenous activation, it was noted that human liver microsomes induced a more strongly positive response than S9 mix. In Chinese hamster V79H3 cells (which do not express genes for CYP enzymes), acrylamide did not induce mutations at the HPRT locus (Tsuda et al. 1993). Acrylamide did not induce reverse mutations in multiple strains of *Salmonella typhimurium* or in *Escherichia coli* WP2 *uvrA*$^-$ with or without metabolic activation (Hashimoto and Tanii 1985; Jung et al. 1992; Knaap et al. 1988; Lijinsky and Andrews 1980; Müller et al. 1993; Tsuda et al. 1993; Zeiger et al. 1987). One exception was a weakly positive result in a few trials of TA98 and TA100, but only with S9 activation (Zeiger et al. 1987). Acrylamide did not cause DNA damage in *S. typhimurium* strain OY1002/2E1 (a strain that expresses human CYP2E1, reductase, and *O*-acetyl-transferase) in the absence of exogenous metabolic activation, or in *S. typhimurium* strain TA1535/pSK1002 (a strain that does not express human CYP2E1, reductase, or *O*-acetyl-transferase) either with or without exogenous metabolic activation (Koyama et al. 2011b).

Clastogenic effects associated with *in vitro* exposure to acrylamide include: chromosomal aberrations in mammalian cells (Knaap et al. 1988; Moore et al. 1987; Oliveira et al. 2009; Tsuda et al. 1993); polyploidy, sister chromatid exchanges, and spindle disturbances in Chinese hamster cells (Adler et al. 1993; Knaap et al. 1988; Martins et al. 2007; Tsuda et al. 1993; Warr et al. 1990); and micronuclei in human hepatoma G2 cells (Jiang et al. 2007). Although acrylamide did not induce micronuclei in one *in vitro* assay using seminiferous tubule segments from rats (Lähdetie et al. 1994), micronuclei were induced in spermatids of rodents exposed to acrylamide *in vivo* (Collins et al. 1992; Lähdetie et al. 1994; Russo et al. 1994; Xiao and Tates 1994). Koyama et al. (2011b) exposed human lymphoblastoid cell lines (TK6, AHH-1, and h2E1v2 lines) to acrylamide at concentrations in the range of 5–15 mM (TK6 cells) or up to 3 mM (AHH-1 and h2E1v2 cells). The assay of TK6 cells included tester groups with and without exogenous S9 mix; acrylamide induced micronuclei in the absence, but not the presence, of S9. A weak induction of micronuclei was observed in the AHH-1 and h2E1v2 cell preparations that were assayed only in the absence of exogenous metabolic activation.

Table 3-10. Genotoxicity of Acrylamide *In Vitro*

Species (test system)	End point	Result: Activation: With	Result: Activation: Without	Test conditions	Reference
Prokaryotic					
Bacterial gene mutation					
Salmonella. typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	10–10,000 µg/plate +/- S9 activation Weakly positive in TA98 and TA100 in only a few trials with activation; all other strains negative	Zeiger et al. 1987
S. typhimurium TA97, TA98, TA100, TA1535	Reverse mutation	–	–	100–10,000 µg/plate +/- S9 activation	Zeiger et al. 1987
S. typhimurium TA98, TA100, TA102, TA1535, TA1537	Reverse mutation	–	–	1–100 mg/plate +/- S9 activation	Knaap et al. 1988
S. typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	0.5–50 mg/plate +/- S9 activation	Tsuda et al. 1993
S. typhimurium TA1535	Reverse mutation	–	–	Up to 5 mg/plate +/- S9 activation	Müller et al. 1993; Jung et al. 1992
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	–	–	Up to 1 mg/plate +/- S9 activation	Lijinsky and Andrews 1980
S. typhimurium TA98, TA100, TA 1535, TA1537, TA1538	Reverse mutation	–	–	0.5–5,000 µg/plate +/- S9 activation	Hashimoto and Tanii 1985
Escherichia coli WP2 *uvrA*$^-$	Reverse mutation	–	–	0.5–50 mg/plate +/- S9 activation	Tsuda et al. 1993
Klebsiella. pneumoniae ur$^-$ pro$^-$	Fluctuation	No data	–	2–10 mg/L	Knaap et al. 1988
DNA damage and repair and DNA adduct formation					
S. typhimurium TA1535/pSK1002	DNA damage	–	–	2–10 mM +/- S9 activation	Koyama et al. 2011b
S. typhimurium OY1002/2E1	DNA damage	No data	–	2–10 mM	Koyama et al. 2011b
Bacillus subtilis	DNA damage	+	+	1–50 mg/ disk +/- S9 activation	Tsuda et al. 1993
Mammalian gene mutation assays					
Mouse lymphoma L5178Y TK$^{+/-}$, tk locus	Gene mutation	+	+	10 mM	Barfknecht et al. 1988
Mouse lymphoma L5178Y TK$^{+/-}$, tk locus	Gene mutation	No data	+	0–0.85 mg/mL No activation Clastogenic response	Moore et al. 1987

Table 3-10. Genotoxicity of Acrylamide *In Vitro*

Species (test system)	End point	Result: Activation: With	Result: Activation: Without	Test conditions	Reference
Mouse lymphoma L5178Y TK$^{+/-}$, tk locus	Gene mutation	No data	+	0–18 mM No activation	Mei et al. 1988
Mouse lymphoma L5178Y TK$^{+/-}$, tk and HPRT loci	Gene mutation	–	–	0.5–7.5 mg/mL +/- S9 activation Mutation only at cytotoxic concentrations	Knaap et al. 1988
Mouse lymphoma L5178Y TK$^{+/-}$, HPRT locus	Gene mutation	+	+	0.1–0.5 mg/mL Cocultivated activation	Knaap et al. 1988
Chinese hamster V79H3, HPRT locus	Gene mutation	No data	–	1–7 mM No activation	Tsuda et al. 1993
Human lymphoblastoid cell line (TK6)	Gene mutation	(+)	(+)	5–15 mM +/- S9 activation	Koyama et al. 2011b
Human lymphoblastoid cell line (TK6)	Gene mutation	+	(+)	5–15 mM +/- human liver microsomal activation	Koyama et al. 2011b
Human lymphoblastoid cell line (AHH-1)	Gene mutation	No data	(+)	Up to 3 mM	Koyama et al. 2011b
Human lymphoblastoid cell line (h2E1v2)	Gene mutation	No data	(+)	Up to 3 mM	Koyama et al. 2011b
Human promyelocytic leukemia HL-60 and NB4, HPRT locus	Gene mutation	No data	+	0–700 mg/L	Ao et al. 2008
Chromosomal alteration in mammalian cells					
Chinese hamster V79H3	Chromosomal aberration	No data	+	0.5–5 mM No activation	Tsuda et al. 1993
Chinese hamster V79	Chromosomal aberration	+	+	0.1–3 mg/mL +/- S9 activation	Knaap et al. 1988
Chinese hamster V79	Chromosomal aberration	No data	–	0–2,000 µM No activation	Martins et al. 2007
Chinese hamster V79	Chromosomal aberration	No data	+	2 mM	Oliveira et al. 2009
Mouse lymphoma L5178Y TK$^{+/-}$	Chromosomal aberration	No data	+	0.65–0.85 mg/mL No activation	Moore et al. 1987
Chinese hamster V79H3	Polyploidy	No data	+	0.5–5 mM	Tsuda et al. 1993
Chinese hamster LUC2 p5	Polyploidy	No data	+	0.0125–0.5 mg/mL	Warr et al. 1990

Table 3-10. Genotoxicity of Acrylamide *In Vitro*

Species (test system)	End point	Result: Activation: With	Result: Activation: Without	Test conditions	Reference
Chinese hamster V79	Spindle disturbance	No data	+	0.01–1 mg/mL	Adler et al. 1993
Chinese hamster DON:Wg3h	Spindle disturbance	No data	+	0.2–2 mg/mL	Warr et al. 1990
Chinese hamster LUC2 p5	Spindle disturbance	No data	+	0.01–1 mg/mL	Warr et al. 1990
Rat seminiferous tubular segments	Micronucleus	No data	–	5–50 µg/mL	Lähdetie et al. 1994
Human hepatoma G2	Micronucleus	No data	+	0–2.5 mM	Jiang et al. 2007
Human lymphoblastoid cell line (TK6)	Micronucleus	–	+	5–15 mM +/- S9 activation	Koyama et al. 2011b
Human lymphoblastoid cell line (TK6)	Micronucleus	–	+	5–15 mM +/- human liver microsomal activation	Koyama et al. 2011b
Human lymphoblastoid cell line (AHH-1)	Micronucleus	No data	(+)	Up to 3 mM	Koyama et al. 2011b
Human lymphoblastoid cell line (h2E1v2)	Micronucleus	No data	(+)	Up to 3 mM	Koyama et al. 2011b
Sister chromatid exchange					
Chinese hamster V79	Chromatid exchange	+	+	0.1–1 mg/mL	Knaap et al. 1988
Chinese hamster V79	Chromatid exchange	No data	+	0.5–2.5 mg/mL	Tsuda et al. 1993
Chinese hamster V79	Chromatid exchange	No data	+	0–2,000 µM	Martins et al. 2007
DNA damage and repair and DNA adduct formation					
Human hepatoma G2	DNA breakage	No data	+	0–20 mM	Jiang et al. 2007
Human hepatoma G2	Oxidative DNA damage	No data	+	0–20 mM	Jiang et al. 2007
F344 Rat primary hepatocytes	Unscheduled DNA synthesis	No data	–	0.01–1 mM	Butterworth et al. 1992
Human mammary epithelial	Unscheduled DNA synthesis	No data	+	1–10 mM	Butterworth et al. 1992
Chinese hamster V79	DNA adducts (N7-GA-Gua, N3-GA-Ade)	No data	+	0–2,000 µM	Martins et al. 2007

Table 3-10. Genotoxicity of Acrylamide *In Vitro*

Species (test system)	End point	Result: Activation: With	Result: Activation: Without	Test conditions	Reference
Mouse lymphoma L5178Y $TK^{+/-}$	DNA adducts (N7-GA-Gua, N3-GA-Ade)	No data	–	0–20 mM	Mei et al. 2008b
Big Blue mouse embryonic fibroblasts	DNA adducts (N7-GA-Gua, N1-GA-Ade, N3-GA-Ade)	No data	+	0, 0.0032, 0.320. and 16 mM	Besaratinia and Pfeifer 2004
Human bronchial epithelial	DNA adducts (N7-GA-Gua, N1-GA-Ade, N3-GA-Ade)	No data	+	0, 0.320 and 3.2 mM	Besaratinia and Pfeifer 2004
Human lymphoblastoid cell line (TK6)	DNA adducts (N7-GA-Gua)	+	+	Up to 15 mM +/- human liver microsomal activation	Koyama et al. 2011b
Human lymphoblastoid cell line (AHH-1)	DNA adducts (N7-GA-Gua)	No data	–	0.7–2.8 mM	Koyama et al. 2011b
Human lymphoblastoid cell line (h2E1v2)	DNA adducts (N7-GA-Gua)	No data	–	0.7–2.8 mM	Koyama et al. 2011b
Cell transformation					
Mouse C3H/10T1/2	Morphological transformation	No data	+	25–200 µg/mL	Banerjee and Segal 1986
Mouse NIH/$3T_3$	Morphological transformation	No data	+	2–200 µg/mL	Banerjee and Segal 1986
Mouse C3H/10T1/2	Morphological transformation	No data	–	10–300 µg/mL	Abernethy and Boreiko 1987
Mouse BALB/c $3T_3$	Morphological transformation	No data	+	0.5–2 mM	Tsuda et al. 1993
Syrian hamster embryo	Morphological transformation	No data	+	0.1–0.7 mM	Park et al. 2002
Syrian hamster embryo	Morphological transformation	No data	–	0.001–10 mM	Kaster et al. 1998

– = negative result; + = positive result; (+) = weakly positive result; DNA = deoxyribonucleic acid

DNA damage associated with *in vitro* exposure to acrylamide includes DNA damage in *Bacillus subtilis* (Tsuda et al. 1993); DNA breakage and oxidative DNA damage in human hepatoma G2 cells (Jiang et al. 2007); unscheduled DNA synthesis in human mammary epithelial cells (Butterworth et al. 1992); and glycidamide-DNA adducts in Chinese hamster V79 cells (Martins et al. 2007), mouse embryonic fibroblasts (Besaratinia and Pfeifer 2004), human bronchial epithelial cells (Besaratinia and Pfeifer 2004), and a TK6 human lymphoblastoid cell line (Koyama et al. 2011b). DNA adducts were not detected in AHH-1 or h2E1v2 human lymphoblastoid cell lines (Koyama et al. 2011b). DNA adducts were not detected when mouse lymphoma cells were incubated with up to 20 mM acrylamide, but glycidamide concentrations in the range of 0.5–4 mM induced DNA adducts in these cells (Mei et al. 2008b). Acrylamide *in vitro* exposure also caused cell morphological transformations in several mouse cell lines and Syrian hamster embryo cells (Abernethy and Boreiko 1987; Banerjee and Segal 1986; Park et al. 2002; Tsuda et al. 1993).

3.4 TOXICOKINETICS

Acrylamide and its principal and toxicologically significant (epoxide) metabolite, glycidamide, react with various biologically significant targets. The chemical basis for these interactions is strongly associated with the degree of electrophilicity (electron deficiency) with nucleophilic centers (i.e., unshared electrons). Electrophiles and nucleophiles are generally characterized as being either "hard" or "soft" corresponding to a spectral range of high or low charge densities or electronegativity for reactivity (Pearson and Songstad 1967). Due to its ά,β-unsaturated structure and ready capacity to undergo Michael-type additions, acrylamide may be classified as a "soft" electrophile. Soft electrophiles like acrylamide react readily with soft nucleophiles such as the thiol groups of proteins or glutathione. Glycidamide, on the other hand, has a relatively high positive charge density, and acts as a hard electrophile, more capable of reacting with centers of high electronegativity (i.e., hard nucleophiles) such as the purine and pyrimidine bases in DNA (Dearfield et al. 1995; Lopachin and DeCaprio 2005). Hemoglobin adducts have been used as biomarkers of exposure to acrylamide and are based on the assumption that a measured adduct level represents a steady-state level from a continuous exposure to acrylamide over the previous 120 days, which is the average life span of a red blood cell. Hemoglobin adduct levels provide a direct measure of the total amount of acrylamide and its reactive metabolite, glycidamide, in the blood over a given time period, which is quantified as the area under the curve (AUC in amount-unit time/volume). AUC is the integral of "concentration" (e.g., mg or mmol/L) × "time" (e.g., minutes or hours). Under the reasonable assumption that the amount of parent or reactive toxicant in

blood indicates the amount available to bind to tissue macromolecules or DNA, hemoglobin adducts provide a relevant internal metric for use in estimating the risk of acrylamide toxicity.

3.4.1 Absorption

Detection of hemoglobin adducts of acrylamide in exposed workers and volunteers provides qualitative evidence of absorption. Controlled human studies of the formation of hemoglobin adducts of acrylamide following oral and dermal exposure provide some information regarding the extent of absorption via these exposure routes. Available animal data indicate that acrylamide is readily and rapidly absorbed following inhalation and oral exposure, and somewhat less readily absorbed following dermal exposure.

3.4.1.1 Inhalation Exposure

Hagmar et al. (2001) measured hemoglobin adducts of acrylamide in a group of 210 tunnel construction workers who were occupationally exposed for 2 months to a chemical grouting agent containing acrylamide and N-methylolacrylamide. Within 1 month after construction work was completed, blood samples were drawn for the analysis of adducts of acrylamide with N-terminal valines (acrylamideVal) in hemoglobin. Workers were expected to have experienced both inhalation and dermal exposure. Quantitative exposure data were limited to two personal air samples showing concentrations of 0.27 and 0.34 mg/m^3 for the sum of acrylamide and N-methylolacrylamide; further analysis suggested that the air contained a 50:50 mixture of these compounds. Hemoglobin adduct levels for 18 nonsmoking unexposed reference subjects varied between 0.02 and 0.07 nmol/g globin. The frequency distribution of adduct levels in the 210 tunnel workers was as follows: 47 with <0.08 nmol/g globin; 89 with 0.08–0.29 nmol/g; 36 with 0.3–1.0 nmol/g; and 38 with 1.0–17.7 nmol/g. Adduct levels were determined in blood samples collected at intervals up to 5 months after cessation of exposure from five workers with initial levels ranging from about 2.2 to 4.4 nmol/g. Adduct levels decreased to background levels within 120 days, consistent with the approximate 120-day life of red blood cells.

Hemoglobin adduct levels were measured in 41 Chinese workers who were exposed to acrylamide for 0.1–8 years (Bergmark et al. 1993). AcrylamideVal hemoglobin levels were measured. Workers were involved in the production of acrylamide (via the hydration of acrylonitrile) and polyacrylamide. The adduct levels in exposed workers ranged from 0.3 to 34 nmol acrylamide/g hemoglobin. AcrylamideVal levels were not detected in blood samples of 10 control workers from the same city who had not been occupationally exposed to acrylamide (or acrylonitrile). Blood samples from 5 of the 41 exposed workers were also analyzed for hemoglobin adducts of glycidamide (glycidamideVal), a principal metabolite of

acrylamide in animals (see also Section 3.4.3, Metabolism). A statistically significant linear relationship was observed between levels of acrylamideVal and glycidamideVal in these five workers; the ratio between acrylamideVal and glycidamideVal was approximately 3:10. Average levels of acrylamide in air samples were 1.52 and 0.73 mg/m^3 for workplaces involved with polymerization and synthesis processes, respectively. Workers involved in these processes showed average acrylamideVal levels of 7.3±3.4 (n=12, polymerization) and 14.7±10.6 nmol/g hemoglobin (n=14, synthesis).

Bergmark et al. (1993) made the following assumptions based on a central assumption that these workers may have experienced only inhalation exposure: (1) adducts are stable during the life of erythrocytes; (2) the lifespan of human erythrocytes is about 120 days (17 weeks); (3) the second-order reaction rate constant for the reaction of acrylamideVal in human hemoglobin is $4.4x10^{-6}$ [L $(g\ Hb)^{-1}$hour $^{-1}$] (based on *in vitro* experiments); (4) the human ventilation rate is 0.2 L $minute^{-1}$ kg bw^{-1}; and (5) inhaled acrylamide is 100% absorbed. Using these assumptions, the predicted levels of acrylamideVal were only 0.93 and 0.44 nmol/g hemoglobin, respectively. The large disparity between the observed and predicted adduct levels resulted in the conclusion that dermal contact may have been the predominant source of absorption in these workers.

Animal studies indicate that inhaled acrylamide is readily absorbed (Sumner et al. 2003). Male F344 rats and B6C3F1 mice were exposed to approximately 3 ppm of a mixture of [^{13}C]-labeled acrylamide and [^{14}C]-labeled acrylamide vapor via nose-only inhalation for 6 hours. Selected rats and mice were sacrificed immediately following the exposure period for determination of [^{14}C] content in tissues, an indicator of the extent of absorption of inhaled acrylamide. The remaining rats and mice were monitored for 24-hour elimination of acrylamide and metabolites via urine, feces, and expired air. Immediately following the 6-hour exposure period, approximately 18 and 8 µmol of [^{14}C]-equivalents were recovered from tissues and carcasses of the rats and mice, respectively. At the end of the 24-hour postexposure period, 42% of the total recovered radioactivity was in urine, feces, and nose-tube and cage washes of rats; <3% was in exhaled air; and 56% remained in the body. In mice, 51% was recovered in urine, feces, and nose-tube and cage washes; <3% was in exhaled air, and 46% remained in the body. Fractional absorption could not be determined from the presented data because ventilation rates were apparently not measured.

