# TOXICOLOGICAL PROFILE FOR ACRYLAMIDE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

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# DISCLAIMER

Use of trade names is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry, the Public Health Service, or the U.S. Department of Health and Human Services.

# UPDATE STATEMENT

A Toxicological Profile for Acrylamide Draft for Public Comment was released in September, 2009. This edition supersedes any previously released draft or final profile.

Toxicological profiles are revised and republished as necessary. For information regarding the update status of previously released profiles, contact ATSDR at:

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Division of Toxicology and Human Health Sciences
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1600 Clifton Road NE
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# FOREWORD

This toxicological profile is prepared in accordance with guidelines* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the toxic substances each profile describes. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The profiles focus on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. A health effects summary describes the adequacy of information to determine a substance's health effects. ATSDR identifies data needs that are significant to protection of public health.

Each profile:

(A) Examines, summarizes, and interprets available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;

(B) Determines whether adequate information on the health effects of each substance is available or being developed to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and

(C) Where appropriate, identifies toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are federal, state, and local health professionals; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staff of the Centers for Disease Control and Prevention and other federal scientists also have reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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*Legislative Background

The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the National Priorities List, in an effort to "…establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

# QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances will find the following information helpful for fast answers to often-asked questions.

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***Primary Chapters/Sections of Interest***

**Chapter 1: Public Health Statement**: The Public Health Statement can be a useful tool for educating patients about possible exposure to a hazardous substance. It explains a substance's relevant toxicologic properties in a nontechnical, question-and-answer format, and it includes a review of the general health effects observed following exposure.

**Chapter 2: Relevance to Public Health**: The Relevance to Public Health Section evaluates, interprets, and assesses the significance of toxicity data to human health.

**Chapter 3: Health Effects**: Specific health effects of a given hazardous compound are reported by type of health effect (death, systemic, immunologic, reproductive), by route of exposure, and by length of exposure (acute, intermediate, and chronic). In addition, both human and animal studies are reported in this section.
***NOTE***: Not all health effects reported in this section are necessarily observed in the clinical setting. Please refer to the Public Health Statement to identify general health effects observed following exposure.

**Pediatrics**: Four new sections have been added to each Toxicological Profile to address child health issues:

**Section 1.6** **How Can (Chemical X) Affect Children?**
**Section 1.7** **How Can Families Reduce the Risk of Exposure to (Chemical X)?**
**Section 3.7** **Children's Susceptibility**
**Section 6.6** **Exposures of Children**

**Other Sections of Interest:**

**Section 3.8** **Biomarkers of Exposure and Effect**
**Section 3.11** **Methods for Reducing Toxic Effects**

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***ATSDR Information Center***

***Phone:*** 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY) ***Fax:*** (770) 488-4178
***E-mail:*** cdcinfo@cdc.gov ***Internet***: http://www.atsdr.cdc.gov

The following additional material can be ordered through the ATSDR Information Center:

*Case Studies in Environmental Medicine: Taking an Exposure History*—The importance of taking an exposure history and how to conduct one are described, and an example of a thorough exposure history is provided. Other case studies of interest include *Reproductive and Developmental Hazards; Skin Lesions and Environmental Exposures; Cholinesterase-Inhibiting Pesticide Toxicity*; and numerous chemical-specific case studies.

*Managing Hazardous Materials Incidents* is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident. Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposures*—is a guide for health care professionals treating patients exposed to hazardous materials.

*Fact Sheets (ToxFAQs)* provide answers to frequently asked questions about toxic substances.

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***Other Agencies and Organizations***

*The National Center for Environmental Health* (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015.

*The National Institute for Occupational Safety and Health* (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 200 Independence Avenue, SW, Washington, DC 20201 • Phone: 800-356-4674 or NIOSH Technical Information Branch, Robert A. Taft Laboratory, Mailstop C-19, 4676 Columbia Parkway, Cincinnati, OH 45226-1998 • Phone: 800-35-NIOSH.

*The National Institute of Environmental Health Sciences* (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212.

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***Referrals***

*The Association of Occupational and Environmental Clinics* (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

*The American College of Occupational and Environmental Medicine* (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266.

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## THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.

2. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific Minimal Risk Levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.