3.4.1.2 Oral Exposure

Fennell and coworkers evaluated metabolism and hemoglobin adduct formation (Fennell et al. 2005b) and kinetics of elimination of urinary metabolites of acrylamide (Fennell et al. 2006) following oral administration of [1,2,3-$^{13}C_3$]-acrylamide to 24 adult male volunteers exposed under controlled conditions. All volunteers were aspermic (i.e., clinically sterile because of the potential for adverse effects of acrylamide on sperm), and had not used tobacco products for the prior 6 months. The health of the volunteers was continually monitored. Acrylamide was administered in an aqueous solution (single dose of 0.5, 1, or 3 mg/kg) to the volunteers. For the 3 mg/kg dose group, approximately 40% of the administered dose was recovered as urinary metabolites in the 24-hour urine. Approximately 86% of the urinary metabolites were derived from GSH conjugation and excreted as N-acetyl-S-(3-amino-3-oxopropyl)cysteine and its S-oxide; the remainder included glycidamide, glyceramide, and low levels of N-acetyl-S-(3-amino-2-hydroxy-3-oxopropyl)cysteine. Mean levels of hemoglobin adducts of acrylamide ($^{13}C_3$-acrylamideVal) and glycidamide ($^{13}C_3$-glycidamideVal) in the 3 mg/kg group at 24 hours postadministration were 2,479 and 1,076 fmol/mg globin, respectively. These findings demonstrate that orally-administered acrylamide is rapidly and readily absorbed by the gastrointestinal tract.

Fuhr et al. (2006) evaluated the toxicokinetics of acrylamide in six young healthy volunteers after the consumption of a meal containing 0.94 mg of acrylamide. Urine was collected up to 72 hours thereafter. Unchanged acrylamide; its mercapturic acid metabolite, N-acetyl-S-(2-carbamoylethyl)cysteine (AAMA), its epoxy derivative, glycidamide; and the respective metabolite of glycidamide, N-acetyl-S-(2-hydroxy-2-carbamoylethyl)cysteine (GAMA), were quantified in the urine by liquid chromatography-mass spectrometry. Toxicokinetic variables were obtained by noncompartmental methods. Overall, approximately 60% of the dose was recovered in the urine. Although no glycidamide was found, unchanged acrylamide, AAMA, and GAMA accounted for urinary excretion of approximately 4.5, 50, and 6% of the dose, respectively. These results indicate that most of the acrylamide ingested with food is absorbed in humans.

Boettcher et al. (2006a) investigated the human metabolism of acrylamide to AAMA and GAMA in a healthy male volunteer who received a single dose of about 1 mg deuterium-labeled (d(3)) acrylamide, representing 13 µg/kg body weight, in drinking water. Urine samples before dosing and within 46 hours after the dose were analyzed for d(3)-AAMA and d(3)-GAMA. Total recovery of AAMA and GAMA in the 24-hour urine was about 51% of the administered dose, which provides additional demonstration of the rapid and extensive absorption of ingested acrylamide.

Boettcher et al. (2006b) reported the influence of an acrylamide-free diet on the excretion of urinary mercapturic acid metabolites derived from acrylamide in three healthy volunteers who fasted for 48 hours. Urinary acrylamide mercapturic acid metabolites were considerably reduced after 48 hours of fasting, with levels even well below the median level in nonsmokers. These results indicate that the acrylamide in the diet is the main source of environmental acrylamide exposure in humans, apart from smoking.

Bjellaas et al. (2007a) reported urinary mercapturic acid derivatives of acrylamide in a clinical study comprised of 53 subjects. Median intakes (range) of acrylamide were estimated based on 24-hour dietary recall as 21 (13–178) µg for nonsmokers and 26 (12–67) µg for smokers. The median dietary exposure to acrylamide was estimated to be 0.47 (range 0.17–1.16) µg/kg body weight per day. The median (range) total excretion of acrylamide in urine during 24 hours was 16 (7–47) µg for nonsmokers and 74 (38–106) µg for smokers. In a multiple linear regression analysis, a statistically significant correlation was found between the urinary excretion of acrylamide metabolites and intake of aspartic acid, protein, starch, and coffee.

Studies in rats and mice indicate that orally administered acrylamide is rapidly and extensively absorbed by the gastrointestinal tract (Dixit et al. 1982; Doerge et al. 2005b, 2005c; Fennell et al. 2005a; Kadry et al. 1999; Miller et al. 1982; Ramsey et al. 1984). The time course of urinary elimination of radioactivity from male F344 rats during a 7-day period following single oral doses of 10 mg/kg [2,3-^{14}C]-acrylamide was essentially the same as that observed with male F344 rats following single intravenous dosing at 10 mg/kg [2,3-^{14}C]-acrylamide (Miller et al. 1982). This observation suggests that 100% of the oral dose was absorbed. The time courses of urinary elimination of radioactivity were similar for groups of rats given single 1, 10, or 100 mg/kg oral doses of radiolabeled acrylamide, suggesting that the extent of absorption was not affected by dose level in the tested range. The rapidity of absorption was demonstrated by observations that peak plasma levels of radioactivity were attained by 1 hour after administration and that 53–67% of administered radioactivity was detected in the urine collected within 24 hours of administration (Miller et al. 1982). Similar results indicating rapid and extensive oral absorption were reported for studies in which male Sprague-Dawley rats were given single 50 mg/kg oral doses of [1-^{14}C]-acrylamide (Kadry et al. 1999). Radioactivity was detected in blood 5 minutes after administration, and peak plasma levels of radioactivity occurred at 38 minutes after administration. Approximately 51% of administered radioactivity was detected in urine collected within 24 hours of administration (Kadry et al. 1999). Fennell et al. (2005a) administered 3 mg/kg of [1,2,3-$^{13}C_3$]-acrylamide by gavage to male F344 rats. The total

amount of acrylamide metabolites recovered in urine by 24 hours after dosing was 50%, which is similar to that reported by Miller et al. (1982) and Kadry et al. (1999).

Doerge and coworkers evaluated the toxicokinetics of acrylamide and glycidamide in serum and tissues of male and female Fischer 344 rats (Doerge et al. 2005c) and B6C3F1 mice (Doerge et al. 2005b) following single 0.1 mg/kg acrylamide dosing by intravenous injection or gavage or a comparable dose of acrylamide from a feeding exposure for 30 minutes. Study groups also received an equimolar amount of glycidamide from either intravenous injection or gavage dosing. Following oral dosing, acrylamide was rapidly absorbed, widely distributed to tissues, and efficiently converted to glycidamide in the rats and mice. Oral glycidamide dosing also resulted in rapid absorption and wide distribution to tissues in the rats and mice. Evaluation of livers from the orally-treated rats at 10 hours posttreatment revealed significantly higher glycidamide-DNA adduct levels in both the acrylamide- and glycidamide-treated groups of male and female rats, relative to controls. The glycidamide-DNA adduct level in the glycidamide-treated male rats was significantly (2-fold) higher than that in the acrylamide-treated male rats; there was no significant difference in glycidamide-DNA adduct levels from the acrylamide- and glycidamide-treated female rats. In the mice, treatment with glycidamide produced a 1.5-fold increase in glycidamide-DNA adduct formation compared to that produced from treatment with acrylamide; there were no apparent gender-related differences in glycidamide-DNA adduct formation in the mice. For the rats, oral administration of acrylamide from the diet attenuated acrylamide bioavailability to 32–44% of the intravenous dose, and aqueous gavage resulted in approximately 60–98% of the acrylamide bioavailability from the intravenous dose. For the mice, oral administration of acrylamide from the diet attenuated acrylamide bioavailability to 23% of the intravenous dose, and aqueous gavage attenuated acrylamide bioavailability to 32–52%. For the rats and mice, oral exposure to acrylamide resulted in higher relative internal glycidamide levels compared with levels following an intravenous injection, likely due to a first-pass effect but possibly the result of some other kinetic change.

3.4.1.3 Dermal Exposure

Fennell and coworkers evaluated metabolism and hemoglobin adduct formation (Fennell et al. 2005b) and kinetics of elimination of urinary metabolites of acrylamide (Fennell et al. 2006) following dermal administration of [1,2,3-$^{13}C_3$]-acrylamide to 24 adult male volunteers exposed under controlled conditions. All volunteers were aspermic (i.e., clinically sterile because of the potential for adverse effects of acrylamide on sperm) and had not used tobacco products for the prior 6 months. The health of the volunteers was continually monitored. Acrylamide was administered as three daily 24-hour occluded

dermal doses of 3 mg/kg. Mean levels of hemoglobin adducts of acrylamide ($^{13}C_3$-acrylamideVal) and glycidamide ($^{13}C_3$-glycidamideVal) increased from 116 and 55 fmol/mg globin, respectively, following the first exposure period to 464 and 316 fmol/mg globin, respectively, following the last exposure period. Based on total amount administered, formation of acrylamideVal after dermal exposure was much lower than after oral administration (4.9 vs. 74.7 nmol/g globin/mmol acrylamide/kg). Approximately 4.5% of the administered dose was recovered as urinary metabolites through day 4. Dermal exposure also resulted in much lower formation of glycidamideVal (9.7% of that formed following oral exposure).

Animal studies confirm that acrylamide is readily absorbed following dermal exposure (Ramsey et al. 1984; Sumner et al. 2003). In male F344 rats, an average of 22% of an occluded dermal dose of 162 mg/kg [2,3-^{14}C]-labeled acrylamide was absorbed during a 24-hour exposure period (Sumner et al. 2003). Following dermal administration of [1,3-^{14}C]-labeled acrylamide to other F344 rats, peak plasma concentrations of acrylamide occurred at approximately 2 and 5 hours postadministration, respectively (Ramsey et al. 1984). The peak concentration at the high dose was about 20-fold higher than that of the low dose.

Results of an *in vitro* study describe dermal absorption of acrylamide. Marty and Vincent (1998) applied [^{14}C]-labeled acrylamide (in an aqueous gel of 2% polyacrylamide) to biopsied human abdominal skin for 24 hours at acrylamide concentrations of 1.28 or 2 ppm. Approximately 28 and 21% of the applied doses, respectively, was recovered in the receptor fluid. Between 1.6 and 3.4% of applied doses was recovered in dermis and epidermis. The authors estimated total absorption of acrylamide to be 33.2 and 26.7% at low and high concentration, respectively, based on radioactivity recovered from the receptor phase, epidermis, and dermis.

3.4.2 Distribution

Results from several animal studies indicate that, following absorption, radioactivity from radiolabeled acrylamide is widely distributed with no specific accumulation in any tissues other than red blood cells (Barber et al. 2001a; Crofton et al. 1996; Edwards 1975; Hashimoto and Aldridge 1970; Ikeda et al. 1985; Kadry et al. 1999; Marlowe et al. 1986; Miller et al. 1982; Ramsey et al. 1984) and late-staged spermatids (Sega et al. 1989). Results of intravenous injection and gavage studies in pregnant animals indicate that acrylamide and/or its metabolites readily cross the placenta and are distributed within the developing fetus in a manner similar to that of the pregnant mother (Ferguson et al. 2010; Ikeda et al. 1983, 1985; Marlowe et al. 1986). In a recent study designed to assess hemoglobin adduct levels of acrylamide in blood

samples of pregnant mothers and umbilical cord blood of neonates, the concentration in umbilical cord blood was approximately 50% of that found in the blood of the mother, indicating that acrylamide readily passes from mother to developing fetus (Schettgen et al. 2004a).

3.4.2.1 Inhalation Exposure

Immediately following a 6-hour inhalation exposure of male F344 rats to 3 ppm of [^{14}C]/[^{13}C]-acrylamide vapor, the rank of acrylamide equivalent concentrations was: blood cells (~7 µg/g) > testes, skin, liver, and kidneys (~6 µg/g) > brain, spleen, lung, and epididymis (~4 µg/g) (Sumner et al. 2003). Acrylamide equivalent concentrations in similarly-treated male B6C3F1 mice were: testes (~14 µg/g) > skin and liver (~11 µg/g) > epididymis (~8 µg/g) > brain (~7 µg/g) > lung and blood (~6 µg/g) > fat (~5 µg/g) (Sumner et al. 2003).

3.4.2.2 Oral Exposure

Following 13 daily oral doses of [1,3-^{14}C]-labeled acrylamide at 0.05 or 30 mg/kg/day, tissue concentrations of acrylamide in male F344 rats were similar among tissues with the exception of red blood cells, which showed higher concentrations (Ramsey et al. 1984), presumably due to the hemoglobin binding of acrylamide to cysteine and the formation of acrylamideVal and/or glycidamideVal hemoglobin adducts. Mean concentrations (µg equivalents [^{14}C]-acrylamide per gram of tissue) at the high dose were 383.7 µg/g in red blood cells, 87.74 µg/g in liver, 70.43 µg/g in kidneys, 70.60 µg/g in epididymis, 67.14 µg/g in testis, 54.00 µg/g in sciatic nerve, 53.52 µg/g in brain, 47.56 µg/g in carcass, 39.11 µg/g in skin, and 16.45 µg/g in plasma. At the low dose, the mean concentration in red blood cells was 1.26 µg/g (approximately 61% of the total recovered dose) compared with a range of 0.07–0.13 µg/g in other tissues.

In Sprague-Dawley rats given single 50 mg/kg oral doses of [1-^{14}C]-labeled acrylamide, tissue concentrations of radioactivity at 28 and 144 hours postadministration were indicative of wide distribution of acrylamide metabolites among tissues with no evidence for accumulation in toxicity targets (Kadry et al. 1999). These results suggest the binding of acrylamide in the absence of its accumulation in erythrocytes or neural tissue. At 28 hours, brain, thyroid, testes, adrenal, pancreas, thymus, liver, kidney, heart, and spleen showed a narrow range of mean concentrations, approximately 0.05–0.10% of the initial dose/g. Higher concentrations were noted in the skin, bone marrow, stomach, and lung (0.15–0.18% of the initial dose/g); only the gastric contents showed a markedly higher concentration (1.37% of the initial dose/g. At 144 hours after administration, tissue concentrations were uniformly low for tissues including

the gastric contents, ranging from 0.01 to 0.05% of the initial dose/g, with the exception of skin, bone marrow, and lung, which exhibited mean concentrations of 0.06, 0.08, and 0.19% of the initial dose/g, respectively.

3.4.2.3 Dermal Exposure

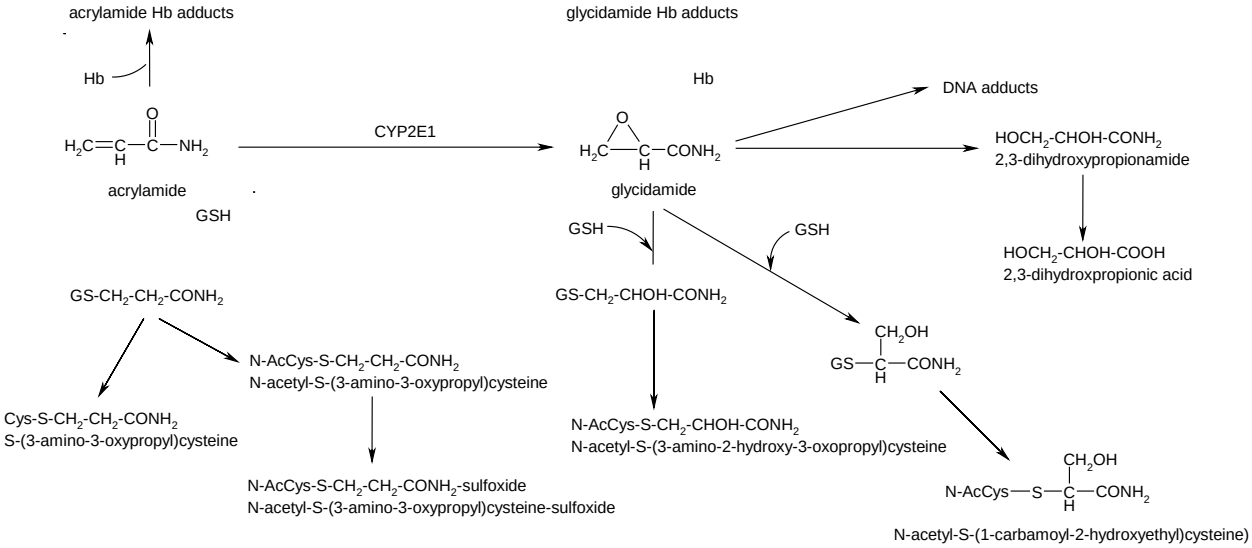
Following 24-hour dermal exposure of male F344 rats to 162 mg/kg [^{14}C]-labeled acrylamide, the highest concentration of acrylamide equivalents (excluding skin at the application site) was found in blood cells (71 µg/g), followed by skin at nondosing sites (~28 µg/g); liver, spleen, testes, and kidneys (~21 µg/g); lungs, thymus, brain, and epididymis (~14 µg/g); and fat (<4 µg/g) (Sumner et al. 2003).

3.4.3 Metabolism

Results from rat and mouse studies indicate that acrylamide is rapidly metabolized and excreted predominantly in the urine as metabolites (Dixit et al. 1982; Edwards 1975; Miller et al. 1982; Ramsey et al. 1984; Sumner et al. 1992, 1999, 2003; Twaddle et al. 2004). Figure 3-3 depicts a metabolic scheme for acrylamide adapted from reports of Calleman (1996), IARC (1994), and Sumner et al. (1992, 1999). According to the metabolic scheme, acrylamide reacts readily with glutathione to form a glutathione conjugate, which is further metabolized to N-acetyl-S-(3-amino-3-oxopropyl)cysteine or S-(3-amino-3-oxopropyl)cysteine. Another initial step, catalyzed by CYP2E1, involves oxidation of acrylamide to the epoxide derivative, glycidamide. Glycidamide can react with glutathione to form conjugates that are further metabolized to N-acetyl-S-(3-amino-2-hydroxy-3-oxopropyl)cysteine or N-acetyl-S-(1-carbamoyl-2-hydroxyethyl)cysteine. Glycidamide may also undergo hydrolysis, perhaps catalyzed by epoxide hydrolases, leading to the formation of 2,3-dihydroxypropionamide and 2,3-dihydroxypropionic acid.

Both acrylamide and glycidamide react with nucleophilic sites in macromolecules (including hemoglobin and DNA) in Michael-type additions (Bergmark et al. 1991, 1993; Segerbäck et al. 1995; Solomon et al. 1985). In rats, the binding index of acrylamide to cysteine in hemoglobin was extremely high (6,400 pmol/g Hb/µmol acrylamide/kg); the binding index of glycidamide to cysteine was 1,820 pmol/g Hb/µmol glycidamide/kg. The lower binding index for glycidamide may be the result of lower reactivity toward hemoglobin-cysteine and shorter half-life in blood. Rate constants of 0.0054 and 0.021 $M^{-1}s^{-1}$ for reactions of acrylamide with human serum albumin and glutathione, respectively, were reported by Tong et al. (2004), suggesting that these reactions account for most of the elimination of absorbed acrylamide.

Figure 3-3. Metabolic Scheme for Acrylamide and Its Metabolite Glycidamide*



*Processes involving several steps are represented with broken arrows.

GSH = reduced glutathione; Hb = hemoglobin; N-AcCys = N-acetylcysteine

Sources: Calleman 1996; Fennell et al. 2006; IARC 1994; Sumner et al. 1992, 1999

Doerge et al. (2005a) measured DNA adducts following a single intraperitoneal administration of acrylamide to adult B6C3F1 mice and F344 rats at 50 mg/kg. Glycidamide-derived DNA adducts of adenine and guanine formed in all tissues examined, including target tissues identified in rodent carcinogenicity bioassays and nontarget tissues. Dosing rats and mice with an equimolar amount of glycidamide (61 mg/kg) typically produced higher levels of DNA adducts than those observed from the acrylamide dose. Gamboa da Costa et al. (2003) measured DNA adduct formation in selected tissues of adult and whole body DNA of 3-day-old neonatal mice treated with acrylamide and glycidamide. In adult mice, DNA adduct formation was observed in liver, lung, and kidney; glycidamide treatment produced modestly higher adduct levels than acrylamide treatment. However, glycidamide-treated neonates exhibited 5–7-fold higher whole-body DNA adduct levels than the acrylamide-treated neonates.

Results from studies of CYP2E1 null and wild-type mice demonstrate the importance of CYP2E1 in catalyzing the oxidative formation of glycidamide from acrylamide. Following oral administration of single 50 mg/kg doses of [1,2,3-^{13}C]-acrylamide to wild-type mice, considerable amounts of metabolites derived from glycidamide were found in the 24-hour urine (Sumner et al. 1999). Approximately 22% of the excreted metabolites were derived from glutathione conjugation with glycidamide (N-acetyl-S-[3-amino-2-hydroxy-3-oxopropyl]cysteine and N-acetyl-S-[1-carbamoyl-2-hydroxyethyl]cysteine) and 28% were derived from glycidamide and its hydrolysis products (2,3-dihydroxypropionamide and 2,3-dihydroxypropionic acid). In contrast, no evidence was found for the formation of these metabolites in the 24-hour urine of CYP2E1 null mice or wild-type mice treated with the CYP2E1 inhibitor, aminobenzotriazole. The wild-type and CYP2E1-null mice excreted a similar percentage of the administered dose in the urine within 24 hours (about 30%), suggesting that the CYP2E1-null mice compensated for the CYP2E1 deficiency by metabolizing more of the administered acrylamide via direct conjugation with glutathione.

Ghanayem et al. (2005c) investigated the formation of hemoglobin adducts of acrylamide and glycidamide and DNA adducts of glycidamide in wild-type and CYP2E1-null mice following the administration of a single 50 mg/kg dose of acrylamide via intraperitoneal injection. At 6 hours posttreatment, mean plasma levels of acrylamide and glycidamide were 0.84 and 33.0 µM, respectively, in the wild-type mice and 115 and 1.7 µM, respectively, in the CYP2E1-null mice. Levels of hemoglobin adducts of acrylamide were approximately 2-fold higher in the CYP2E1-null mice compared to the wild-type mice. Levels of hemoglobin adducts of glycidamide were approximately 33-fold lower in CYP2E1-null mice compared to the wild type mice. Although only traces of DNA adducts of glycidamide were seen in the CYP2E1-null mice, levels were 52–66 times higher in the wild type mice. These results

demonstrate the importance of CYP2E1 in the epoxidation of acrylamide to glycidamde and the formation of hemoglobin adducts and glycidamide-DNA adducts.

N-Acetyl-S-(3-amino-3-oxopropyl)cysteine has been identified as the major urinary metabolite of acrylamide in male F344 rats exposed to oral doses of 1–100 mg/kg [2,3-^{14}C]-labeled acrylamide (Miller et al. 1982) and in male F344 rats and B6C3F1 mice exposed to oral doses of 50 mg/kg [1,2,3-^{13}C]-acrylamide (Sumner et al. 1992).

Following oral administration of [1,2,3-^{13}C]-acrylamide (50 mg/kg) to rats and mice, glycidamide and glycidamide-derived metabolites accounted for about 33% (rats) and 59% (mice) of the total metabolites excreted in the urine within 24 hours, indicating that acrylamide is transformed to glycidamide to a greater extent in mice than in rats (Sumner et al. 1992). Similar results were reported in a study of metabolites in urine collected for 24 hours after 6-hour inhalation exposure (nose only) of rats and mice to 3 ppm [^{14}C]/[^{13}C]-acrylamide where glycidamide and glycidamide-derived metabolites accounted for 36% (rats) and 73% (mice) of total metabolites excreted in the urine within 24 hours (Sumner et al. 2003). Following dermal application of 138 mg/kg [1,2,3-^{13}C]-acrylamide, male F344 rats excreted approximately 2% of the applied dose in the 24-hour urine as GSH-acrylamide derived metabolites (~50% of the urinary metabolites), glycidamide (~17%), and GSH-glycidamide derived metabolites (~31%) (Sumner et al. 2003).

Figure 3-3 does not include a possible minor pathway in which CO_2 may be released from the hydrolysis products of glycidamide, because of conflicting results from several studies. Following intravenous administration of 100 mg/kg [1-^{14}C]-labeled acrylamide to male albino Porton rats, about 6% of the injected dose of radioactivity was exhaled as CO_2 in 8 hours (Hashimoto and Aldridge 1970), but following administration of [2,3-^{14}C]-labeled acrylamide to male Fischer 344 rats, no radioactivity was detected in exhaled breath (Miller et al. 1982). Sumner et al. (1992) hypothesized that these results may be consistent with the existence of a minor pathway involving metabolism of 2,3-dihydroxypropionamide to glycerate and hydroxypyruvate with the subsequent release of CO_2 and production of glycoaldehyde, but they did not detect two-carbon metabolites in urine of mice exposed to [1,2,3-^{13}C]-acrylamide. In other experiments, no exhaled $^{14}CO_2$ was detected following 50 mg/kg oral dosing of [1-^{14}C]-labeled acrylamide to male Sprague-Dawley rats (Kadry et al. 1999), whereas 3–4% of intravenously injected [1,3-^{14}C]-acrylamide (2 or 100 mg/kg) was detected as $^{14}CO_2$ in exhaled breath of male Fischer 344 rats (Ramsey et al. 1984).

Species differences in acrylamide metabolism are apparent. Twaddle et al. (2004) administered acrylamide at approximately 50 mg/kg via gavage to adult male and female B6C3F1 mice. Serum concentrations of acrylamide and glycidamide were taken at 0.5, 1, 2, 4, and 8 hours postdosing. Livers were removed from control and acrylamide-treated mice and analyzed for glycidamide-derived DNA adducts. The results indicated no systematic sex differences in acrylamide or glycidamide serum levels at each time point. Twaddle et al. (2004) estimated an acrylamide half-life of elimination from plasma at 0.73 hours. This value in mice can be compared to an estimate of 2 hours in F344 rats following a subchronic oral administration of 2.8 mM acrylamide in drinking water for 34 days or subacute intraperitoneal doses at 50 mg/kg/day for 11 days (Barber et al. 2001). Miller et al. (1982) estimated a 1.7-hour half-life for acrylamide in rat blood following a 10 mg/kg intravenous dose. Twaddle et al. (2004) reported an elimination half-life of 1.9 hours for the acrylamide-treated mice, which is identical to that measured by Barber et al. (2001) in rats. Barber et al. (2001) also reported a glycidamide/ acrylamide-areas under the curve (AUC) ratio of 0.18 for Sprague-Dawley rats treated with 20 mg/kg acrylamide by gavage. This contrasts to the observation of equal AUCs for glycidamide and acrylamide in B6C3F1 mice (Twaddle et al. 2004). Since rats and mice had a comparable glycidamide elimination half-life, this approximately 5-fold difference in internal exposure to glycidamide for mice compared with rats is considered to be the result of an increased rate of glycidamide formation in the mouse. Based on quantitation of metabolites in the urine of acrylamide-treated rats and mice, Sumner et al. (1992) estimated that acrylamide is converted to glycidamide to a greater extent in the mouse (59%) than in the rat (33%).

The metabolism of acrylamide has also been investigated in controlled human studies (Boettcher et al. 2006a; Fennell et al. 2005b, 2006; Fuhr et al. 2006).

Fennell and coworkers evaluated metabolism and hemoglobin adduct formation (Fennell et al. 2005b) and kinetics of elimination of urinary metabolites (Fennell et al. 2006) following oral and dermal administration of [1,2,3-$^{13}C_3$)-acrylamide and/or [2,3-^{14}C]-acrylamide to 24 adult male volunteers. Metabolism of the administered acrylamide was investigated by ^{13}C nuclear magnetic resonance (NMR) spectroscopy (Fennell et al. 2005b) and by liquid chromatography-tandem mass spectroscopy (Fennell et al. 2006). Urinary metabolites accounted for approximately 40% of a 3 mg/kg oral dose of acrylamide and 4.5% of a 3 mg/kg/day repeated (3 consecutive days) dermal dose (Fennell et al. 2006). Approximately 86% of the recovered urinary metabolites were derived from glutathione conjugation and excreted as N-acetyl-S-(3-amino-3-oxopropyl)cysteine and its S-oxide. Glycidamide, glyceramide (2,3-dihydroxypropionamide), and low levels of N-acetyl-S-(3-amino-2-hydroxy-3-oxopropyl)cysteine

were also detected in urine (Fennell et al. 2005b). On oral administration, a linear dose response was observed for acrylamideVal and glycidamideVal in hemoglobin. The main pathway of metabolism in humans was via direct glutathione conjugation, forming N-acetyl-S-(3-amino-3-oxopropyl)cysteine and its S-oxide, which has not been reported previously (Fennell et al. 2006). Epoxidation to glycidamide was the other important pathway, with glyceramide formed as a major metabolite in humans. Glycidamide was detected in low amounts. The glutathione conjugation of glycidamide, which is a major pathway in rodents, appeared to occur at very low levels in humans. Metabolism via glycidamide in humans was approximately 12% of the total urinary metabolites. This is considerably lower than the amount of glycidamide-derived metabolites reported for oral administration of acrylamide in rats (28% at 50 mg/kg, [Sumner et al. 2003]) and in mice (59% at 50 mg/kg [Sumner et al. 1992]). It should be noted that glyceramide has only been quantitated in human urine by NMR spectroscopy in combination with administration of [1,2,3-$^{13}C_3$]-acrylamide.

The Fennell et al. (2005b) study also provided data on the amount of hemoglobin adducts derived from acrylamide and glycidamide following administration of a defined dose of acrylamide to adult male volunteers. Both acrylamideVal and glycidamideVal increased linearly with increasing dose of acrylamide administered orally, suggesting that saturation of the conversion of acrylamide to glycidamide is not reached in the dosing range of 0.5–3.0 mg/kg. The ratio of glycidamideVal:acrylamideVal produced by administration of acrylamide was similar to the ratio of the background adducts prior to exposure. Compared with the equivalent oral administration in rats (3 mg/kg), the ratio of glycidamideVal:acrylamideVal was lower in humans (0.44 compared to 0.84 for the rat), and the absolute amounts of acrylamideVal and glycidamideVal formed were approximately 2.7- and 1.4-fold higher, respectively, in humans compared to rats.

Fennell et al. (2005b) calculated the expected amount of adduct that would accumulate in men from continuous exposure based on the amount of adduct formed/day of exposure, and from the lifespan of the erythrocyte. Oral intake of 1 µg/kg acrylamide/day (1.05 fmol acrylamideVal/mg globin/day) for the lifespan of the erythrocyte (120 days) was estimated to result in the accumulation of adducts to 63 fmol/mg globin. Daily dermal exposure to 1 µg/kg acrylamide (0.18 fmol acrylamideVal/mg globin/day) for the lifespan of the erythrocyte (120 days) would result in the accumulation of adducts to 10.8 fmol acrylamideVal/mg globin. With workplace exposure of 5 days/week, this would decrease to approximately 7.8 fmol acrylamideVal/mg globin.

Some investigators have reported on formation of AAMA and GAMA in humans following controlled administration of acrylamide (Boettcher et al. 2006a; Fuhr et al. 2006). However, it should be noted that GAMA and AAMA sulfoxide metabolites are isomeric, which means that the formation of GAMA may be overestimated since the sulfoxide can account for a major portion of the metabolism of acrylamide. Boettcher et al. (2006a) investigated the human metabolism of acrylamide to AAMA and GAMA in a healthy male volunteer who received a single dose of about 1 mg deuterium-labeled (d(3)) acrylamide, representing 13 μg/kg body weight, in drinking water. Urine samples before dosing and within 46 hours after the dose were analyzed for d(3)-AAMA and d(3)-GAMA using liquid chromatography-mass spectrometry (LC-MS). Total recovery in urine after 24 hours was about 51% as the sum of AAMA and GAMA and was similar to recoveries in rats (53–66%) given a gavage dose of 0.1 mg/kg (Doerge et al. 2007). After 2 days, AAMA accounted for 52% of the total acrylamide dose, and was the major metabolite of acrylamide in humans. GAMA accounted for 5%, and appeared as a minor metabolite of acrylamide. A urinary ratio of 0.1 was observed for GAMA/AAMA compared to previously reported values of 0.2 for rats and 0.5 for mice (Doerge et al. 2005b, 2005c). The authors concluded that the metabolic fate of acrylamide in humans was more similar to that in rats than in mice as previously demonstrated in terms of hemoglobin adducts. Fuhr et al. (2006) evaluated the urinary levels of acrylamide, AAMA, glycidamide, and GAMA (using LC-MS) in six young healthy volunteers after the consumption of a meal containing 0.94 mg of acrylamide. Urine was collected up to 72 hours thereafter. No glycidamide was found. Unchanged acrylamide, AAMA, and GAMA accounted for urinary excretion of approximately 4.5, 50, and 6% of the dose, respectively. Conjugation with glutathione exceeded the formation of the reactive metabolite glycidamide. The data suggest 2- and 4-fold lower relative internal exposures for glycidamide from dietary acrylamide in humans compared with rats and mice, respectively.

Hemoglobin adducts of acrylamide and glycidamide and urinary metabolites have been used as biomarkers of exposure to acrylamide (Bergmark 1997; Bergmark et al. 1993; Boettcher et al. 2005; Calleman et al. 1994). Refer to Section 3.8. for detailed information regarding biomarkers of exposure to acrylamide.

3.4.4 Elimination and Excretion

Available human and animal data indicate that urinary excretion of acrylamide metabolites is the primary route of elimination of absorbed acrylamide (Barber et al. 2001a; Boettcher et al. 2006a; Doerge et al. 2007; Fennell et al. 2005b, 2006; Fuhr et al. 2006; Hashimoto and Aldridge 1970; Kadry et al. 1999; Miller et al. 1982; Ramsey et al. 1984; Sumner et al. 1992, 1999, 2003).

3.4.4.1 Inhalation Exposure

No human data were located regarding excretion and elimination following inhalation exposure to acrylamide. Sumner et al. (2003) exposed male F344 rats and B6C3F1 mice nose-only to a mixture of [1,2,3-^{13}C]-acrylamide (90%) and [2,3-^{14}C]-acrylamide (10%) vapors at an average analytical concentration of 1.17 ppm for 6 hours. During a 24-hour postexposure period, the distribution of the inhaled dose in the rats was: 31% in the urine, 56% retained in the body, 3% in the feces, and 2% as exhaled organic volatiles and $^{14}CO_2$. The distribution of the inhaled dose in the mice was: 27% in the urine, 46% in tissues, 5% in feces, 2% as organic volatiles, and 1% as$^{14}CO_2$.

3.4.4.2 Oral Exposure

Approximately 34% of an orally-administered 3 mg/kg dose of acrylamide to adult male volunteers was recovered in the total urinary metabolites within 24 hours of administration (Fennell et al. 2005b). In a male volunteer who received a single dose of about 1 mg deuterium-labeled acrylamide (representing 13 µg/kg body weight) in drinking water, elimination via urinary AAMA and GAMA followed a two-phase pattern (Boettcher et al. 2006a). Elimination half-lives of both AAMA and GAMA were estimated to be approximately 3.5 hours for the first phase and >10 hours up to few days for the second phase. After 2 days, urinary AAMA and GAMA accounted for 52 and 5%, respectively, of the total acrylamide dose. The ratio GAMA/AAMA was approximately 0.2.

Fuhr et al. (2006) measured acrylamide and metabolite levels in a 72-hour urine collection from six young healthy volunteers after the consumption of a meal containing 0.94 mg of acrylamide. Unchanged acrylamide, AAMA, and GAMA accounted for urinary excretion of approximately 4, 50, and 10%, respectively, of the administered dose; glycidamide was not detected. Estimated elimination half-lives for the unchanged acrylamide, AAMA, and GAMA were 2.4, 17.4, and 25.1 hours, respectively.

Heudorf et al. (2009) reported median levels of 36 µg AAMA/L and 13 µg GAMA/L in the urine of 110 children (63 boys and 47 girls; 5–6 years of age). Children who reported higher regular consumption of French fries had significantly higher urinary levels of acrylamide metabolites. Based on the urinary levels of AAMA and GAMA, mean estimated acrylamide dietary intakes were 1.13 µg/kg/day (creatinine excretion based model) and 0.81 µg/kg/day (volume based model). The ratio GAMA/AAMA was approximately 0.4, which is 2-fold higher than that observed by Boettcher et al. (2006a) in adults. Based

on this finding, Heudorf et al. (2009) suggest that acrylamide may undergo oxidative metabolism to a greater extent in children than adults.

In male Fischer 344 rats given oral (1, 10, or 100 mg/kg) doses of [2,3-^{14}C]-acrylamide, about 60 and 70% of the administered radioactivity was excreted in urine collected within 24 hours and 7 days, respectively (Miller et al. 1982). Less than 2% of radioactivity in the urine was accounted for by acrylamide. Elimination of radioactivity from tissues was biphasic with half-lives of about 5 and 8 hours, respectively. The elimination time course of parent compound from tissues exhibited single phase exponential elimination with a half-life of about 2 hours. Fecal excretion accounted for 4.8 and 6% of administered radioactivity at 24 hours and 7 days, respectively. Bile-duct cannulated rats given single intravenous 10 mg/kg doses of [2,3-^{14}C]-acrylamide excreted about 15% of the administered radioactivity as metabolites within about 6 hours; <1% of radioactivity in the bile was in the form of acrylamide (Miller at al. 1982). These results are consistent with the existence of enterohepatic circulation of metabolites.

Doerge et al. (2007) administered acrylamide to male and female rats and mice at 0.1 mg/kg by oral gavage or via the diet and measured the percentages of parent compound and metabolites in the 24-hour urine. For rats and mice combined, the percentage of total dose excreted as the sum of acrylamide and its metabolites (glycidamide, AAMA, and GAMA) averaged 49%. Excretion of glycidamide and glycidamide-derived species was typically greater in mice than rats.

No radiolabeled CO_2 was captured from rats given [2,3-^{14}C]-acrylamide orally (Kadry et al. 1999; Miller et al. 1982). However, intravenous injection of rats with a 100 mg/kg dose of [1-^{14}C]-labeled acrylamide (Hashimoto and Aldridge 1970) or a 2 mg/kg dose of [1,3-^{14}C]-labeled acrylamide (Ramsey et al. 1984) resulted in the capture of about 6 and 4%, respectively, of the radioactivity as CO_2 in the expired air during the subsequent 6–8 hours.

3.4.4.3 Dermal Exposure

Fennell et al. (2006) exposed 24 adult male volunteers to dermal applications of [1,2,3-$^{13}C_3$]-acrylamide under controlled conditions. Acrylamide was administered as three daily 24-hour occluded dermal doses of 3 mg/kg. Approximately 4.5% of the administered dose (12.35% of the absorbed dose) was recovered as urinary metabolites through day 4. Urinary recovery included $^{13}C_3$-acrylamide (~4% of total urinary recovery) and metabolites $^{13}C_3$-cysteine-S-propionamide ($^{13}C_3$-CP; ~0.8%), $^{13}C_3$-N-acetyl cysteine-

S-propionamide ($^{13}C_3$-NACP; ~70%), $^{13}C_3$-NACP sulfoxide (~22.5%), $^{13}C_3$-GAMA3 (~2.6%), and $^{13}C_3$-GAMA2 (~0.4%). Glycidamide was not detected in the majority of the urine samples. $^{13}C_3$-Acrylamide and $^{13}C_3$-CP were detected in 2–4 hour urine samples collected on the first day. $^{13}C_3$-NACP and its sulfoxide were first detectable in the urine between 4 and 8 hours posttreatment. The mercapturic acids of glycidamide ($^{13}C_3$-GAMA2 and $^{13}C_3$-GAMA2) were first detectable in the urine between 8 and 16 hours posttreatment. The metabolite, glycidamide, was not detectable in the majority of the urine samples collected over the 4-day period.