3. Data Needs Review. The Environmental Toxicology Branch reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.

4. Green Border Review. Green Border review assures the consistency with ATSDR policy.

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# PEER REVIEW

A peer review panel was assembled for acrylamide. The panel consisted of the following members:

1. Herman Bolt, M.D., Ph.D., Professor Emeritus, Leibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany

2. Timothy Fennell, Ph.D., Senior Research Chemist, RTI International, Drug Metabolism and Pharmacokinetics Department, Research Triangle Park, NC

3. James Klaunig, Ph.D., Professor of Public Health, Director, Center for Environmental Health, Indiana University School of Medicine, Indianapolis, IN

These experts collectively have knowledge of acrylamide's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

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# 1. PUBLIC HEALTH STATEMENT

This public health statement tells you about acrylamide and the effects of exposure to it.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites are then placed on the National Priorities List (NPL) and are targeted for long-term federal clean-up activities. Acrylamide has been found in at least 3 of the 1,699 current or former NPL sites. Although the total number of NPL sites evaluated for this substance is not known, the possibility exists that the number of sites at which acrylamide is found may increase in the future as more sites are evaluated. This information is important because these sites may be sources of exposure and exposure to this substance may be harmful.

When a substance is released either from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment. Such a release does not always lead to exposure. You can be exposed to a substance only when you come in contact with it. You may be exposed by breathing, eating, or drinking the substance, or by skin contact.

If you are exposed to acrylamide, many factors will determine whether you will be harmed. These factors include the dose (how much), the duration (how long), and how you come in contact with it. You must also consider any other chemicals you are exposed to and your age, sex, diet, family traits, lifestyle, and state of health.

## 1.1 WHAT IS ACRYLAMIDE?

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|---|---|
| **White or colorless, odorless crystalline solid** | Acrylamide can violently react when melting. When heated, acrid fumes may be released. |
| **Used in industry** | Acrylamide is used to make polyacrylamide, which is mainly used in treating effluent from water treatment plants and industrial processes. |

## 1.2 WHAT HAPPENS TO ACRYLAMIDE WHEN IT ENTERS THE ENVIRONMENT?

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| **Most commonly found in water** | Acrylamide may enter drinking water if polyacrylamide is used in the treatment process. It can be found in soils, but is rarely found in air. |

| | |
|---|---|
| **Rapidly broken down in soil and water** | If acrylamide enters soil or water, it will be broken down quickly by bacteria. |

## 1.3 HOW MIGHT I BE EXPOSED TO ACRYLAMIDE?

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| **Water and soil** | Drinking water can sometimes contain acrylamide. It can enter drinking water from the treatment process of municipal supplies as well as from substances used to construct dams and wells. Acrylamide breaks down quickly in water and soil, but there is still a chance of exposure if you live near a plastics or dye plants. |
| **Inhalation and dermal contact** | If you smoke, or breathe second-hand tobacco smoke, you might be exposed to acrylamide. Tobacco smoke is a major source of acrylamide exposure within the general population. People involved in the production or use of acrylamide and acrylamide-containing products are exposed if they breathe in air that contains acrylamide. They may also be exposed by coming into skin contact with acrylamide. |
| **Food** | Acrylamide is formed in foods that are rich in carbohydrates (particularly potatoes) when they are fried, grilled, or baked at normal cooking temperatures. Levels of acrylamide in these foods increase with higher temperatures and longer cooking times. Protein-based foods (such as meats) probably contain low amounts of acrylamide. Ingestion of foods that contain acrylamide is a primary source of exposure. |

## 1.4 HOW CAN ACRYLAMIDE ENTER AND LEAVE MY BODY?

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| **May enter your body through food, drinking water, breathing, and skin contact** | Acrylamide can enter your body when you eat foods or drink water containing acrylamide. Breathing tobacco smoke may cause some level of acrylamide to enter your lungs. Acrylamide can also enter your body if it comes in contact with your skin. Dermal contact with acrylamide can occur if you work in the manufacture of acrylamide or polyacrylamide gels. |
| **Leaves through bodily fluids** | Once in your body, acrylamide enters your body fluids. Acrylamide and its breakdown products leave your body mostly through urine; small amounts may leave through feces, exhaled air, and breast milk. |