Sumner et al. (2003) exposed male F344 rats to [2,3-^{14}C]-labeled acrylamide under occluded dermal conditions for 24 hours. During a 24-hour postexposure period, approximately 8% of the applied dose (36% of the absorbed dose) was excreted in the urine, 3% as volatiles and $^{14}CO_2$ in exhaled air, <1% in feces, and53% remained in tissues.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

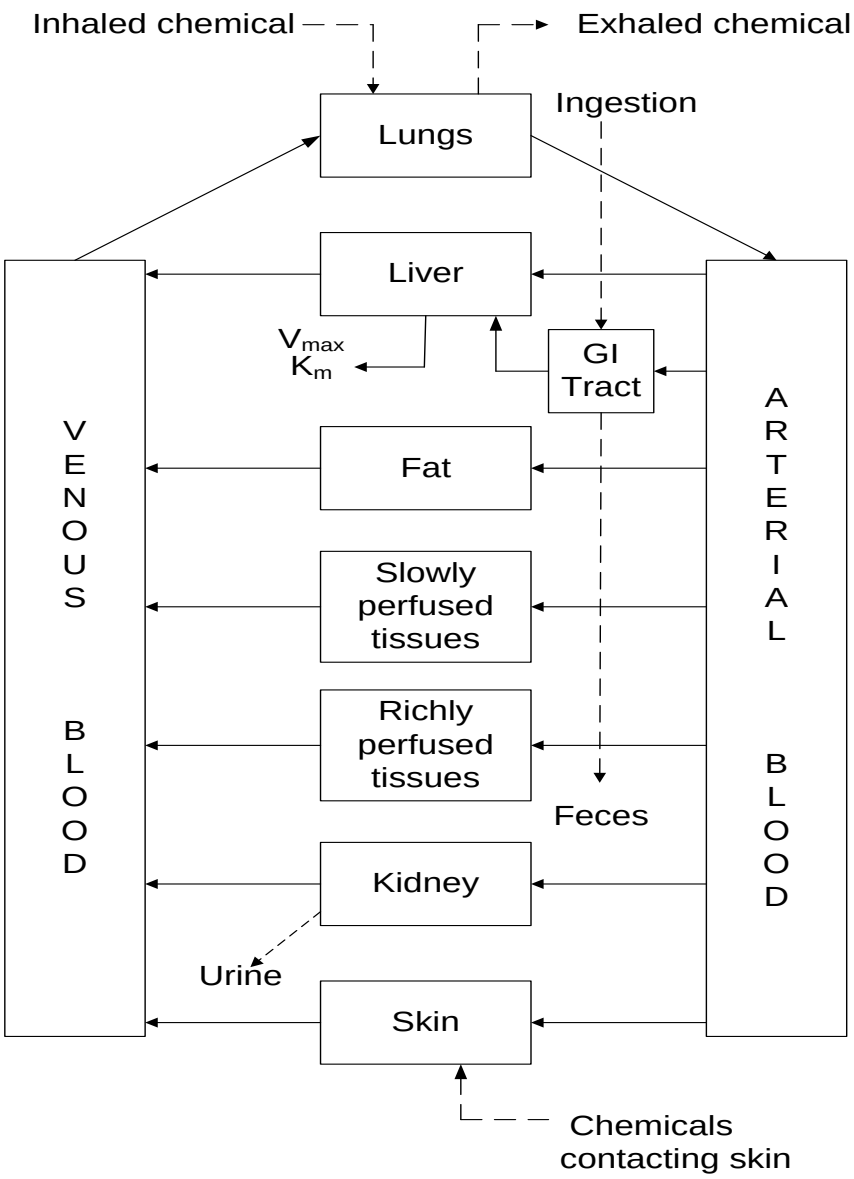
The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

If PBPK models for acrylamide exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

Physiologically based pharmacokinetic (PBPK) models for acrylamide are available (Calleman et al. 1992, 1993; Kirman et al. 2003; Sweeney et al., 2010; Walker et al. 2007; Young et al. 2007).

Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: adapted from Krishnan and Andersen 1994

Calleman et al. (1992, 1993) Model

Calleman et al. (1992, 1993) developed a nonlinear dosimetric model to simultaneously determine hemoglobin adduct formation by acrylamide and its genotoxic metabolite, glycidamide, in rats. The model is not useful for human health risk assessment.

Kirman et al. (2003) Model

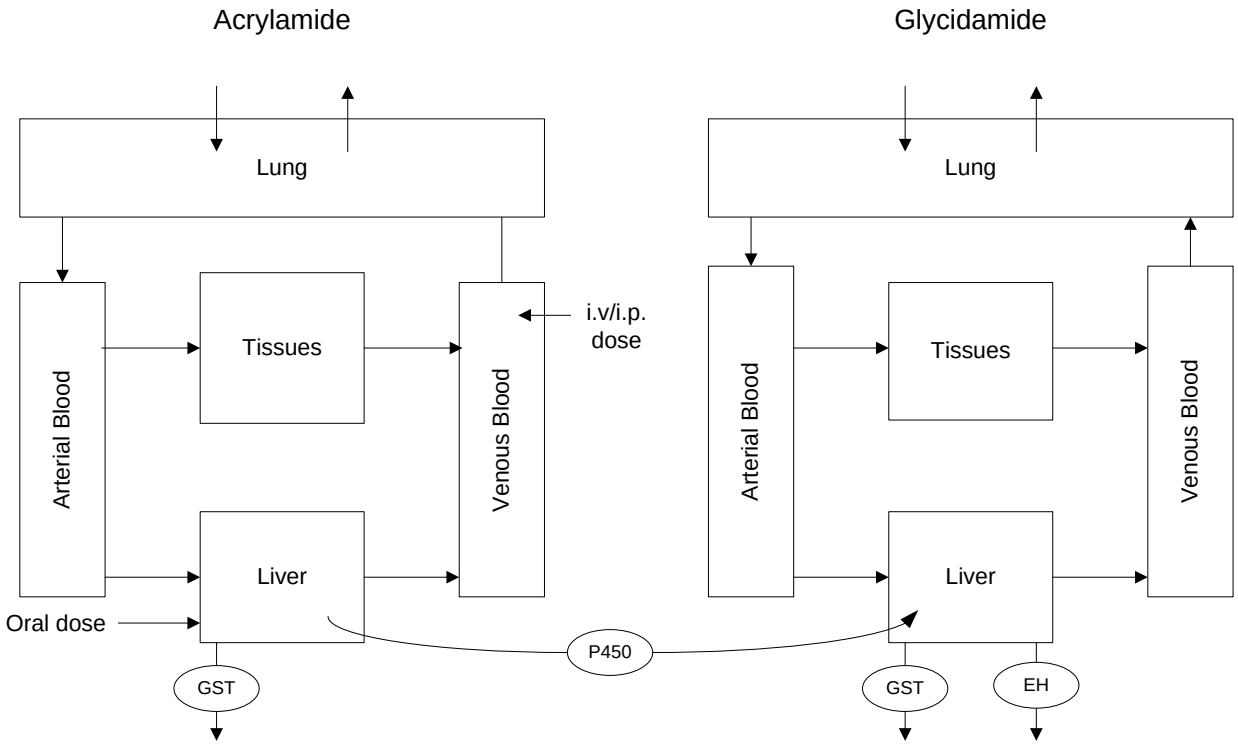
Kirman et al. (2003) developed a PBPK model (Figure 3-5) to predict the behavior of acrylamide and its epoxide metabolite, glycidamide, in the rat for intravenously-, intraperitoneally-, or orally-administered acrylamide. The model includes components for both acrylamide and glycidamide, each consisting of five compartments (arterial blood, venous blood, liver, lung, and all other tissues). The acrylamide component is linked to the glycidamide portion of the model via metabolism in the liver. Model parameters are listed in Table 3-11. Rat physiological parameters were selected from measured data (Brown et al. 1997; Kedderis et al. 1996). Partition coefficients for acrylamide were estimated based on an algorithm derived by Poulin and Krishnan (1995, 1996a, 1996b) using specific chemical properties. Partition coefficients for glycidamide were derived by application of a proportionality constant of 3.2 to the values calculated for acrylamide (Kedderis et al. 1996). Metabolism of acrylamide and glycidamide are represented only in the liver. Estimated values for metabolism and tissue binding were based on fitting of the model to metabolism and urinary elimination data from Miller et al. (1982), Ramsey et al. (1984), Raymer et al. (1993), and Sumner at al. (1992). Depletion and resynthesis of glutathione were included in the model structure (D'Souza et al. 1988).

The PBPK model of Kirman et al. (2003) was developed to predict the behavior of acrylamide and glycidamide in the rat. The model has only been partially validated in rats and is not useful for human risk assessment.

Sweeney et al. (2010) Model

Sweeney et al. (2010) extended the Kirman et al. (2003) model to include the following functionality: (1) separate compartments representing brain, fat, liver, kidney, slowly perfused tissues, and other richly perfused tissues; (2) production and urinary excretion of AAMA, GAMA, and glycidamide; (3) a human model; and (4) calibration and evaluation of the model based on newer data not available at the time the Kirman et al. (2003) model was developed. The structure of the Sweeney et al. (2010) is shown in

Figure 3-5. Schematic of the Kirman et al. (2003) Physiologically Based Pharmacokinetic (PBPK) Model for Acrylamide



EH = epoxide hydrolase; GST = glutathione *S*-transferase; i.p. = intraperitoneal injection; i.v. = intravenous injection;

Source: Kirman et al. 2003

Table 3-11. Original Model Parameter Values for Rats in the Kirman et al. (2003) Physiologically Based Pharmacokinetic Model

Parameter group	Parameter	Symbol (units)	Value	Reference/source
Rat physiology	Body weight	BW (kg)	Study specific	Study specific
	Cardiac output	QCC (L/hour-kg)	14	Kedderis et al. 1996
	Alveolar ventilation	QPC (L/hour-kg)	14	Kedderis et al. 1996
	Liver blood flow	QLC (fraction QCC)	0.25	Kedderis et al. 1996
	Fraction arterial	FABC (fraction VB)	0.35	Kedderis et al. 1996
	Fraction venous	FVBC (fraction VB)	0.65	Kedderis et al. 1996
	Liver volume	VLC (fraction BW)	0.04	Brown et al. 1997
	Tissue volume	VTC (fraction BW)	0.87	Calculated (0.91-VLC)
	Tissue blood flow	QTC (fraction QCC)	0.75	Calculated (1-QLC)
	Volume blood	VBC (fraction BW)	0.06	Brown et al. 1997
	Hemoglobin concentration	HGB (g/L)	1.5	Kedderis et al. 1996
	Fraction blood cells	FBC (fraction VB)	0.44	Kedderis et al. 1996
	Fraction blood serum	FBS (fraction VB)	0.56	Kedderis et al. 1996
Absorption	Absorption rate from gastrointestinal tract (oral dose) or intraperitoneal cavity (intraperitoneal dose)	KA (/hour)	5	Model simulations fit to Miller et al. 1982; Ramsey et al. 1984; Raymer et al. 1993
	Infusion time (intravenous dose)	TINF (hour)	0.003	Model simulations fit to Miller et al. 1982; Ramsey et al. 1984; Raymer et al. 1993

Table 3-11. Original Model Parameter Values for Rats in the Kirman et al. (2003) Physiologically Based Pharmacokinetic Model

Parameter group	Parameter	Symbol (units)	Value	Reference/source
Partition coefficients	Blood:air, AA	PB1 (unitless)	31,000,000	Estimated
	Liver:blood, AA	PL1 (unitless)	0.83	Poulin and Krishnan 1995, 1996a, 1996b
	Tissue:blood, AA	PL1	0.95	Poulin and Krishnan 1995, 1996a, 1996b
	Blood:air, GA	PB2 (unitless)	98,000,000	Poulin and Krishnan 1995, 1996a, 1996b
	Liver:blood, GA	PL2 (unitless)	2.7	Poulin and Krishnan 1995, 1996a, 1996b
	Tissue:blood, GA	PT2 (unitless)	3.0	Poulin and Krishnan 1995, 1996a, 1996b
Metabolism	Cytochrome P-450 oxidation rate, AA	VMAXC1 (mg/hour-kg)	1.6	Model simulations fit to Miller et al. 1982; Raymer et al. 1993; Sumner et al. 1992
	Cytochrome P-450, Michaelis-Menten constant, AA	KMC1 (mg/L)	10	Model simulations fit to Miller et al. 1982; Raymer et al. 1993; Sumner et al. 1992
	Epoxide hydrolase hydrolysis rate, GA	VMAXC2 (mg/hour-kg)	1.9	Model simulations fit to Miller et al. 1982; Raymer et al. 1993; Sumner et al. 1992
	Epoxide hydrolase, Michaelis-Menten constant, GA	KMC2 (mg/L)	100	Model simulations fit to Miller et al. 1982; Raymer et al. 1993; Sumner et al. 1992
	Reaction with glutathione, AA	KGSTC1 (L/mmol GSH-hr)	0.55	Model simulations fit to Miller et al. 1982; Raymer et al. 1993; Sumner et al. 1992
	Reaction with glutathione, GA	KGSTC2 (L/mmol GSH-hour)	0.8	Model simulations fit to Miller et al. 1982; Raymer et al. 1993; Sumner et al. 1992

Table 3-11. Original Model Parameter Values for Rats in the Kirman et al. (2003) Physiologically Based Pharmacokinetic Model

Parameter group	Parameter	Symbol (units)	Value	Reference/source
Tissue binding	Binding to hemoglobin, AA	KHGB1 (L/gHGB)	0.5	Model simulations fit to Miller et al. 1982, Raymer et al. 1993
	Binding to hemoglobin, GA	KHGB2 (L/gHGB-hour)	0.25	Model simulations fit to Miller et al. 1982; Raymer et al. 1993
	Binding to liver macromolecules, acrylamide	KFEEL1 (/hour)	0.2	Model simulations fit to Miller et al. 1982; Raymer et al. 1993
	Binding to liver macromolecules, GA	KFEEL2 (/hour)	0.1	Model simulations fit to Miller et al. 1982; Raymer et al. 1993
	Binding to tissue macromolecules, AA	KFEET1 (/hour)	0.08	Model simulations fit to Miller et al. 1982; Raymer et al. 1993
	Binding to tissue macromolecules, GA	KFEET1 (/hour)	0.04	Model simulations fit to Miller et al. 1982; Raymer et al. 1993
	Binding to blood macromolecules, AA	KFEEB1 (/hour)	0.01	Model simulations fit to Miller et al. 1982; Raymer et al. 1993
	Binding to blood macromolecules, GA	KFEEB2 (/hour)	0.005	Model simulations fit to Miller et al. 1982; Raymer et al. 1993
	Protein turnover	KPT (/hour)	0.008	Model simulations fit to Miller et al. 1982; Raymer et al. 1993
Glutathione	GSH production rate	KGSHP (mmol/hour)	0.025	D'Souza et al. 1988
	GSH loss rate	KGSHL (/hour)	0.35	D'Souza et al. 1988
	Initial GSH concentration in liver	GSHL0 (mmol/L)	7.0	D'Souza et al. 1988

AA = acrylamide; GA = glycidamide; GSH = glutathione

Source: Kirman et al. 2003

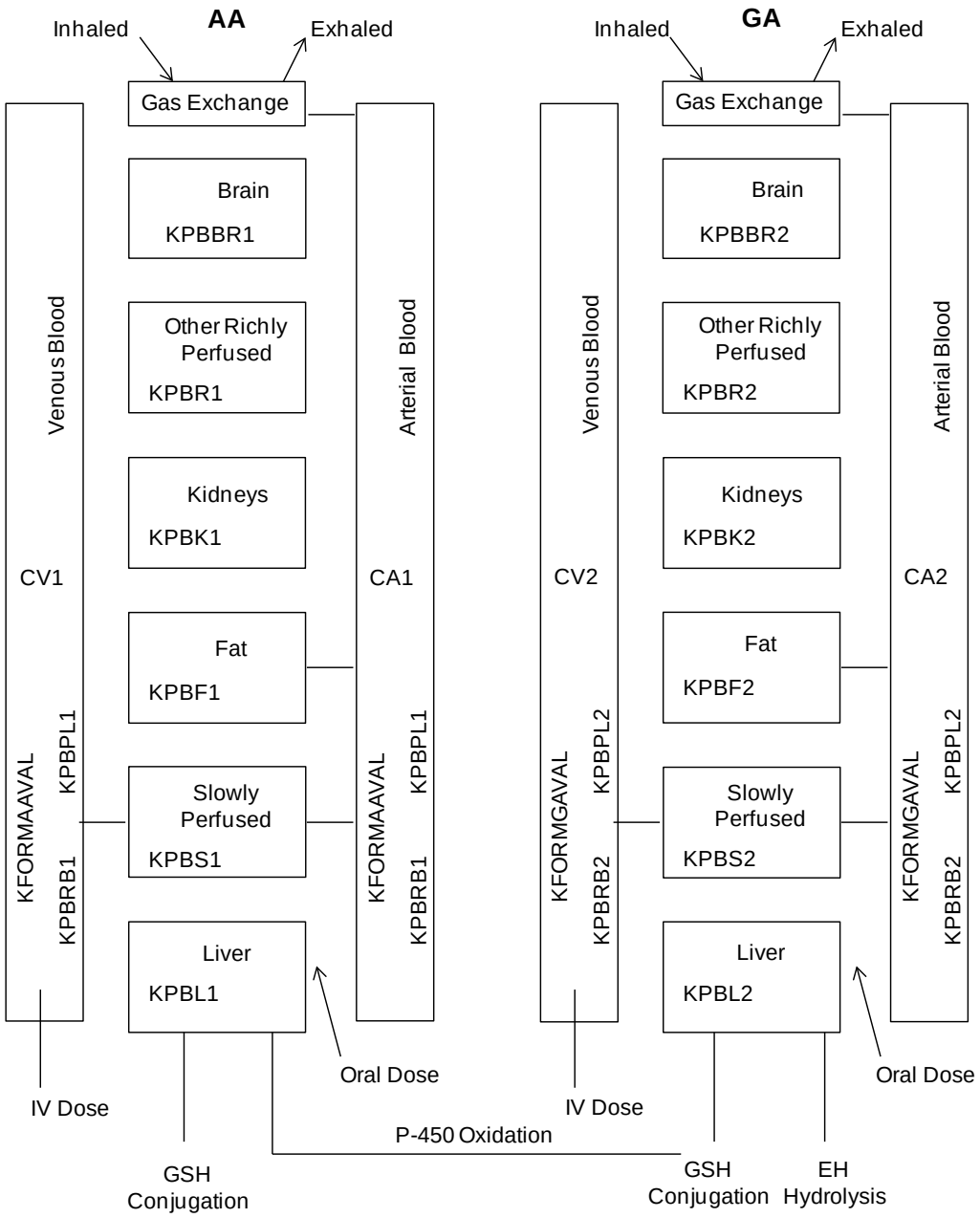
Figure 3-6; parameters are listed in Table 3-12. Like the Kirman et al. (2003) model, the Sweeney et al. (2010) model consists of separate models for acrylamide and glycidamide that are connected at the liver compartments where acrylamide is assumed to undergo oxidation to glycidamide by cytochrome P-450 (K_m, V_{max}). Conjugation of acrylamide and glycidamide with GSH mediated by glutathione transferase (K_m, V_{max}) and hydrolysis of glycidamide mediated by epoxide hydrolase (K_m, V_{max}) are also assumed to occur solely in the liver. The conjugation and epoxide hydrolase reactions yield the urinary metabolites AAMA, GAMA, and glyceramide (first-order clearance). Values for metabolism parameters were derived from measurements made in rat hepatocytes (e.g., K_m) or optimized based on data for blood or urinary kinetics of acrylamide, glycidamide, or metabolites AAMA and GAMA (e.g., V_{max}, first-order clearance). Tissue:blood partition coefficients were based on *in vivo* measurements of concentration ratios measured in rats exposed to acrylamide or glycidamide (Doerge et al. 2005c). Partition coefficients estimated for rats were adopted for the human model. Data used to derive model parameters are presented in Table 3-12. The rat and human models were calibrated and then validated with different sets of data. Sources of data used to calibrate and validate the models are described in Sweeney et al. (2010). Sweeney et al. (2010) also describe the results of sensitivity analyses conducted to ascertain the sensitivity of internal dose metrics (e.g., blood AUC for glycidamide) to variation in model parameters.