## 1.5 HOW CAN ACRYLAMIDE AFFECT MY HEALTH?

**This section looks at studies concerning potential health effects in animal and human studies.**

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| **Nervous system effects** | Nervous system effects such as muscle weakness, numbness in hands and feet, sweating, unsteadiness, and clumsiness were reported in some acrylamide workers. However, most people are not exposed to acrylamide levels high enough to cause these effects. |
| **Reproductive effects** | Acrylamide reduces the ability of male animals to produce offspring and could cause similar effects in humans, but not likely at exposure levels experienced by most people. |
| **Cancer** | Acrylamide has caused several types of cancer in animals. We do not know whether acrylamide causes cancer in humans. The EPA, International Agency for Research on Cancer (IARC), National Toxicology Program (NTP), and the Department of Health and Human Services have concluded that acrylamide is likely to be carcinogenic to humans. |

## 1.6 HOW CAN ACRYLAMIDE AFFECT CHILDREN?

**This section discusses potential health effects in humans from exposures during the period from conception to maturity at 18 years of age.**

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| **Effects in children** | Acrylamide is expected to affect children in the same manner as adults. It is not known whether children are more susceptible than adults to the effects of acrylamide. |
| **Developmental effects** | Effects such as decreased body weight, decreased startle responses, indicators of repressed learning ability and motivation, delayed development of motor skills, and decreased levels of some chemicals involved in transmission of brain signals were seen in some animals exposed to acrylamide before and after birth. There are no reports of acrylamide causing developmental effects in humans. |

## 1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO ACRYLAMIDE?

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| **Limit exposure to tobacco and second-hand smoke** | Tobacco smoke contains acrylamide. Avoid smoking or breathing in second-hand smoke. |

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| **Reduce consumption of foods that contain acrylamide** | Avoid eating a lot of carbohydrate-rich foods that are cooked at high temperatures (e.g., French fries). Foods with higher protein content appear to have lower amounts of acrylamide. Avoid overcooking foods. |

## 1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO ACRYLAMIDE?

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| **Can be measured in blood and urine** | Acrylamide and its breakdown products can be measured in blood and urine. These measurements may be useful in estimating how much acrylamide has entered the body. However, assays to identify exposure to acrylamide are not readily available to clinicians. |

## 1.9 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

The federal government develops regulations and recommendations to protect public health. Regulations can be enforced by law. The EPA, the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA) are some federal agencies that develop regulations for toxic substances. Recommendations provide valuable guidelines to protect public health, but cannot be enforced by law. The Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH) are two federal organizations that develop recommendations for toxic substances.

Regulations and recommendations can be expressed as “not-to-exceed” levels. These are levels of a toxic substance in air, water, soil, or food that do not exceed a critical value. This critical value is usually based on levels that affect animals; they are then adjusted to levels that will help protect humans. Sometimes these not-to-exceed levels differ among federal organizations because they used different exposure times (an 8-hour workday or a 24-hour day), different animal studies, or other factors.

Recommendations and regulations are also updated periodically as more information becomes available. For the most current information, check with the federal agency or organization that provides it.

Some regulations and recommendations for acrylamide include the following:

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| **Levels in drinking water set by EPA** | The EPA has determined that exposure to acrylamide in drinking water at concentrations of 1.5 mg/L for one day or 0.3 mg/L for 10 days is not expected to cause any adverse effects in a child. |
| **Levels in workplace air set by OSHA** | OSHA set a legal limit of 0.3 $mg/m^3$ for acrylamide in air averaged over an 8-hour work day. |

## 1.10 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns, please contact your community or state health or environmental quality department, or contact ATSDR at the address and phone number below.

ATSDR can also tell you the location of occupational and environmental health clinics. These clinics specialize in recognizing, evaluating, and treating illnesses that result from exposure to hazardous substances.

Toxicological profiles are also available on-line at www.atsdr.cdc.gov and on CD-ROM. You may request a copy of the ATSDR ToxProfiles™ CD-ROM by calling the toll-free information and technical assistance number at 1-800-CDCINFO (1-800-232-4636), by e-mail at cdcinfo@cdc.gov, or by writing to:

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Human Health Sciences
1600 Clifton Road NE
Mailstop F-57
Atlanta, GA 30333
Fax: 1-770-488-4178

Organizations for-profit may request copies of final Toxicological Profiles from the following:

National Technical Information Service (NTIS)
5285 Port Royal Road
Springfield, VA 22161
Phone: 1-800-553-6847 or 1-703-605-6000
Web site: http://www.ntis.gov/

# 1. PUBLIC HEALTH STATEMENT

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# 2. RELEVANCE TO PUBLIC HEALTH

## 2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO ACRYLAMIDE IN THE UNITED STATES

Acrylamide is an industrial chemical used mainly in the production of polyacrylamides, which are used primarily as flocculants for clarifying drinking and treating municipal and industrial effluents. In the oil industry, acrylamide is used as a flow control agent to enhance oil production from wells. Acrylamide and polyacrylamides are also used in the production of dyes and organic chemicals, contact lenses, cosmetics and toiletries, permanent-press fabrics, textiles, pulp and paper products; also in ore processing, sugar refining, and as a chemical grouting agent and soil stabilizer for the construction of tunnels, sewers, wells, and reservoirs. In 2008, approximately 141,000 metric tons of acrylamide were produced in the United States.

Acrylamide may be released to the environment during production and use of polyacrylamides, which are used as clarifiers in water treatment. Residual monomer in the polyacrylamide is the main source of drinking water contamination by acrylamide. It can be released to soil and land from plastics and dye industries and from acrylamide-containing grouting agents used in reservoirs and wells. Acrylamide is rarely found in the atmosphere.

Acrylamide is not considered highly persistent in the environment. It is expected to be highly mobile in soil and water and is highly susceptible to biodegradation in both media. It can be removed from soils by hydrolysis, though it is typically not present in soil or the atmosphere. Acrylamide is not expected to significantly bioconcentrate.

Acrylamide is sometimes present in drinking water due to leaching of monomer during treatment processes. Acrylamide was identified in food samples cooked at high temperatures. Concentrations of acrylamide in food vary with the type of food, cooking method, temperature, water content, food thickness, and length of heating. Carbohydrate-rich foods typically contain the highest levels of acrylamide.

Exposure to acrylamide occurs mainly through ingestion, dermal contact, and inhalation. Ingestion of foods containing acrylamide appears to be one of the most common methods of exposure for the general public. Average estimated intake of acrylamide from food sources ranged from 0.3 to 0.8 μg/kg bw/day. Children may be susceptible to food-borne exposure 2–3 times that of adults on a body weight basis.

Ingestion of contaminated drinking water can result in exposure to acrylamide. Inhalation of tobacco smoke, including second-hand smoke, can result in inhalation exposure for both adults and children. In the body, acrylamide can cross the placenta and result in exposure to unborn children. Breast milk has been shown to contain acrylamide in mothers with diets high in acrylamide-containing foods. Occupational exposure to acrylamide is primarily due to dermal contact when handling bags and drums of the chemical, followed by inhalation of dust or aerosols.

## 2.2 SUMMARY OF HEALTH EFFECTS

**Neurological Effects.** Ataxia and skeletal muscle weakness are hallmark symptoms of acrylamide-induced central-peripheral neuropathy. Available information in humans includes case reports and cross-sectional studies in which reported symptoms of neuropathy were primarily associated with occupational exposure potential via both inhalation and dermal contact. Numerous animal studies confirm the neurological effects of acrylamide exposure. Available information in animals primarily involves oral exposure. Clinical signs can be elicited following single oral dosing in the range of 100–200 mg/kg; lower dose levels require repeated dosing to elicit clinical signs. Histopathological evidence of acrylamide-induced peripheral neuropathy has been observed in rats receiving oral doses as low as 1 mg/kg/day for 3 months; the observed degenerative effects in peripheral nerve fibers at such dose levels have been shown to be completely reversible within a few months following the cessation of exposure.

**Reproductive Effects.** Pre- and postimplantation losses and decreased numbers of live fetuses have been observed in rats and mice receiving repeated oral doses of acrylamide in the range of 3–60 mg/kg/day. Results of dominant lethality testing and crossover trials indicate that acrylamide-induced reproductive effects are male mediated. Other reported effects in acrylamide-treated laboratory animals include decreases in sperm concentration and mobility, degenerative effects in spermatids and germinal epithelium of testis and epididymis, and testicular atrophy, which were observed at repeated oral doses as low as 3–5 mg/kg/day in some studies.