The various improvements and extensions made to the Kirman et al. (2003) model, including development of a human model and calibration and evaluation of the rat and human models against newer data from rat bioassays and human studies, enabled application of the Sweeney et al. (2010) model to interspecies dose-response extrapolation. Tardiff et al. (2010) applied the Sweeney et al. (2010) model to derive tolerable daily intakes for acrylamide. The rat model was used to estimate internal doses (e.g., blood AUC for acrylamide or glycidamide) corresponding to external drinking water doses in 2-year rat bioassays (e.g., Friedman et al., 1995; Johnson et al., 1986), from which internal dose-response relationships were derived. The human model was used to estimate human equivalent external doses corresponding to rat BMDLs.

Young et al. (2007) Model

Young et al. (2007) produced a PBPK/toxicodynamic (TD) model to predict the behavior of acrylamide, glycidamide, and their respective glutathione conjugates in rats, mice, and humans (Figure 3-7). The model was developed using PostNatal, a windows-based program from the U.S. FDA's National Center for Toxicological Research (NCTR). The program controls up to four PBPK model units (depicted as PBPK 1–4 in Figure 3-7) under one shell with multiple input and output options. Each PBPK unit is

Figure 3-6. Structure of the Physiologically Based Pharmacokinetic Model for Acrylamide and Glycidamide



AA = acrylamide; EH = epoxide hydrolase; GA = glycidamide; GSH = glutathione; IV = intravenous; KFORMAAVAL = Rate of formation of adducts of AA with the N-terminal valine of hemoglobin; KFORMGAVAL = Rate of formation of adducts of GA with the N-terminal valine of hemoglobin; KPBBR = Rate of binding of AA in brain; KPBF = Rate of binding of AA in fat; KPBK = Rate of binding of AA in kidney; KPBL = Rate of binding of AA in liver; KPBPL = Rate of binding of AA in plasma; KPBR = Rate of binding of AA in richly perfused tissues; KPBRB = Rate of binding of AA in red blood cells; KPBS = Rate of binding of AA in slowly perfused tissues

Source: Sweeney et al. 2010

Table 3-12. Model Parameter Values in the Sweeney et al. (2010) Physiologically Based Pharmacokinetic Model

Parameter (abbreviation)	Units	Male F344 rat	Human[a]	Source/comment
Body weight	kg	0.25	70	Default values; study-specific values used when available
Cardiac output, normalized to body weight (QCC)	L/(hour $kg^{0.74}$)	14	14	Kirman et al. 2003
Alveolar ventilation, normalized to body weight (QPC)	L/(hour $kg^{0.74}$)	14	14	QPC = QCC
Blood flow to the liver (fraction of cardiac output) (QLC)	None	0.18	0.175	Brown et al. 1997
Blood flow to the kidneys (fraction of cardiac output) (QKC)	None	0.13	0.175	Brown et al. 1997
Blood flow to the brain (fraction of cardiac output) (QBrC)	None	0.02	0.114	Brown et al. 1997
Blood flow to fat (fraction of cardiac output) (QFC)	None	0.087	0.085	Brown et al. 1997
Blood flow to slowly perfused tissues (fraction of cardiac output) (QSC)	None	0.34	0.249	Brown et al. 1997, muscle and skin
Blood flow to other richly perfused tissues (fraction of cardiac output) (QRC)	None	0.25	0.202	QRC = 1 – (QLC + QKC + QBrC + QFC + QSC)
Liver volume (fraction of body weight) (VLC)	None	0.037	0.026	Brown et al. 1997
Kidney volume (fraction of body weight) (VKC)	None	0.0073	0.0044	Brown et al. 1997
Brain volume (fraction of body weight) (VBrC)	None	0.006	0.02	Brown et al. 1997
Fat volume (fraction of body weight) (VFC)	None	0.087	0.21	Schoeffner et al. 1999
Slowly perfused tissue volume (fraction of body weight) (VSC)	None	0.59	0.511	Brown et al. 1997, muscle and skin
Unperfused tissue volume (fraction of body weight) (VUC)	None	0.05	0.09	Brown et al. 1997

Table 3-12. Model Parameter Values in the Sweeney et al. (2010) Physiologically Based Pharmacokinetic Model

Parameter (abbreviation)	Units	Male F344 rat	Human[a]	Source/comment
Volume of other richly perfused tissues (fraction of body weight) (VRC)	None	0.1487	0.1386	VRC = 1 – (VLC + VKC + VBrC + VFC + VSC + VBC + VUC)
Blood volume (fraction of body weight) (VBC)	None	0.074	0.079	Brown et al. 1997
Arterial blood volume (fraction of total blood volume) (VABC)	None	0.35	0.35	Kirman et al. 2003
Red blood cell volume (fraction of blood volume) (FBC)	None	0.44	0.44	Kirman et al. 2003
Liver:blood partition coefficient for AA (PL1)	None	0.4		Doerge et al. 2005c; liver:blood concentration ratio
Kidney:blood partition coefficient for AA (PK1)	None	0.8		Miller et al. 1982; kidney:blood concentration ratio
Brain:blood partition coefficient for AA (PBr1)	None	1.2		Doerge et al. 2005c; brain:blood concentration ratio
Fat:blood partition coefficient for AA (PF1)	None	0.2		Estimated from Doerge et al. 2005c; mammary:blood concentration ratio and mammary tissue composition (percent lipid; Duck 1990)
Slowly perfused tissues:blood partition coefficient for AA (PS1)	None	0.69		Doerge et al. 2005c; muscle:blood concentration ratio
Other richly perfused tissues:blood partition coefficient for AA (PR1)	None	0.4		Same as liver
Blood:air partition coefficient for AA (PB1)	None	31,000,000		Kirman et al. 2003
Liver:blood partition coefficient for GA (PL2)	None	0.5		Doerge et al. 2005c; liver:blood concentration ratio
Kidney:blood partition coefficient for GA (PK2)	None	1		Average of liver:blood and brain:blood partition coefficients
Brain:blood partition coefficient for GA (PBr2)	None	1.4		Doerge et al. 2005c; brain:blood concentration ratio
Fat:blood partition coefficient for GA (PF2)	None	0.2		Estimated from Doerge et al. 2005c; mammary:blood concentration ratio and mammary tissue composition (percent lipid; Duck 1990)

Table 3-12. Model Parameter Values in the Sweeney et al. (2010) Physiologically Based Pharmacokinetic Model

Parameter (abbreviation)	Units	Male F344 rat	Human[a]	Source/comment
Slowly perfused tissues:blood partition coefficient for GA (PS2)	None	1		Doerge et al. 2005c; muscle:blood concentration ratio
Other richly perfused tissues:blood partition coefficient for GA (PR2)	None	0.5		Same as liver
Blood:air partition coefficient for GA (PB2)	None	98,000,000		Kirman et al. 2003
Maximum rate of AA epoxidation to GA (body weight normalized) ($V_{max}C1$)	mg/(hour $kg^{0.7}$)	1.5 (gavage, diet, ip); 0.43 (iv)	0.5 (oral)	Rat: fit to Doerge et al. 2005c; human: fit to Fennell et al. 2005b
KM for epoxidation of AA to GA (KMC1)	mg/L	10		Kirman et al. 2003; fit to Sumner et al. 1992
Maximum rate of AA conjugation with GSH (body weight normalized) ($V_{max}GC1$)	mg/(hour $kg^{0.7}$)	13 (gavage, diet, ip); 8.2 (iv)	22	Rat: fit to Doerge et al. 2005c, 2007; human: fit to Fennell et al. 2005b, Kopp and Dekant 2009
KM for conjugation of AA with GSH (KMGC1)	mg/L	100		Kurebayashi and Ohno 2006 (Sprague-Dawley rat hepatocytes)
Maximum rate of GA hydrolysis (body weight normalized) ($V_{max}C2$)	mg/(hour $kg^{0.7}$)	1.3	20	Rat: fit to Doerge et al. 2005c, 2007; human: fit to Fennell et al. 2005b
KM for hydrolysis of GA to glyceramide (KMGC2)	mg/L	100		Kirman et al. 2003
Maximum rate of GA conjugation with GSH (body weight normalized) ($V_{max}GC2$)	mg/(hour $kg^{0.7}$)	19	20	Rat: fit to Doerge et al. 2005c, 2007; human, fit to Kopp and Dekant 2009
KM for conjugation of GA with GSH (KMGC1)	mg/L	100		Kurebayashi and Ohno 2006 (Sprague-Dawley rat hepatocytes)
Urinary elimination of GA (fraction of kidney blood flow) (KUC2)	None	0.025		Fit to Doerge et al. 2005c, 2007
Urinary elimination of AAMA (KUAAMA)	$Hour^{-1}$	0.13		Fit to Kopp and Dekant 2009
Urinary elimination of GAMA (KUGAMA)	$Hour^{-1}$	0.077		Fit to Kopp and Dekant 2009
Urinary elimination of glyceramide (KUGAOH)	$Hour^{-1}$	0.077		Assumed equal to KUGAMA

Table 3-12. Model Parameter Values in the Sweeney et al. (2010) Physiologically Based Pharmacokinetic Model

Parameter (abbreviation)	Units	Male F344 rat	Human[a]	Source/comment
Rate of binding of AA in liver (KPBL1)	$Hour^{-1}$	0.55	0.25	Rat: fit to Miller et al. 1982; human: adjusted to fit Kopp and Dekant 2009 urinary recovery
Rate of binding of AA in kidney (KPBK1)	$Hour^{-1}$	0.27	0.13	Rat: fit to Miller et al. 1982; human: adjusted to fit Kopp and Dekant 2009 urinary recovery
Rate of binding of AA in brain (KPBBr1)	$Hour^{-1}$	0.093	0.041	Rat: fit to Miller et al. 1982; human: adjusted to fit Kopp and Dekant 2009 urinary recovery
Rate of binding of AA in fat (KPBF1)	$Hour^{-1}$	0.10	0.045	Rat: fit to Miller et al. 1982; human: adjusted to fit Kopp and Dekant 2009 urinary recovery
Rate of binding of AA in slowly perfused tissues (KPBS1)	$Hour^{-1}$	0.079	0.036	Rat: fit to Miller et al. 1982; human: adjusted to fit Kopp and Dekant 2009 urinary recovery
Rate of binding of AA in richly perfused tissues (KPBR1)	$Hour^{-1}$	0.093	0.041	Assumed equal to rate of binding in brain
Rate of binding of AA in plasma (KPBPl1)	$Hour^{-1}$	0.0065	0.003	Rat: fit to Miller et al. 1982; human: adjusted to fit Kopp and Dekant 2009 urinary recovery
Rate of binding of AA in red blood cells (KPBRB1)	$Hour^{-1}$	0.34	0.15	Rat: fit to Miller et al. 1982; human: adjusted to fit Kopp and Dekant 2009 urinary recovery
Ratio of AA binding rate to GA binding rate for tissues and blood components	None	2		Kirman et al. 2003, Bergmark et al. 1991
Loss rate for bound material in liver (KPTL)	$Hour^{-1}$	0.017		Fit to Miller et al. 1982
Loss rate for bound material in kidney (KPTK)	$Hour^{-1}$	0.016		Fit to Miller et al. 1982
Loss rate for bound material in brain (KPTBr)	$Hour^{-1}$	0.0091		Fit to Miller et al. 1982
Loss rate for bound material in fat (KPTF)	$Hour^{-1}$	0.0051		Fit to Miller et al. 1982
Loss rate for bound material in slowly perfused tissues (KPTS)	$Hour^{-1}$	0.0051		Fit to Miller et al. 1982
Loss rate for bound material in other richly perfused tissues (KPTR)	$Hour^{-1}$	0.0091		Assumed equal to loss rate in brain

Table 3-12. Model Parameter Values in the Sweeney et al. (2010) Physiologically Based Pharmacokinetic Model

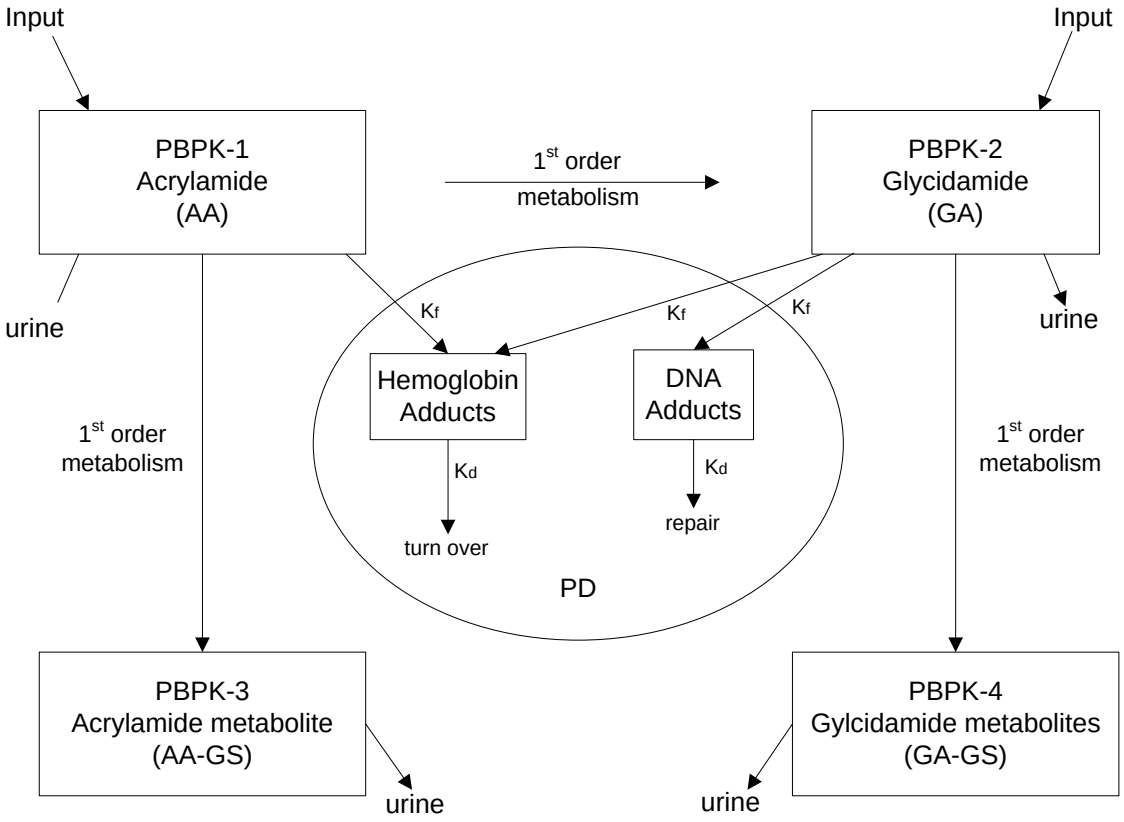
Parameter (abbreviation)	Units	Male F344 rat	Human[a]	Source/comment
Loss rate for bound material in plasma (KPTPl)	$Hour^{-1}$	0.012		Fit to Miller et al. 1982
Loss of bound material in red blood cells				See Section 4 in Sweeney et al. 2010
Rate of formation of adducts of AA with the N-terminal valine of hemoglobin (KFORMAAVAL)	fmol adduct/mg globin per mM hour AA	7,500		Tareke et al. 2006
Rate of formation of adducts of GA with the N-terminal valine of hemoglobin (KFORMGAVAL)	fmol adduct/mg globin per mM hour GA	34,000		Tareke et al. 2006
Rate of AAVal clearance from red blood cells (KREMAAVAL)	$Hour^{-1}$	0.00231	NA	Tareke et al. 2006
Rate of GAVal clearance from red blood cells (KREMGAVAL)	$Hour^{-1}$	0.00231		Tareke et al. 2006
Absorption rate for AA (KA) from gavage/water; diet	$Hour^{-1}$	0.46; 0.27		Fit to Doerge et al. 2005c
Absorption rate for GA (KA2)	$Hour^{-1}$	1.1		Fit to Doerge et al. 2005c
Infusion duration (intravenous) (TINF)	Hour	0.003		Estimate

AA = acrylamide; AAMA = N-acetyl-S-(2-carbamoylethyl)cysteine; GA = glycidamide; GAMA = N-acetyl-S-(2-hydroxy-2-carbamoylethyl)cysteine; NA = not applicable

[a]Where no value is listed under “human”, the human value was assumed to be the same as the rat value.

Source: Sweeney et al. 2010

Figure 3-7. Schematic of the Young et al. (2007) Physiologically Based Pharmacokinetic/Toxicodynamic (PBPK/TD) Model For Acrylamide and Glycidamide*



*Block diagram for the metabolism of AA to GA and further metabolism of both to their glutathione conjugates. All pharmacokinetic and pharmacodynamic (PD) processes are first order.

comprised of 28 organ/tissue/fluid components maintained independently or connected through metabolic pathways. Pharmacodynamic components for liver glycidamide-DNA adducts and hemoglobin adducts with acrylamide and glycidamide are included in the model as well. Dose administration can be either acrylamide or glycidamide. All metabolism and urinary elimination are considered first order.

Physiological parameters were assigned values from within the PostNatal program based on animal species, gender, and total body weight. Serum acrylamide and glycidamide concentrations and urinary elimination levels for male and female rats and mice were simulated from intravenous and oral administration of 0.1 or 0.12 mg/kg glycidamide. Adduct formation and decay rates were determined from a 6-week exposure to 1 mg/kg acrylamide in the drinking water followed by 6 weeks of nontreatment.

The data used to calibrate the Young et al. (2007) model for rats and mice include acrylamide serum levels in rats following intraperitoneal administration (Raymer et al. 1993); plasma acrylamide and glycidamide levels, and acrylamide and glycidamide hemoglobin adduct levels following relatively high (50 mg/kg bw) repeat intraperitoneal dosing in rats for 11 days or 2.8 mM of acrylamide in drinking water for 47 days (Barber et al. 2001); urinary excretion profile and acrylamide and glycidamide hemoglobin adduct levels following dosing via intraperitoneal injection (50 mg/kg), gavage (50 mg/kg), dermal application (150 mg/kg), or inhalation (3 ppm for 6 hours) (Sumner et al. 2003); and serum and tissue levels of acrylamide and glycidamide, and liver glycidamide-DNA adduct data in rats and mice following relatively low-dose administration via intravenous injection (acrylamide and glycidamide at 0.1–0.12 mg/kg), gavage (acrylamide and glycidamide at 0.12 and 50 mg/kg), diet (~0.1 mg/kg over 30 minutes), and drinking water (~1 mg/kg acrylamide over 42 days) (Doerge et al. 2005a, 2005b, 2005c). The single and multiple oral data from Barber et al. (2001) were combined with the urinary elimination data of Sumner et al. (1992, 2003) and simulated with the model. The Raymer et al. (1993) data were also combined with the urinary elimination data of Sumner et al. (1992, 2003) and simulated in a similar manner. The NCTR tissue data (Doerge et al. 2005a, 2005b, 2005c) were used to develop partition coefficients. Only those tissues specifically analyzed for acrylamide or glycidamide were partitioned differently from the blood compartment. Values for the human parameters were calibrated against urinary excretion data (Fennell et al. 2005b; Fuhr et al. 2006) and hemoglobin adduct data from a dietary exposure (Boettcher et al. 2005).