**Cancer.** Available cohort mortality studies of occupationally-exposed workers have not found associations between acrylamide and mortality from cancer. Available human data on cancer risk from acrylamide in food include case-control studies and reports from ongoing prospective cohort studies. Most of these studies found no significant associations between dietary acrylamide and the risk of cancers; however, risks for selected cancers were slightly elevated in a few instances.

Results of two similarly-designed lifetime studies in orally-exposed rats provide clear evidence of acrylamide-induced carcinogenicity. Reported effects common to both studies include significantly increased incidences of thyroid (follicular cell) tumors, mesotheliomas of the tunica vaginalis testis, and mammary gland tumors. Findings of significantly increased incidences of adrenal pheochromocytomas in male rats and tumors of the oral cavity, central nervous system (glial origin), pituitary, and clitoris or uterus in female rats in the earlier bioassay were not replicated in the second bioassay.

More recent 2-year cancer bioassays in rats and mice exposed to acrylamide via the drinking water confirm the earlier findings of acrylamide carcinogenicity. In these bioassays, treatment-related significantly increased incidences of selected tumors were observed in the epididymis, heart, pancreatic islets, and thyroid gland of male rats; clitoral gland, mammary gland, oral mucosa or tongue, skin, and thyroid gland of female rats; Harderian gland, lung, and forestomach of male mice; and Harderian gland, lung, mammary gland, ovary, and skin of female mice.

Acrylamide was reported to initiate skin tumors in female mice administered repeated oral doses of acrylamide followed by dermal applications of 12-O-tetradecanoylphorbol-13-acetate (TPA, a tumor promoter) to the shaved back.

EPA has characterized acrylamide as "likely to be carcinogenic to humans" based on findings that chronic oral exposure to acrylamide in the drinking water of laboratory animals induced significantly increased incidences of selected tumors in multiple bioassays; that oral, intraperitoneal, or dermal exposure to acrylamide initiated skin tumors in two strains of mice; that intraperitoneal injections of acrylamide induced lung tumors in one strain of mice; and that there is ample evidence for the ability of acrylamide to induce genotoxic effects in mammalian cells. The International Agency for Research on Cancer assigned acrylamide to Group 2A (probably carcinogenic to humans) based on similar assessment. The National Toxicology Program has determined that acrylamide is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals.

**Body Weight Effects.** Depressed body weight, including actual body weight loss, and depressed body weight gain were consistently reported in laboratory animals following oral exposure to acrylamide. For example, adverse effects on body weight have been observed following single or repeated dosing at relatively high dose levels for short durations (≤5 days). In one study of rats administered oral doses as low as 15 mg/kg/day for 5 consecutive days, body weight gain was depressed by approximately 40%; higher doses (30–60 mg/kg/day) caused actual weight loss. An 8-week dosing period to dogs at

7 mg/kg/day resulted in weight loss. Weight loss was reported in cats receiving acrylamide orally for 16 weeks at 15 mg/kg/day.

**Developmental Effects.** Most animal studies found no signs of acrylamide-related overt developmental effects in the offspring of animals whose mothers had received nontoxic doses of acrylamide during the development of their fetuses and pups. However, there is some evidence that relatively low oral doses (in the range of 4–25 mg/kg/day) during pre- and postnatal periods of development may result in signs of delayed motor development, repressed learning ability and motivation, decreased brain levels of selected chemicals, and impaired neurogenesis.

## 2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for acrylamide. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

### *Inhalation MRLs*

No inhalation MRLs were derived due to the lack of appropriate data on the effects of inhalation exposure to acrylamide.

Numerous accounts of associations between exposure to acrylamide and neurological impairment are available (Auld and Bedwell 1967; Bachmann et al. 1992; Calleman et al. 1994; Davenport et al. 1976; Donovan and Pearson 1987; Dumitru 1989; Fullerton 1969; Garland and Patterson 1967; Gjerløff et al. 2001; Hagmar et al. 2001; He et al. 1989; Igisu and Matsuoka 2002; Igisu et al. 1975; Kesson et al. 1977; Mapp et al. 1977; Mulloy 1996; Myers and Macun 1991; Takahashi et al. 1971). Most associations were made from occupational exposure scenarios that likely included inhalation and dermal (and possibly oral) exposure. Acrylamide levels in the workplace air were measured in some instances; however, reliable exposure-response data are not available.

Acrylamide-induced health effects in laboratory animals following inhalation exposure to acrylamide were assessed in two studies that did not include multiple exposure levels. Mortalities and central nervous system effects were observed in dogs exposed to acrylamide dust at a concentration of 15.6 mg/m$^3$ for 6 hours/day for up to 16 days (American Cyanamid Company 1953a). There were no signs of adverse effects in similarly-exposed guinea pigs. No adverse effects were seen in cats exposed to what was described 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$^3$) (Dow Chemical Company 1957).

### *Oral MRLs*

- An MRL of 0.01 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to acrylamide.

Available human data are limited to case reports that include findings 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.

Single oral doses elicit clinical signs of acrylamide-induced neurological effects in laboratory animals at lethal or near-lethal doses (American Cyanamid Company 1953c; Fullerton and Barnes 1966; McCollister et al. 1964; Tilson and Cabe 1979). Repeated oral dosing elicits clinical signs at lower daily dose levels. For example, convulsions and ataxia were noted as early as day 14 in rats administered acrylamide by daily gavage at 25 mg/kg/day for 21 days (Dixit et al. 1981). Daily exposure of male mice to acrylamide in the drinking water for 12 days at an estimated dose of 25.8 mg/kg/day caused decreased rotarod

performance and increased hindlimb splay as early as days 6 and 8, respectively (Gilbert and Maurissen 1982). 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. 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 66–76 mg/kg/day (NTP 2011b).

Histopathological evaluations of nervous tissues following acute-duration oral exposure to acrylamide are limited to electron microscope results from peripheral nerve preparations in subgroups of control and high-dose (20 mg/kg/day) male rats administered acrylamide in the drinking water for up to 93 days (Burek et al. 1980). No signs of acrylamide-induced degenerative effects were observed after 7 days of treatment, but degeneration in axons and myelin sheath were noted after 33 days of treatment. These interim assessments were not performed at lower dose levels and the effect of treatment following 14 days (maximum acute-duration exposure period as defined by ATSDR) was not assessed.

Acrylamide-induced effects on body weight have been reported in rats following acute-duration oral exposure to acrylamide. In one study, repeated-dose oral exposure of rats to doses as low as 15 mg/kg/day for 5 consecutive days resulted in significantly depressed body weight gain (approximately 40% less than controls); higher doses (30–60 mg/kg/day) caused actual weight loss, whereas no effect on body weight was seen at a dose level of 5 mg/kg/day (Tyl et al. 2000b). Another study reported significantly lower mean body weight (magnitude 8%) as early as treatment day 13 in male rats administered acrylamide in the drinking water at a concentration resulting in an estimated dose level of 20 mg/kg/day, but no effect on body weight at an estimated dose level of 5 mg/kg/day (Burek et al. 1980).

Daily oral administration of acrylamide to male rats at 15 mg/kg/day for as little as 5 days followed by mating with unexposed female rats resulted in reduced mating, decreased fertility, and male-mediated increased pre- and postimplantation losses; these effects were not seen at 5 mg/kg/day (Sublet et al. 1989; Tyl et al. 2000b). A similarly-designed study (Tyl et al. 2000b) confirmed the findings of acrylamide-induced increased implantation loss, although, based on pairwise comparisons with controls, statistical significance was achieved only at the highest dose level (60 mg/kg/day). Tyl et al. (2000b) also noted significantly decreased body weight gain during the 5 days of dosing at 15 mg/kg/day and actual weight loss at higher doses, findings not included in the study report of Sublet et al. (1989). Based on the reproducible results for male-mediated infertility in the studies of Sublet et al. (1989) and Tyl et al.