Values for the metabolism and elimination of acrylamide or glycidamide, for acrylamide or glycidamide binding to hemoglobin, and for glycidamide-DNA adduct formation were derived by optimizing the fit of

the simulation results to individual animal data. All rate constants for the metabolic and elimination processes, the binding and decay of acrylamide or glycidamide to hemoglobin, and the binding of glycidamide to liver macromolecule are represented as first order. Although Young et al. (2007) calibrated their model parameter values in a logical sequence against the data identified in the paper, a number of sensitive parameters were allowed to vary when fitting the individual animal data so as to optimize the model fit to each set of data. The authors evaluated the resulting differences among the model parameter values relative to gender and study conditions for insights into the toxicokinetics of acrylamide and glycidamide, and to assess the uncertainty in the model parameter values. Although there are statistically significant differences in some cases in the fitted model parameter values for basic physiological functions such as excretion of acrylamide-GSH conjugates in urine (which varies as much as 4–6 for model fits to different studies), the authors argued that the ranges of values are not exceedingly wide considering that different routes of administration for different chemicals are all being compared, and that there is very little difference for each metabolic rate constant when comparing across gender, dose, and route.

For the purpose of quantitative risk assessment, a PBPK model is generally developed to produce a single set of parameter values that fits at least the most relevant data for a particular application. Evaluating the importance of uncertainty in parameter values also depends upon the choice of the dose metric and its sensitivity to parameters of interest. For useful application of the PBPK model of Young et al. (2007) to human health risk, additional studies are needed to identify a single set of parameters, and to evaluate the sensitivity of various dose metrics to the parameters that are the most uncertain.

Walker et al. (2007) Model

Walker et al. (2007) described an adaptation of the PBPK model of Kirman et al. (2003) to account for: (1) hemoglobin adduct data for rats (Fennell et al. 2003) and (2) extrapolation to adult humans using human adduct data (Fennell et al. 2004, 2005b). The Walker et al. (2007) model incorporates available information regarding children's changing physiology and metabolic capacity for selected processes involved in acrylamide disposition (i.e., CYP2E1, glutathione conjugation, epoxide hydrolase). However, this model has not been calibrated or validated with respect to acrylamide dosimetry in children.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Acrylamide is readily absorbed via all natural routes of exposure (inhalation, oral, dermal) and is distributed throughout the body via the blood. No studies were located regarding mechanisms for acrylamide absorption across lung, gut, or skin; processes may include passive and/or facilitative mechanisms. Acrylamide and its reactive metabolite, glycidamide, readily bind to hemoglobin, but do not accumulate appreciably in any tissues. Acrylamide metabolism is relatively rapid; both parent compound and the epoxide, glycidamide, appear to be involved in acrylamide toxicity. Animal data demonstrate the importance of CYP2E1 in acrylamide metabolism. Metabolism is assumed to take place primarily in the liver. Urinary excretion of conjugated acrylamide derivatives represents the major excretory pathway for acrylamide.

3.5.2 Mechanisms of Toxicity

Neurotoxic Effects. The neurotoxicity of acrylamide has been assessed since the 1950s in numerous animal studies; notable signs and symptoms include hindfoot splay, decreased grip strength, and increasingly impaired mobility with continued exposure (see Sections 3.2.1.4, 3.2.2.4, and 3.2.3.4 for detailed information regarding neurological effects in animals administered acrylamide by inhalation, oral, and dermal exposure routes, respectively). Some of the neurological effects observed in animals can be elicited by administration of acrylamide or its epoxide metabolite (glycidamide). However, in one study of male rats administered acrylamide (25 or 50 mg/kg/day) or glycidamide (50 or 100 mg/kg/day) via intraperitoneal injection for 8 days, only acrylamide elicited poor performance on the hindlimb splay test (Costa et al. 1995). Observations of neurological effects in acrylamide-exposed humans include muscle weakness and other signs of functional impairments (Calleman et al. 1994; Hagmar et al. 2001; He et al. 1989). Early histopathologic investigations performed on acrylamide-intoxicated animals revealed degenerative effects in distal portions of large peripheral nerve fibers and associated myelin sheath (Spencer and Schaumberg 1974, 1977). The degeneration was observed to progress proximally in a process termed “dying back”. Early hypotheses to explain this “dying back” effect involved damage to axonal neurofilaments, impairment of cellular metabolism, effects on axonal transport mechanisms, and disruption of axon cytoskeleton (Cavanaugh 1964; Harris et al. 1994; Harry 1992; Lapadula et al. 1989; LoPachin and Lehning 1994; Padilla et al. 1993; Pleasure et al. 1969; Sickles 1991; Spencer et al. 1979).

Specific mechanisms of acrylamide neurotoxicity have not been clearly elucidated. However, results of numerous studies designed to assess mechanisms of acrylamide-induced neurological effects have led to two major hypotheses: (1) acrylamide-induced disruption of fast axonal transport (Sickles et al. 2002a) and (2) acrylamide-induced disruption of nitric oxide signaling at nerve terminals (LoPachin and Barber 2006; LoPachin et al. 2008). In addition, recent work by Zhu et al. (2008) provides some support to a hypothetical mode of action whereby acrylamide exposure results in increases in reactive oxygen species, damage to cellular macromolecules, and subsequent degeneration of neural tissues.

Disruption of Fast Axonal Transport. The hypothesis that acrylamide induces peripheral neuropathy via disruption of fast axonal transport proposes the binding of acrylamide to kinesin, which leads to impairment of the fast axonal transport system responsible for the distal delivery of macromolecules. This would result in deficiencies in proteins responsible for maintaining axonal structure and function. The particular vulnerability of distal axons and nerve terminals is based on the large axonal volume and transport distance from cell body to distal regions. Kinesin, which plays an integral role in intracellular transport along microtubules, is inhibited by neurotoxic doses of acrylamide. Evidence was provided from results of a microtubule motility assay in which preincubation of purified kinesin with acrylamide produced a dose-dependent loss of numbers of microtubules moving over a bed of kinesin and less-steady locomotory activity (Sickles et al. 1996). These effects were proposed to result from covalent adduction through sulfhydryl alkylation by acrylamide. Comparable effects were produced by glycidamide.

Disruption of Nitric Oxide Signaling at Nerve Terminals. The hypothesis that acrylamide induces peripheral neuropathy via disruption of nitric oxide signaling at nerve terminals was proposed by LoPachin and coworkers (LoPachin and Barber 2006; LoPachin et al. 2008) based on critical review of available data for conjugated α,β-unsaturated carbonyl derivatives, a group of type-2 alkenes that includes acrylamide. The hypothesis is based on evidence that: (1) acrylamide inhibits neurotransmission in peripheral and central synapses and (2) soft electrophiles such as acrylamide form Michael-type adducts with soft nucleophilic sulfhydryl groups on protein cysteine residues.

According to the review of LoPachin and Barber (2006), the functional status of many synaptic processes is determined by proteins whose activity is regulated by the redox state of highly nucleophilic sulfhydryl groups within corresponding catalytic triads. Acrylamide (a soft electrophile) forms adducts with soft nucleophilic sulfhydryl groups on cysteine residues (reviewed in LoPachin and DeCaprio 2005). Results of early electrophysiological studies demonstrated acrylamide-induced neurotransmitter inhibition at peripheral and central synapses (reviewed in LoPachin et al. 2002, 2003). Results of subsequent

mechanistic studies indicate that acrylamide causes decreases in neurotransmitter release, uptake, and storage (Barber and LoPachin 2004; LoPachin et al. 2004, 2006) and that these effects may be mediated by acrylamide-sulfhydryl adduction of specific cysteine residues on functionally critical proteins (Barber and LoPachin 2004). Because the cysteine-acrylamide adduction sites are also sites of nitric oxide nitrosylation (Jaffrey et al. 2001; Stamler et al. 2001), it has been suggested that acrylamide inhibits synaptic activity by disrupting nitric oxide signaling (LoPachin and Barber 2006). LoPachin and coworkers suggest that acrylamide itself, and not its major electrophilic metabolite glycidamide, is responsible for the neurotoxicity because glycidamide is a hard electrophile that forms adducts with hard nucleophiles (nitrogen, carbon, oxygen) consistent with glycidamide adducts on adenine and guanine bases.

Reactive Oxygen Species Hypothesis. Results of Zhu et al. (2008) provide some indication that acrylamide-induced neurotoxicity may involve enhancement of lipid peroxidation and decreased antioxidative capacity, depletion of neural glutathione levels and antioxidant enzyme activities, leading to increased levels of reactive oxygen species, damage to cellular macromolecules, and subsequent degeneration of neural tissues. Time-dependent decreased glutathione levels and anti-reactive oxygen species activities and increased malondialdehyde levels in sciatic nerve preparations were highly correlated with changes in electrophysiological indices of acrylamide-induced neurotoxicity in rats receiving repeated intraperitoneally-injected doses of 40 mg/kg acrylamide.

Bowyer et al. (2009) designed a study to assess whether gene expression or overt histological signs of neurotoxicity in specific regions of the forebrain might be involved in acrylamide-induced neurological effects by administering acrylamide to male Fischer 344 rats via their drinking water for 14 days at concentrations up to 500 µg/mL, which delivered an overtly neurotoxic dose (44 mg/kg/day). The results indicate that the forebrain is not a likely source of acrylamide neurotoxicity in the rat because there were no treatment-related prominent changes in gene expression or histopathological evidence of axonal, dendritic, or cell body damage in the forebrain.

Reproductive Effects. Mechanisms of acrylamide-induced reproductive toxicity are poorly understood. There is some indication that mutagenic effects on male germ cells may play a significant role (see Section 3.3 Genotoxic Effects). Available data provide suggestions that acrylamide-induced male dominant lethal mutations may involve clastogenic events from binding of acrylamide and/or glycidamide to spermatid protamines or spindle fiber proteins and/or direct alkylation of DNA by glycidamide (Adler et al. 2000; Perrault 2003; Sega et al. 1989; Tyl and Friedman 2003; Tyl et al. 2000b).

Tyl and Friedman (2003) also suggested that adverse effects on mounting, sperm motility, and intromission could be related to distal axonopathy resulting from binding of acrylamide to motor proteins. Results of one study using male CYP2E1-null and wild-type mice demonstrate the importance of metabolism in acrylamide-induced germ cell mutations (Ghanayem et al. 2005a). The male mice were administered acrylamide via intraperitoneal injection for 5 consecutive days at dose levels of 0, 12.5, 24, or 50 mg/kg/day and subsequently mated with untreated B6C3F1 female mice. Dose-related increased incidences of dominant lethal mutations and decreases in numbers of pregnant females and proportion of living fetuses were observed in females mated to wild-type male mice. No significant changes in any fertility parameters were seen in females mated to CYP2E1-null male mice. These results demonstrate the importance of CYP2E1-mediated epoxidation to glycidamide for acrylamide-induced germ cell mutations in male mice.

Genotoxic/Carcinogenic Effects. Specific mechanisms whereby acrylamide induces tumors in laboratory animals are not understood at present. However, the weight of evidence supports a mutagenic mode of action (Besaratinia and Pfeiffer 2005, 2007; Dearfield et al. 1995; Moore et al. 1987; Segerbäck et al. 1995). Evidence for a mutagenic mode of action includes findings that: (1) acrylamide is metabolized by CYP2E1 to DNA-reactive glycidamide; (2) acrylamide and glycidamide induce mutations in lymphocyte HPRT and liver cII cells; (3) DNA adducts of glycidamide have been detected in tissues of all relevant tumor targets of acrylamide-and glycidamide-exposed male and female rats and mice; (4) glycidamide is mutagenic to bacteria and male mouse germ cells and male and female mouse somatic cells *in vivo*; (5) acrylamide induces heritable translocations and specific locus mutations in germ cells of exposed male mice; (6) acrylamide induces clastogenic effects in mouse lymphoma assays; and (7) dominant lethal effects in rodents occur at subchronic oral exposure levels comparable to those associated with carcinogenic effects in chronically-exposed rats. These findings support a proposed mode of action whereby acrylamide is metabolized to the relatively long-lived epoxide, glycidamide, which reacts with proteins and DNA, causing mutations that persist in viable somatic cells, resulting in tumor formation. Ghanayem et al. (2005b) observed significant dose-related increases in micronucleated erythrocytes and DNA damage in somatic cells (leukocytes, liver, lung) of acrylamide-treated wild-type mice, but not in CYP2E1-null mice, indicating that genetic damage in somatic cells is dependent on metabolism of acrylamide by CYP2E1. Allen et al. (2005) investigated dose-response relationships from *in vivo* genotoxicity data (chromosomal damage, gene mutations, and recombinations in somatic and germ cells) for acrylamide using three different mathematical models according to end point; the investigation included BMD calculations. The results were not consistent with a genotoxic mode of action for the thyroid tumors reported in 2-year cancer bioassays in rats (Friedman et al. 1995; Johnson et

al. 1984, 1986). Based on these results, Allen et al. (2005) suggested that a nongenotoxic mode of action may be primarily responsible for acrylamide-induced thyroid tumors in rats.

Another hypothetical mode of action involves disruption of hormone levels or hormone signaling for acrylamide-induced tumors in hormonally-sensitive tissues including mammary gland and thyroid or tissues adjacent to hormonally sensitive tissue, such as tunica vaginalis mesothelium (DFG 2009; Dourson et al. 2008; Environ 2002; Haber et al. 2009; Klaunig 2008; Shipp et al. 2006). In support of this hypothetical mode of action, American Cyanamid Company (1991) reported decreases in serum testosterone and thyroid hormone (T_3 and T_4) levels in rats following oral exposure to acrylamide. The induction of cellular proliferation is yet another proposed mode of action for acrylamide carcinogenicity in selected target tissues, although limited supporting data are available. Lafferty et al. (2004) demonstrated that tumorigenic doses of acrylamide administered to male rats induced cell proliferation in thyroid, tunica vaginalis, and adrenal medulla (tumor target tissues), but not in liver or adrenal cortex (nontarget tissues).

Studies designed by Bowyer et al. (2008a) found no evidence to support hormonal disruption as a plausible mechanism for acrylamide-induced thyroid cancer in male Fischer 344 rats. Groups of male rats were administered acrylamide in the drinking water for 14 days at concentrations designed to deliver doses of 2.5, 10, and 50 mg/kg/day. The low dose was selected to represent a dose level that is carcinogenic with lifetime exposure; the high dose is neurotoxic when administered acutely. End points assessed included serum levels of thyroid and pituitary hormones; target tissue expression of genes involved in hormone synthesis, release, and receptors; neurotransmitters in the central nervous system that affect hormone homeostasis; and histopathological evaluation of target tissues. The study authors stated that the negative results are consistent with a genotoxic mechanism of acrylamide carcinogenicity based on metabolism to glycidamide and DNA adduct formation.

3.5.3 Animal-to-Human Extrapolations

Available data from rats and mice indicate that acrylamide is transformed to glycidamide to a greater extent in mice than rats. Following oral administration of radiolabeled acrylamide (50 mg/kg), glycidamide and glycidamide-derived metabolites accounted for about 33% (rats) and 59% (mice) of the total metabolites excreted in the urine within 24 hours (Sumner et al. 1992). Similar results were reported in a study of metabolites in urine collected for 24 hours after 6-hour inhalation exposure (nose only) of rats and mice to 3 ppm acrylamide where glycidamide and glycidamide-derived metabolites accounted for

36 % (rats) and 73 % (mice) of total metabolites excreted in the urine within 24 hours (Sumner et al. 2003).

Available PBPK models for acrylamide (Kirman et al. 2003; Walker et al. 2009; Young et al. 2007) have not been adequately calibrated and validated for useful extrapolation from animals to humans.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

No studies were located regarding endocrine disruption in humans after exposure to acrylamide.

One repeated-dose oral study in rats reported acrylamide-induced decreases in serum testosterone levels, thyroid hormone (T_3 and T_4) levels, and prolactin (American Cyanamid Company 1991).

No *in vitro* studies were located regarding endocrine disruption of acrylamide.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi

1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

Specific information regarding acrylamide-induced health effects in children was not located.

Neurotoxic end points have been examined in acrylamide-exposed mature and immature animals; however, with respect to possible age-related differences in susceptibility to acrylamide neurotoxicity, results are conflicting. Some reports indicate that young animals may be less susceptible than older ones (Fullerton and Barnes 1966; Kaplan et al. 1973), whereas other reports present evidence that young animals may be more sensitive (Ko et al. 1999; Suzuki and Pfaff 1973). In rats administered acrylamide orally at 100 mg/kg/day, Fullerton and Barnes (1966) noted that 26-week-old rats experienced earlier and more severe neurotoxic effects than 5-week-old rats. In contrast, Ko et al. (1999) reported that oral administration of acrylamide at 91 mg/kg/day resulted in earlier onset and more rapid progression of neuropathy in 3-week-old mice compared to 8-week-old mice. Studies that employed intraperitoneal injection of acrylamide also yielded conflicting results (Kaplan et al. 1973; Suzuki and Pfaff 1973). In rats repeatedly injected with 50 mg/kg acrylamide, earlier and more prominent degenerative histopathologic changes were noted in peripheral nerves of 1-day-old pups compared to adults. Kaplan et al. (1973) found the opposite in rats repeatedly injected with the same dose; 14-week-old rats experienced

impaired rotarod performance earlier than 5-week-old rats (although the younger rats recovered more slowly).

Cancer is also a possible human health effect based on evidence that carcinogenic responses in rats chronically exposed to acrylamide throughout adulthood are likely mediated through a mutagenic mode of action that could occur in humans. In the absence of direct evidence that early-life exposure leads to increased risk for cancer, EPA (2005b) assumes that increased risk occurs with early-life exposure to agents that act through a mutagenic mode of action. For acrylamide, no human or animal studies were located that examined whether early-life exposure to acrylamide increased the risk for cancer, compared with exposure during adulthood alone; however, there is evidence that acrylamide acts through a mutagenic carcinogenic mode of action. CYP2E1, which catalyzes acrylamide to its DNA-reactive metabolite glycidamide, is not expressed in the developing fetus, but attains levels of expression during early postnatal periods that are similar to levels in adults (Johnsrud et al. 2003).

There is no information regarding developmental health effects as a result of acrylamide exposure in humans. Developmental effects observed in acrylamide-exposed rats include decreased brain levels of catecholamines, decreased auditory startle response, deficient motor skills, decreased open field activity, decreased performance in operant testing of cognitive motivation and learning ability, and decreased body weight (Field et al. 1990; Ferguson et al. 2010; Garey and Paule 2007, 2010; Garey et al. 2005; Husain et al. 1987; Wise et al. 1995). Decreased brain levels of catecholamine resulted from various lengths of exposure via lactation or gavage (Husain et al. 1987). Decreased auditory startle response followed exposure during gestation (Wise et al. 1995), and decreased performance in an operant test of cognitive motivation followed either exposure during gestation, lactation, or through 12 weeks of age (Garey and Paule 2007). Garey and Paule (2010) reported decreased performance in an incremental repeated acquisition task (a measure of learning ability) followed exposure during gestation, lactation and up to 8 months of age. Delayed pinnae detachment (a developmental landmark) and deficient negative geotaxis and rotarod performance were reported in F344 rat pups that had been exposed via their mothers (10 mg acrylamide/kg/day by gavage) during gestation followed by gavage of the pups at the same dose until postnatal day 22; these effects were not seen at doses ≤5 mg/kg/day (Garey et al. 2005). In a similarly-designed study that included 5 mg/kg/day as the highest dose tested, there were no effects on pup developmental landmarks or most behavioral tests; however, the high-dose pups exhibited 30–49% less open field activity than controls (Ferguson et al. 2010).

Acrylamide has been shown to distribute across the placenta in exposed pregnant animals. Two hours following intravenous administration of acrylamide to pregnant beagle dogs, concentrations of radioactivity in blood, brain, heart, and lung were similar in both maternal and fetal tissues (Ikeda et al. 1983, 1985; Marlowe et al. 1986). Ferguson et al. (2010) reported similar levels of acrylamide in the serum of rat dams and their fetuses at 60 minutes postadministration on gestation day 20 in a study where the dams had been treated from gestation day 6. Humans are unlikely to be exposed by intravenous routes, but these results show that acrylamide could cross the placenta if exposure was great enough to achieve comparable maternal blood levels.