(2000b) and the lack of supporting information regarding the body weight effects, the male-mediated infertility was selected as the critical effect for deriving an acute-duration oral MRL for acrylamide. As discussed in detail in Appendix A, a physiologically based pharmacokinetic (PBPK) rat model for acrylamide and glycidamide (Sweeney et al. 2010) was used to estimate rat dose metrics for blood time-weighted average (TWA) acrylamide and glycidamide (a readily-formed metabolite of acrylamide in aqueous environment) doses for each of the administered acrylamide dose levels in the study of Sublet et al. (1989). All dichotomous models in the EPA Benchmark Dose Software (Version 2.1.2) were fit to incidence data for fraction of nonpregnant females (number of nonpregnant females/number of sperm-positive females) using the PBPK-modeled rat blood TWA acrylamide dose as the dose metric and using the TWA glycidamide dose as the dose metric. As described in detail in Appendix A, the best-fitting model for each dose metric provided a $BMDL_{10}$ of 0.00177669 mM for rat blood TWA acrylamide and a $BMDL_{10}$ of 0.00220167 mM for rat blood TWA glycidamide. Using these BMDLs, a human PBPK model (Sweeney et al. 2010) predicted a human equivalent dose (HED) of 0.31 mg acrylamide/kg/day based on blood TWA acrylamide and a HED of 5.25 mg acrylamide/kg/day based on blood TWA glycidamide. Both acrylamide and glycidamide are widely distributed by the blood and both are reactive. However, based on uncertainty regarding the proximal toxicant(s) responsible for acrylamide-induced reproductive toxicity in the male rat, a conservative public health approach was taken and the lowest HED of 0.31 mg acrylamide/kg/day (based on blood TWA acrylamide) was selected as the point of departure (POD) for deriving an acute-duration oral MRL for acrylamide. A total uncertainty factor of 30 (3 for interspecies extrapolation using a PBPK model and 10 for human variability) was applied to the HED of 0.31 mg/kg/day, resulting in an acute-duration oral MRL of 0.01 mg/kg/day for acrylamide.

- An MRL of 0.001 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to acrylamide.

No human data are available regarding health effects associated with intermediate-duration oral exposure to acrylamide.

Results of available animal studies demonstrate that neurological effects in males and females and reproductive effects in males are the most sensitive noncancer effects associated with intermediate-duration oral exposure to acrylamide (Burek et al. 1980; Chapin et al. 1995; Johnson et al. 1984, 1985, 1986; NTP 2011b; Sakamoto and Hashimoto 1986; Smith et al. 1986; Tyl et al. 2000a, 2000b; Zenick et al. 1986). The lowest dose level reported to induce male-mediated implantation losses was 2.8 mg/kg/day in male Long-Evans rats receiving acrylamide from the drinking water for 80 days; this study identified a no-observed-adverse effect level (NOAEL) of 1 mg/kg/day (Smith et al. 1986). Ultrastructural

degenerative peripheral nerve changes were observed at a dose level as low as 1 mg/kg/day in male F344 rats receiving acrylamide from the drinking water for up to 93 days; this study identified a NOAEL of 0.2 mg/kg/day (Burek et al. 1980). Available data suggest that neurological effects represent a more sensitive point of departure than reproductive effects for deriving intermediate- and chronic-duration oral MRLs for acrylamide. Therefore, degenerative nerve change was selected as the critical effect for deriving an intermediate-duration oral MRL for acrylamide. The study of Burek et al. (1980) was selected as the principal study because it identified the lowest lowest-observed-adverse-effect-level (LOAEL) for the critical effect. A NOAEL/LOAEL approach was selected because results of the ultrastructural evaluations included only 3 of 10 rats/group and were reported only as the total numbers of fields (per group) with ultrastructural changes as axolemma invaginations or Schwann cells without axons and/or with degenerating myelin. The distribution of fields exhibiting ultrastructural changes among the three rats within a particular dose group was not included in the study report. The lack of adequate quantitative data precludes the utilization of benchmark dose (BMD) analysis to derive an intermediate-duration oral MRL for acrylamide. As described in detail in Appendix A, a rat PBPK model for acrylamide and glycidamide (Sweeney et al. 2010) was used to predict the rat blood TWA acrylamide dose and TWA glycidamide dose associated with the rat NOAEL of 0.2 mg/kg/day.