Sörgel et al. (2002) reported the detection of low levels of acrylamide (3.17–18.8 ng/mL) in breast milk samples taken from mothers (n=2) during 8 hours following the ingestion of potato chips (estimated acrylamide dose of 0.8–1 mg); predose acrylamide in the breast milk was below the level of quantification (5 ng/mL).

In studies of offspring of nursing rat dams receiving acrylamide orally at 25 mg/kg/day throughout lactation, the dams exhibited severe toxic effects (some mortalities, body weight loss, and hindlimb splay in 90% of the dams) (Friedman et al. 1999). Starting at lactation day 4, offspring showed progressively decreased body weight, compared with control offspring (at lactation day 21, mean body weight of exposed pups was about 43% of the control pup weight); many of the pups died or became moribund during the lactational period without exhibiting signs of peripheral neuropathy (Friedman et al. 1999). In surviving acrylamide-exposed male pups, grip strength was decreased at postnatal day (PND) 30, compared with controls, but was not significantly different from control values at PNDs 60 and 90 (Friedman et al. 1999). The absence of milk in the stomachs of exposed pups that died or became moribund during lactation suggests that inadequate milk supply caused these effects in the offspring. An earlier study by Husain et al. (1987) reported complete hindlimb paralysis in male offspring of comparably-exposed rat dams of the same strain, but neither of the studies examined breast milk for the presence of acrylamide. Free acrylamide was not detected in the serum of Sprague-Dawley rat dams receiving acrylamide from the drinking water from gestation day 6 through postnatal day 21 at concentrations resulting in mean daily acrylamide doses as high as 14.56 mg/kg (Takahashi et al. 2009). No free acrylamide was detected in stomach contents and serum of the pups at postnatal day 14. However, dose-related increases in acrylamide-Hb adduct levels of both dams and their pups were noted; the levels in the pups were ≥10-fold lower than those of their respective dams.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of the U.S. population to environmental chemicals using biomonitoring. This report is available at http://www.cdc.gov/exposurereport/. The biomonitoring data for acrylamide from this report are discussed in Section 6.5. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to acrylamide are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by acrylamide are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or

other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Acrylamide

Several biomarkers of exposure to acrylamide have been reported in the literature. Unchanged acrylamide, its mercapturic acid metabolite, AAMA, its epoxy derivative, glycidamide, and the respective metabolite of glycidamide, GAMA, were quantified in the urine of six volunteers after the consumption of a meal containing 0.94 mg of acrylamide (Fuhr et al. 2006). Urinary mercapturic acid derivatives of acrylamide and/or glycidamide were quantified in other human studies as well (Bjellaas et al. 2007a; Boettcher et al. 2005, 2006a, 2006b; Huang et al. 2011a, 2011b). Results of epidemiological studies support the use of hemoglobin adducts of acrylamide and/or glycidamide as biomarkers of exposure to acrylamide (Bergmark et al. 1993; Boettcher et al. 2005; Calleman et al. 1994; Fennell et al. 2005b, 2006; Hagmer et al. 2001; Olesen et al. 2008). Results of animal studies indicate similar biomarkers of exposure to acrylamide. For example, Doerge and coworkers demonstrated the usefulness of metabolites, glycidamide-hemoglobin adducts, and glycidamide-DNA adducts as biomarkers of exposure to acrylamide in rats and mice (Doerge et al. 2005a, 2005b, 2005c; Tareke et al. 2006). Metabolites can be measured for assessment of recent exposure. Hemoglobin adducts provide a biomarker of exposure for longer periods. See the introductory paragraph in Section 3.4 (Toxicokinetics) for a more detailed discussion of hemoglobin adduct levels as biomarkers of exposure.

It should be noted that hemoglobin adducts of N-methylolacrylamide are indistinguishable from hemoglobin adducts of acrylamide (Fennell et al. 2003; Paulsson et al. 2002). Furthermore, assays to identify exposure to acrylamide are not readily available to clinicians.

3.8.2 Biomarkers Used to Characterize Effects Caused by Acrylamide

Glycidamide-derived DNA adduct formation has been quantified in rats and mice exposed to acrylamide (Doerge et al. 2005a; Gamboa da Costa et al. 2003). There are no other known biomarkers of effect that are considered to be specific to acrylamide exposure.

3.9 INTERACTIONS WITH OTHER CHEMICALS

Nesterova et al. (1999) demonstrated an enhanced effect of acrylamide-induced clastogenicity in male mice administered acrylamide in combination with Verapamil (a calcium antagonist). No other information was located regarding health effects attributed to interactions between acrylamide and other substances.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to acrylamide than will most persons exposed to the same level of acrylamide in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of acrylamide, or compromised function of organs affected by acrylamide. Populations who are at greater risk due to their unusually high exposure to acrylamide are discussed in Section 6.7, Populations with Potentially High Exposures.

No human data were located regarding populations that would be particularly sensitive to acrylamide toxicity.

Available animal data demonstrate increased susceptibility of males to acrylamide-induced reproductive effects expressed as male-mediated implantation loss, reduced number of fetuses, and testicular atrophy. The dominant lethal effects observed in male, but not female, rodents may be caused by acrylamide-induced alkylation of sperm protamine during spermiogenesis (Adler et al. 2000; Generoso et al. 1996; Perrault 2003; Sega et al. 1989; Sublet et al. 1989). Key determinants of male reproductive performance such as copulatory behavior (Zenick et al. 1986) and sperm motility (Sublet et al. 1989; Tyl et al. 2000b) may also be adversely affected by acrylamide.

Available animal data do not suggest gender-related differences in susceptibility to acrylamide neurotoxicity. Male and female rats experience similar neurological effects at comparable dose levels (Burek et al. 1980; Friedman et al. 1995; Fullerton and Barnes 1996; Johnson et al. 1984, 1986). Results of animal cancer bioassays do not indicate any gender-related differences in susceptibility to acrylamide carcinogenicity. Chronic exposure of F344 rats to acrylamide in drinking water induced increased incidences of thyroid follicular cell tumors in both genders, scrotal sac mesotheliomas in males, and mammary gland fibroadenomas in females (Friedman et al. 1995; Johnson et al. 1986).

No human data were located regarding age-related differences in susceptibility to acrylamide toxicity in humans. Conflicting reports are available from animal studies. Some reports indicate that young animals may be more susceptible than older ones (Ko et al. 1999; Suzuki and Pfaff 1973), whereas other reports suggest decreased susceptibility in young animals (Fullerton and Barnes 1966; Kaplan et al. 1973). It should be noted that CYP2E1, which catalyzes acrylamide to its DNA-reactive metabolite glycidamide, is not expressed in the developing fetus, but attains levels of expression during early postnatal periods that are similar to levels in adults (Johnsrud et al. 2003); however, the toxicological significance of this finding has not been demonstrated. Refer to Section 3.7 (Children's Susceptibility) for a detailed discussion of children's susceptibility to acrylamide toxicity.

Genetic polymorphisms in the acrylamide metabolizing P-450 enzyme CYP2E1 have been identified in humans (Hanioka et al. 2003). Polymorphisms in CYP2E1 could conceivably confer a differential risk to acrylamide toxicity and carcinogenicity. There is currently no quantitative estimate of differences in acrylamide or glycidamide tissue or blood levels that might result from CYP2E1 polymorphisms at high or low levels of acrylamide exposure. It is also noted that, since both acrylamide and glycidamide can exert toxic effects, different catalytic activities of CYP2E1 may result in different spectra of adverse effects.

Because the acrylamide metabolite, glycidamide, is DNA reactive, individual differences in DNA repair and detoxification mechanisms might influence susceptibility to acrylamide toxicity.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to acrylamide. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to acrylamide. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to acrylamide:

Currance PL, Clements B, Bronstein AC. 2007. Tri-ortho-cresyl phosphate (TOCP) and related compounds. In: Emergency care for hazardous materials exposure. 3rd ed. St. Louis, MO: MosbyJems, 482-484.

Goldfrank LR, Flomenbaum NE, Lewin NA, et al. 1998. Goldfrank's toxicologic emergencies. Stamford, CT: Appleton & Lange, 322-324, 475.

Leikin JB, Paloucek FP. 2002. Leikin & Paloucek's poisoning & toxicology handbook. 3rd ed. Hudson, OH: Lexi-Comp, Inc., 193-194.

Palmer RB. 2004. Acrylic acid and derivatives. In: Dart RC, ed. Medical toxicology. 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins, 1358-1368.

The following methods for reducing the toxic effects of acrylamide are applicable to numerous organic chemicals; they are not unique to acrylamide.

3.11.1 Reducing Peak Absorption Following Exposure

Rapid absorption of acrylamide can occur following exposure via inhalation, oral, and dermal routes. In the case of inhalation exposure, recommendations include removal from the site of exposure, establishment of a patent airway in the patient, and accompaniment by suction, ventilation, or administration of oxygen by a nonbreathing mask, if necessary (Currance et al. 2007). In the presence of airborne acrylamide, an impervious body protector may shield from absorption via dermal exposure (Leikin and Paloucek 2002). To reduce percutaneous absorption, immediate decontamination with mild liquid soap and large quantities of water has been recommended (Currance et al. 2007; Goldfrank et al. 1998). To reduce absorption resulting from oral exposure, administration of activated charcoal (Leikin and Paloucek 2002; Palmer 2004), particularly within 1 hour of ingestion (Leikin and Paloucek 2002) has been recommended. Because of its potential for central nervous system depression and seizures, ipecac-induced emesis has been discouraged for treatment of acrylamide ingestion (Currance et al. 2007). Use of gastric lavage has not been proven helpful and is not recommended (Palmer 2004).

3.11.2 Reducing Body Burden

No data were located regarding methods for reducing the body burden of absorbed acrylamide.

Animal studies indicate that acrylamide and its metabolites do not accumulate in any tissue other than red blood cells (Barber et al. 2001a; Crofton et al. 1996; Edwards 1975; Hashimoto and Aldridge 1970; Ikeda et al. 1985; Kadry et al. 1999; Marlowe et al. 1986; Miller et al. 1982; Ramsey et al. 1984) and late staged spermatids (Sega et al. 1989) and that elimination of acrylamide and its metabolites is relatively rapid (Fuhr et al. 2006; Leikin and Paloucek 2002).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

The peripheral nervous system is the primary target of acrylamide toxicity in humans and animals. Neurotoxic effects have been observed following inhalation, oral, and dermal exposures. Administration of pyridoxine has been suggested as a possible treatment to delay the onset of neurotoxic effects (Leikin and Paloucek 2002). N-Acetylcysteine has also been utilized, but is of unproven benefit (Leikin and Paloucek 2002).

Administration of 2-cyclopentyl-5-(5-isoquinolylsulfonyl)-6-nitro-1*H*-benzo[D] imidazole to acrylamide-exposed rodents reduced acrylamide-induced behavioral deficits. This effect is suggestive of a therapeutic potential for peripheral neuropathy (Nakagawa-Yagi et al. 2001).

3.12 ADEQUACY OF THE DATABASE

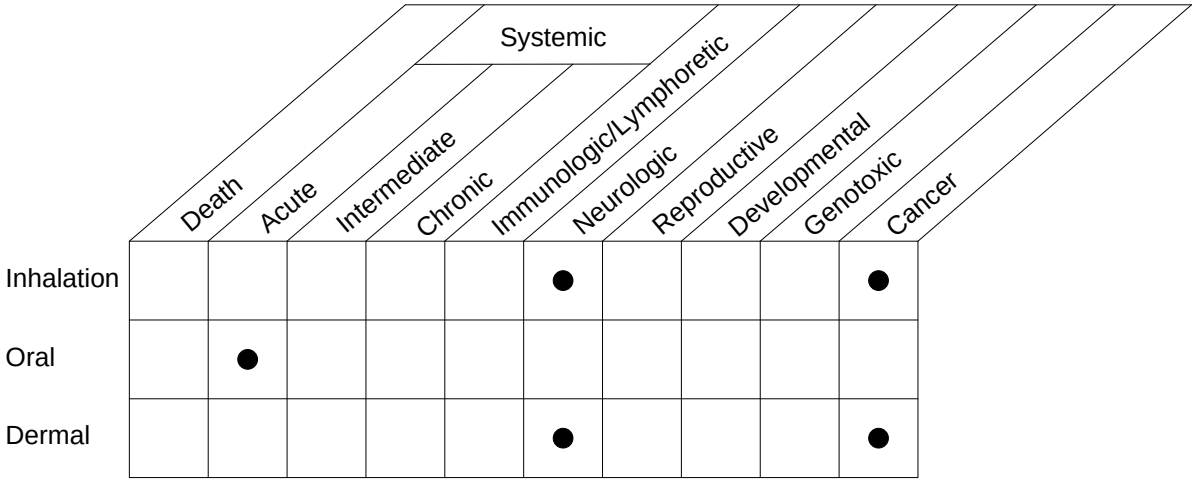
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of acrylamide is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of acrylamide.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

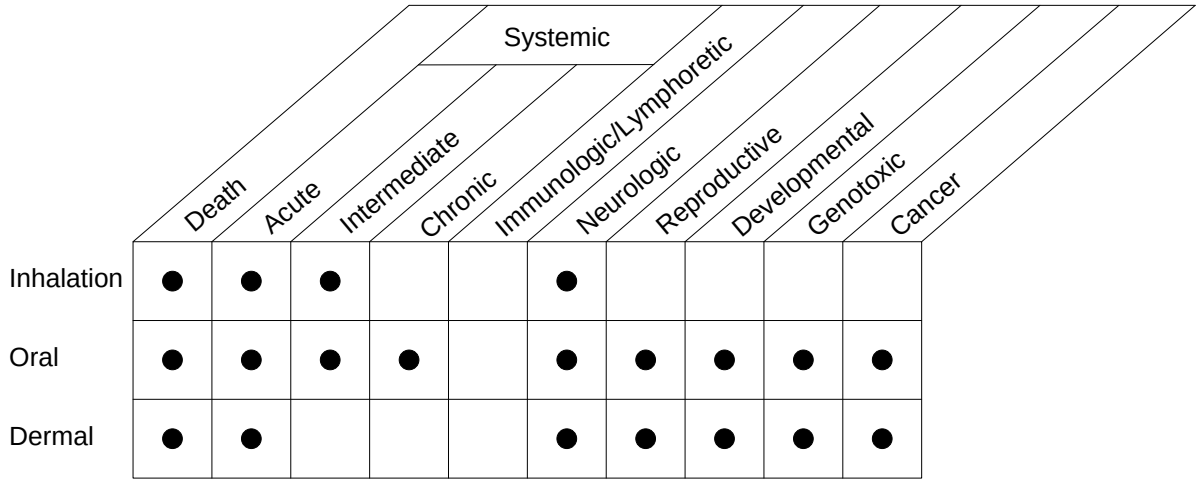
3.12.1 Existing Information on Health Effects of Acrylamide

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to acrylamide are summarized in Figure 3-8. The purpose of this figure is to illustrate the existing information concerning the health effects of acrylamide. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying*

Figure 3-8. Existing Information on Health Effects of Acrylamide



Human



Animal

● Existing Studies

Substance-Specific Data Needs Related to Toxicological Profiles (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. Available human information is limited to a single case in which intentional ingestion of 18 g of acrylamide resulted in clinical signs of peripheral neuropathy (Donovan and Pearson 1987). Limited acute-duration animal data are available for inhalation and dermal exposure routes. Clinical signs of neurological effects were observed in laboratory animals exposed to acrylamide dust at 15.6 mg/m^3, a lethal or near-lethal concentration (American Cyanamid Company 1953a). Acute-duration dermal exposure elicited indications of male-mediated decreased fertility in rabbits at 50 mg/kg/day (Gutierrez-Espeleta et al. 1992), clinical signs of neurological effects in rats at doses ≥200 mg/kg (American Cyanamid Company 1973), and slight initial weight loss and slight dermal irritation in rabbits at doses ≥500 mg/kg (American Cyanamid Company 1951; Dow Chemical Company 1957).

Numerous reports are available regarding the effects of acute-duration oral exposure in animals. Acute oral LD_{50} values range from 107 to 413 mg/kg (American Cyanamid Company 1951, 1973, 1977; Dow Chemical Company 1957; Fullerton and Barnes 1966; Hashimoto et al. 1981; McCollister et al. 1964; Tilson and Cabe 1979; Union Carbide Corporation 1947). All mice (4/sex) given acrylamide in the drinking water at a concentration resulting in an estimated dose of 150 mg/kg/day were sacrificed moribund on the 10th day of treatment (NTP 2011b). Symptoms of acrylamide-induced neurotoxicity were elicited by single oral doses at lethal or near-lethal levels (100–200 mg/kg) (American Cyanamid Company 1953c; Fullerton and Barnes 1966; McCollister et al. 1964; Tilson and Cabe 1979) and at lower dose levels (25–77 mg/kg/day) during repeated oral dosing for ≥14 days (Dixit et al. 1981; Gilbert and Maurissen 1982; NTP 2011b; Tyl et al. 2000b). Single oral dosing at 63–150 mg/kg resulted in depressed body weight gain or actual weight loss (Dow Chemical Company 1957; Sakamoto et al. 1988); similar effects on body weight were elicited during repeated oral dosing at lower dose levels (15–20 mg/kg/day) (Burek et al. 1980; Tyl et al. 2000b). Male-mediated implantation losses were noted following acute-duration repeated oral dosing of rats at levels as low as 15–45 mg/kg/day (Sublet et al. 1989; Tyl et al. 2000b; Working et al. 1987). Results from the study of Sublet et al. (1989) served as the basis for deriving an acute-duration oral MRL for acrylamide.

Additional acute-duration studies of animals exposed by inhalation could be designed to assess exposure concentration-response relationships and provide a basis for an acute-duration inhalation MRL for acrylamide, although the general population is not likely to encounter acutely hazardous airborne concentrations of acrylamide. Additional dermal studies using multiple dose levels could be designed to more extensively characterize the hazards of dermal exposure to acrylamide.

Intermediate-Duration Exposure. Information in humans is available from numerous case reports in which acrylamide exposure was associated with signs of impaired neurological performance in central and peripheral nervous systems that include impaired motor function and muscle weakness (Auld and Bedwell 1967; Davenport et al. 1976; Dumitru 1989; Fullerton 1969; Garland and Patterson 1967; Gjerløff et al. 2001; Igisu et al. 1975; Kesson et al. 1977; Mapp et al. 1977; Mulloy 1996; Takahashi et al. 1971). Human data are also available from cross-sectional studies that included self-reported symptoms and neurological evaluations of acrylamide-exposed workers with potential for inhalation and dermal (and possibly oral) exposure (Bachmann et al. 1992; Calleman et al. 1994; Hagmar et al. 2001; He et al. 1989; Myers and Macun 1991). In the cross-sectional studies, workers were exposed for time periods as short as 1 month (but predominantly for more than 1 year); typical signs and symptoms of acrylamide-induced neuropathy were reported. However, the human studies do not include meaningful exposure-response data, and relative contributions of inhalation, dermal, and oral exposure routes could not be determined.

Most available intermediate-duration animal studies employed the oral exposure route. Treatment-related deaths were noted at repeated doses in the range of 25–50 mg/kg/day (American Cyanamid Company 1953b, 1991; Fullerton and Barnes 1966; Schulze and Boysen 1991). Several studies reported acrylamide-induced adverse effects on body weight (American Cyanamid Company 1953b, 1979, 1991; Burek et al. 1980; Field et al. 1990; Friedman et al. 1999; Ko et al. 1999; NTP 2011b; Post and McLeod 1977a; Satchell and McLeod 1981; Schulze and Boysen 1991; Shi et al. 2011; Tanii and Hashimoto 1983; Wang et al. 2010a; Wise et al. 1995). One study reported 12% decreased maternal weight gain in rats administered acrylamide at a dose as low as 7.5 mg/kg/day during gestation days 6–20 (Field et al. 1990). A 28-day drinking water study reported acrylamide-associated decreases in serum testosterone and thyroid hormones T_3 and T_4 in male rats (American Cyanamid Company 1991). Male-mediated implantation losses were noted following intermediate-duration repeated oral dosing of rats or mice at levels as low as 2.8–13.3 mg/kg/day (Chapin et al. 1995; Sakamoto and Hashimoto 1986; Smith et al. 1986; Tyl et al. 2000a; Zenick et al. 1986). The developmental toxicity of acrylamide has been assessed to some extent (American Cyanamid Company 1979; Field et al. 1990; Ferguson et al. 2010; Garey and

Paule 2007, 2010; Garey et al. 2005; Husain et al. 1987; Sakamoto and Hashimoto 1986; Wise et al. 1995). NTP (2011b) reported congestion and pigmentation in the spleen and erythroid cell hyperplasia in the bone marrow of male and female F344/N rats receiving acrylamide from the drinking water for 13 weeks at estimated doses in the range of 22–26 mg/kg/day. Adverse effects on sperm parameters have been observed in laboratory animals following oral exposure to acrylamide for intermediate durations at doses in the range of 4–10 mg/kg/day (Kermani-Alghoraishi et al. 2010; Takami et al. 2011; Wang et al. 2010a; Yuxin et al. 2011).

Numerous studies reported clinical signs and other indicators (e.g., hindlimb splay) of neurological effects associated with intermediate-duration oral exposure in laboratory animals at doses in the range of 5.7–100 mg/kg/day (American Cyanamid Company 1953b, 1953c, 1959, 1991; Burek et al. 1980; Dixit et al. 1981; Dow Chemical Company 1981; Eskin et al. 1985; Field et al. 1990; Friedman et al. 1999; Fullerton and Barnes 1966; Gorzinski et al. 1979; Hashimoto et al. 1981; Hersch et al. 1989a, 1989b; Hopkins 1970; Ko et al. 1999, 2000, 2002; Leswing and Ribelin 1969; Maurissen et al. 1983; McCollister et al. 1964; Merigan et al. 1985; NTP 2011b; Ogawa et al. 2011; Post and McLeod 1977a; Satchell and McLeod 1981; Shi et al. 2011; Tanii and Hashimoto 1983; Tilson and Cabe 1979; Wise et al. 1995; Zenick et al. 1986). In general, the higher the dose level, the earlier the appearance of neurological signs. Some studies included histopathological evaluation of peripheral nerve fibers in acrylamide-treated animals (Burek et al. 1980; Eskin et al. 1985; Gorzinski et al. 1979; Johnson et al. 1985, 1986; NTP 2011b; Schulze and Boysen 1991). One study reported ultrastructural evidence of degenerative effects in sciatic nerve fibers from male F344 rats that had received acrylamide from the drinking water at 1 mg/kg/day for up to 93 days and served as the basis for deriving an intermediate-duration oral MRL for acrylamide (Burek et al. 1980). Shi et al. (2011) reported treatment-related biochemical and histopathologic changes in the cerebellum of rats administered acrylamide by gavage at doses of 15 or 30 mg/kg/day for 4 weeks.

Available intermediate-duration animal data for inhalation and dermal exposure are limited. Death and clinical signs of neurotoxicity were reported in dogs repeatedly exposed to acrylamide dust at 15.6 mg/m^3, for up to 16 days (American Cyanamid Company 1953a) and rabbits administered acrylamide dermally at 50 mg/kg/day for 5 weeks (Rohm and Haas 1975). No adverse effects were observed in cats repeatedly exposed to acrylamide dust at 4.8 mg/m^3 for up to 3 months (American Cyanamid Company 1954).

Additional intermediate-duration studies of animals exposed by inhalation designed to assess exposure concentration-response relationships could serve as the basis for deriving an intermediate-duration inhalation MRL for acrylamide. Additional dermal studies using multiple dose levels could be designed to better characterize the hazards of intermediate-duration dermal exposure to acrylamide.

Chronic-Duration Exposure and Cancer. Reports of chronic occupational exposure in humans describe a spectrum of neurological effects resulting from inhalation (Calleman et al. 1994; Myers and Macun 1991) and dermal effects such as peeling of skin as a result of dermal exposure (Bachmann et al. 1992; Calleman et al. 1994; He et al. 1989; Myers and Macun 1991). Questionnaires, physical examinations, neurological examinations, and ENMG tests provide support from a cross-sectional analysis on factory workers in China (Calleman et al. 1994). This study also utilized hemoglobin adduct levels as a biomarker (Calleman et al. 1994). Neurological effects in rats from chronic oral exposures to acrylamide are documented and provide NOAELs and LOAELs for statistically significantly increased incidences of degenerative effects in peripheral nerve fibers (Friedman et al. 1995; Johnson et al. 1984, 1985, 1986; NTP 2011b).

Epidemiological information includes a number of prospective cohort studies (Hogervorst et al. 2007, 2008a, 2008b, 2009a, 2009b; Larsson et al. 2009a, 2009b, 2009c, 2009d, 2009e; Mucci et al. 2006; Schouten et al. 2009; Wilson et al. 2009b, 2010) and case-control studies (Lin et al. 2010; Michels et al. 2006; Mucci et al. 2003, 2004, 2005; Pelucchi et al. 2006, 2007, 2011a; Wilson et al. 2009a) that assessed the risk of cancer from acrylamide dietary intake. Most studies found no statistically significant associations between acrylamide in food and risks of cancers of the oro-hypopharynx, larynx, or thyroid gland (Schouten et al. 2009); esophagus, stomach, or pancreas (Hirvonen et al. 2010; Hogervorst et al. 2008b); colon or rectum (Hirvonen et al. 2010; Hogervorst et al. 2008b; Larsen et al. 2009c; Mucci et al. 2006); bladder or prostate (Hirvonen et al. 2010; Hogervorst et al. 2008a; Larsson et al. 2009e); lung (Hogervorst et al. 2009b); brain (Hogervorst et al. 2009a); breast (Hogervorst et al. 2007; Larsson et al. 2009d; Wilson et al. 2009b, 2010); endometrium (Hogervorst et al. 2007; Larsson et al. 2009a); ovarian epithelium (Larsson et al. 2009b); or lymphomas (Hirvonen et al. 2010).

However, Wilson et al. (2010) reported increased risk for endometrial cancer among “high” acrylamide consumers in the Nurses’ Health Study. Hirvonen et al. (2010) reported increased risk for lung cancer within a cohort of 27,111 male smokers identified through the Finnish Cancer Registry. Two prospective studies of a Dutch population reported increased risks of postmenopausal endometrial and ovarian cancer (Hogervorst et al. 2007) and renal cell cancer (Hogervorst et al. 2008a) with increasing dietary acrylamide

in prospective studies of a Dutch population, but in these studies, estimations of dietary acrylamide levels in foods on the market at baseline in 1986 were based on food samples analyzed since 2001 and questionnaires did not include details regarding specifics of food preparation. A Swedish nationwide, population-based case-control study reported a significant association between dietary acrylamide intake and risk of esophageal cancer (Lin et al. 2010). Results of another case-control study indicate a possible association between diet before puberty and subsequent risk of breast cancer; this particular study, however, is of limited use due to lacking documentation of significant factors such as cooking methods and accurate recall (Michels et al. 2006). Olesen et al. (2008) reported a significant positive correlation between acrylamide-hemoglobin adduct levels and ER+ breast cancer, but the conclusions are also only of limited use due to the small study size and uncertainty regarding extrapolation of exposure.

Lifetime cancer bioassays of F344 rats (Johnson et al. 1984, 1986; Friedman et al. 1995; NTP 2011b) and B6C3F1 mice (NTP 2011b) are available; results of Friedman et al. (1995) served as the basis for a chronic-duration oral MRL for acrylamide. The NCTR (2009) is performing carcinogenicity bioassays of B6C3F1 mice that were neonatally exposed to acrylamide or glycidamide; the results of these bioassays are not presently available to the public.

Genotoxicity. The genotoxicity of acrylamide has been studied both *in vivo* and *in vitro*. Studies are limited almost exclusively to laboratory rodents and nonmammalian species with the exception of a few *in vitro* assays of human cells. Results indicate that acrylamide is genotoxic and most potent in its ability to induce clastogenic effects (including heritable translocations in offspring of acrylamide-exposed male rodents mated with untreated females), DNA damage, and gene mutations (including male-germ-cell-mediated dominant lethal mutations and heritable specific-locus mutations).

Genotoxicity assessments for acrylamide and its metabolite, glycidamide, in rats and mice are currently underway at NTCR (2009). Post-public comment drafts of this ATSDR Toxicological Profile for Acrylamide will include available results from these assessments.

Reproductive Toxicity. The reproductive toxicity of acrylamide has been studied almost exclusively in orally-exposed rats and mice. Pre- and postimplantation losses and decreased numbers of live fetuses were noted at repeated doses in the range of 3–60 mg/kg/day (Chapin et al. 1995; Sakamoto and Hashimoto 1986; Smith et al. 1986; Sublet et al. 1989; Tyl et al. 2000a, 2000b; Working et al. 1987; Zenick et al. 1986).

Results of dominant lethality testing and crossover trials indicate that acrylamide induces male-mediated reproductive effects at repeated oral doses in the range of 2.8–19 mg/kg/day (Chapin et al. 1995; Sakamoto and Hashimoto 1986; Smith et al. 1986; Tyl et al. 2000a, 2000b; Zenick et al. 1986). Other reported effects include decreased sperm mobility in Long-Evans rats receiving acrylamide at 45 mg/kg/day for 5 days (Sublet et al. 1989), degenerative effects in spermatids of ddY mice dosed at 100 or 150 mg/kg/day for 5 days (Sakamoto et al. 1988), and testicular atrophy in F344 rats at doses as low as 5 mg/kg/day for 90 days (American Cyanamid Company 1991; Burek et al. 1980). In apparent contrast, Tyl et al. (2000b) found no significant effects on sperm parameters in Long-Evans hooded rats following repeated oral dosing at dose levels as high as 60 mg/kg/day. Gross and histopathologic examinations of reproductive organs and tissues from male and female rats receiving acrylamide from the drinking water for up to 2 years at estimated doses as high as 2 mg/kg/day revealed no signs of acrylamide-induced effects (Friedman et al. 1995; Johnson et al. 1984, 1986). Prebreeding exposure of female rats and mice to acrylamide at doses of approximately 14 and 19 mg/kg/day did not adversely affect reproductive performance (Sakamoto and Hashimoto 1986; Zenick et al. 1986).

Adverse effects on sperm parameters have been observed in laboratory animals following repeated oral exposure to acrylamide at doses in the range of 4–10 mg/kg/day (Kermani-Alghoraishi et al. 2010; Takami et al. 2011; Wang et al. 2010a; Yuxin et al. 2011).

The reproductive toxicity of acrylamide appears to have been adequately characterized in laboratory animals. Continued assessment of possible reproductive effects in humans with potential for significant exposure to acrylamide is recommended.

Developmental Toxicity. The developmental toxicity of acrylamide has been assessed only in oral studies of rats and mice. Effects include body weight decreases and decreased auditory startle response in offspring of female Sprague-Dawley rats exposed on gestation days 6–10 (Wise et al. 1995), decreased mean fetal body weight in offspring of CD-1 mouse dams administered acrylamide by gavage during gestation days 6–20 (Field et al. 1990), decreased mean fetal body weight in offspring of Sprague-Dawley rat dams receiving acrylamide from the drinking water from gestation day 6 throughout lactation (Takahashi et al. 2009), decreased brain levels of selected catecholamines (noradrenaline, dopamine, 5-hydroxytryptamine) in pups of rat dams administered acrylamide during lactation only or in young pups receiving the same dose for 5 days (Husain et al. 1987), deficiencies in cognitive motivation, learning ability, and motor skills in F344 rats exposed during gestation and lactation and extending through postpartum developmental periods and/or early adulthood (Garey and Paule 2007, 2010; Garey et al.

2005; Ferguson et al. 2010). No exposure-related fetal malformations or variations (gross, visceral, or skeletal) were found in offspring of Sprague-Dawley rats administered acrylamide at doses of 2.5, 7.5, or 15 mg/kg/day on gestation days 6–20 or in CD-1 mice at doses of 3, 15, or 45 mg/kg/day on gestation days 6–17 (Field et al. 1990).

Section 3.12.3 (Ongoing Studies) lists information regarding an ongoing developmental neurotoxicity study in rats. The results of this study should be evaluated prior to assessing a need for additional developmental toxicity studies of acrylamide.

Immunotoxicity. No human or animal data were located in which acrylamide was considered to be a potential immunological concern. Immunotoxicity studies do not appear necessary at this time.

Neurotoxicity. Information in humans is available from numerous case reports in which acrylamide exposure has been associated with signs of impaired neurological performance in central and peripheral nervous systems that include impaired motor function and muscle weakness (Auld and Bedwell 1967; Davenport et al. 1976; Dumitru 1989; Fullerton 1969; Garland and Patterson 1967; Gjerløff et al. 2001; Igisu et al. 1975; Kesson et al. 1977; Mapp et al. 1977; Mulloy 1996; Takahashi et al. 1971). Human data are also available from cross-sectional studies that included self-reported symptoms and neurological evaluations of acrylamide-exposed workers with potential for inhalation and dermal (and possibly oral) exposure (Bachmann et al. 1992; Calleman et al. 1994; Hagmar et al. 2001; He et al. 1989; Myers and Macun 1991).

Neurological effects associated with oral exposure to acrylamide have been well characterized in laboratory animals and include clinical signs such as twitching, loss of balance, tremors, lethargy, and general weakness and more subtle indicators of functional deficits such as decreased rotarod performance and increased limb or foot splay. Evidence of degenerative lesions in peripheral nerve fibers, as observed by light and electron microscopy, have been detected at oral doses lower than those eliciting clinical signs and other overt indications of functional deficit. See Sections 3.2.1.4, 3.2.2.4, and 3.2.3.4 for detailed information regarding neurological effects in acrylamide-exposed animals. Available animal data appear to adequately characterize acrylamide neurotoxicity; additional neurotoxicity studies do not appear necessary at this time.

Refer to Section 3.12.2 (Developmental Effects) for a summary of information regarding acrylamide-induced neurodevelopmental effects.

Epidemiological and Human Dosimetry Studies. Early epidemiological studies focused on neurological signs and symptoms in workers employed in the manufacture and/or use of acrylamide (Bachmann et al. 1992; Calleman et al. 1994; Hagmar et al. 2001; He et al. 1989; Myers and Macun 1991). Although these studies, as well as available case reports, provide supportive evidence of acrylamide-induced neurotoxicity, they lack information regarding relative contributions of natural exposure routes (inhalation, oral, dermal), exposure-response relationships, and other confounding exposures.

More recent investigations have focused on examining possible associations between acrylamide dietary intake and various cancer end points (Hogervorst et al. 2007, 2008a, 2008b, 2009a, 2009b; Larsson et al. 2009a, 2009b, 2009c, 2009d, 2009e; Lin et al. 2010; Michels et al. 2006; Mucci et al. 2003, 2004, 2005, 2006; Pelucchi et al. 2006, 2007, 2011a; Schouten et al. 2009; Wilson et al. 2009a, 2009b, 2010). These studies provide limited evidence of acrylamide-induced carcinogenicity from estimated intakes through the diet. However, a major deficiency of these studies is the use of questionnaires to estimate prior dietary intake of acrylamide.

Continued epidemiological research should focus on improving methods for estimating acrylamide intake. Additional information regarding biomarkers of exposure to acrylamide and improved PBPK modeling could be beneficial.

Biomarkers of Exposure and Effect.

Exposure. Biomarkers of exposure to acrylamide include unchanged acrylamide, its mercapturic acid metabolite, AAMA, its epoxy derivative, glycidamide, and the respective metabolite of glycidamide, GAMA in urine (Bjellaas et al. 2007a; Boettcher et al. 2005, 2006a, 2006b; Fuhr et al. 2006; Huang et al. 2011a, 2011b) and hemoglobin adducts of acrylamide and glycidamide (Bergmark et al. 1993; Boettcher et al. 2005; Calleman et al. 1994; Fennell et al. 2005b; Hagmer et al. 2001; Olesen et al. 2008). However, N-methylolacrylamide forms hemoglobin adducts that are indistinguishable from hemoglobin adducts of acrylamide. The development of a method to measure levels of the metabolite, glyceramide, in urine could help to determine the relative importance of glycidamide via glutathione conjugation versus hydrolysis.

See Section 3.12.3 (Ongoing Studies) for information regarding assessments currently underway to assess biomarkers of exposure to acrylamide.

Effect. Glycidamide-DNA adducts may be considered a biomarker of effect from exposure to acrylamide (Doerge et al. 2005a; Gamboa da Costa et al. 2003). It is not likely that additional biomarkers of effect specific to acrylamide would be identified.

Absorption, Distribution, Metabolism, and Excretion. Available human data indicate that acrylamide is readily absorbed following oral or dermal exposure; significant absorption of inhaled acrylamide is expected. In animals, acrylamide is readily absorbed following inhalation, oral, or dermal exposure. Absorbed acrylamide is widely distributed (accumulates only in red blood cells), rapidly metabolized in the liver, and excreted in the urine mainly as acrylamide-derived conjugates. Existing PBPK models for acrylamide are not adequate for human health risk assessment. However, as described in Section 3.12.3 (Ongoing Studies) a new PBPK model for acrylamide is presently being developed. Additional pharmacokinetic studies may be necessary to assist in calibration and validation of PBPK models for acrylamide.

Comparative Toxicokinetics. Available data in rats and mice indicate species differences in acrylamide metabolism. Additional comparative toxicokinetic studies may be warranted pending the outcome of the new PBPK model being developed for acrylamide.

Methods for Reducing Toxic Effects. Suggested methods for reducing absorption of acrylamide include removal from exposure source, decontamination of exposed skin with soap and water, and administration of activated charcoal within 1 hour following ingestion. There are no known methods for reducing the body burden following absorption of acrylamide and no proven methods for interfering with the mechanism of action for toxic effects.

Children's Susceptibility. No specific data were located regarding acrylamide-induced health effects in children. The database of information in animals provides conflicting results. Children are not expected to be less susceptible than adults to acrylamide toxicity.

Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Search of the NCTR website revealed the following (NCTR 2009):

Dr. Frederick Beland (principal investigator), Division of Biochemical Toxicology, is comparing the carcinogenicity of acrylamide and its metabolite glycidamide in B6C3F1 mice treated neonatally and in B6C3F1 mice and Fischer 344 rats treated for 2 years. The 2-year study is funded by the National Toxicology Program (NTP).

Dr. Daniel Doerge (principal investigator), Division of Biochemical Toxicology, in collaboration with the Division of Genetic and Reproductive Toxicology and Division of Personalized Nutrition and Medicine and funding from the University of Maryland, is working to (1) develop a PBPK/PD model for acrylamide and glycidamide and (2) determine mutagenicity of acrylamide and glycidamide in Big Blue® rats. Dr. Doerge, in collaboration with the Department of genetic and Reproductive Toxicology and funding from NTP, is studying genotoxicity, mutagenicity, and biomarkers of exposure for acrylamide and glycidamide in rodents.

Dr. Merle Paule (principal investigator), Division of Neurotoxicology, in collaboration with the Division of Biochemical Toxicology and funding from NTP, is assessing developmental neurotoxicity of acrylamide in rats.

Dr. Eden Tareke (principal investigator), Division of Neurotoxicology, is investigating the effects of acrylamide on normal human brain cortical neuronal (HCN-1), PC12, and HepG2 cells *in vitro*.

Search of the Federal Research in Progress database (FEDRIP 2009) revealed the following:

Dr. John Essigmann (principal investigator), Massachusetts Institute of Technology, Cambridge, Massachusetts, is investigating the genotoxic effects of several compounds, including acrylamide, with the goal of developing new biomarkers. The sponsoring organization is the National Institute of Environmental Health Sciences.

Dr. Richard LoPachin (principal investigator), Montefiore Medical Center, Bronx, New York, is performing ongoing research to elucidate specific mechanisms responsible for acrylamide toxicity.