Based on PBPK model-predicted rat blood TWA acrylamide dose metric at the NOAEL of 0.2 mg acrylamide/kg/day, the HED is 0.038 mg acrylamide/kg/day. Based on PBPK model-predicted rat blood TWA glycidamide dose metric at the NOAEL of 0.2 mg acrylamide/kg/day, the HED is 0.28 mg acrylamide/kg/day. Using a conservative approach, derivation of an intermediate-duration oral MRL for acrylamide was performed using the lowest HED of 0.038 mg acrylamide/kg/day. A total uncertainty factor of 30 (3 for interspecies extrapolation using a PBPK model and 10 for human variability) was applied to the HED of 0.038 mg/kg/day, resulting in an intermediate-duration oral MRL of 0.001 mg/kg/day.

- An MRL of 0.001 mg/kg/day has been derived for chronic-duration oral exposure (365 days or more) to acrylamide.

No human data are available to evaluate neurological effects following chronic-duration oral exposure to acrylamide. Three chronic toxicity and carcinogenicity bioassays are available for male and female F344 rats receiving acrylamide from the drinking water for up to 2 years (Friedman et al. 1995; Johnson et al. 1984, 1985, 1986; NTP 2011b). The study of Johnson et al. (1984, 1985, 1986) included dose levels of 0, 0.01, 0.1, 0.5, and 2.0 mg/kg/day. The study of Friedman et al. (1995) included dose levels of 0, 0.1, 0.5, or 2 mg/kg/day in males and 0, 1.0, or 3.0 mg/kg/day in females (Friedman et al. 1995). Both studies

identified a NOAEL of 0.5 mg/kg/day and a LOAEL of 2 mg/kg/day for histopathological evidence of degenerative nerve changes. The study of NTP (2011b) included dose levels of 0, 0.33, 0.66, 1.32, and 2.71 mg/kg/day in males and 0, 0.44, 0.88, 1.84, and 4.02 mg/kg/day in females; this study identified a NOAEL of 1.32 mg/kg/day and a LOAEL of 2.71 mg/kg/day for significantly increased incidences of axonal degeneration in the sciatic nerve of the male rats; the female rats exhibited a NOAEL of 1.84 mg/kg/day and a LOAEL of 4.02 mg/kg/day. All three chronic studies include sufficient incidence data for degenerative changes in peripheral nerves (detected by light microscopy) to warrant BMD analysis.

As discussed in detail in Appendix A, a PBPK rat model for acrylamide and glycidamide (Sweeney et al. 2010) was used to estimate for blood TWA acrylamide dose metric and TWA glycidamide dose metric for each of the administered acrylamide dose levels for male and female rats from each of the three chronic studies (Friedman et al. 1995; Johnson et al. 1986; NTP 2011b). All dichotomous models in the EPA Benchmark Dose Software (Version 2.1.2) were fit to the incidence data for degenerative peripheral nerve changes reported in each of the three chronic studies using PBPK-modeled rat blood TWA acrylamide as the dose metric and using PBPK-modeled rat blood TWA glycidamide as the dose metric; a benchmark response (BMR) of 10% extra risk was selected for the initial BMD modeling exercise. A BMR of 5% extra risk was also used for each model considered to provide the best fit to the male and female data from each of the three chronic studies, using TWA acrylamide as the dose metric and using TWA glycidamide as the dose metric.

A human PBPK model (Sweeney et al. 2010) was used to predict the HED corresponding to the $BMDL_{10}$ and $BMDL_{05}$ values for rat blood TWA acrylamide and TWA glycidamide from the best-fitting models for each of the three chronic studies. A BMR of 5% extra risk is justified because the chronic studies used sufficient numbers of animals (≥38 per dose group, with the exception of 20 animals in the 1.0 mg/kg/day dose group of female rats in the study of Friedman et al. 1995). The lowest PBPK model-predicted HED is 0.042 mg acrylamide/kg/day based on PBPK model-predicted blood TWA acrylamide for the male rats from the study of Friedman et al. (1995). The HED of 0.042 mg/kg/day was selected as the POD for deriving a chronic-duration oral MRL for acrylamide because it represents the most public health protective POD. A total uncertainty factor of 30 (3 for interspecies extrapolation using a PBPK model and 10 for human variability) was applied to the HED of 0.042 mg/kg/day, resulting in a chronic-duration oral MRL of 0.001 mg/kg/day.

## 2. RELEVANCE TO PUBLIC HEALTH

The Fourth National Report on Human Exposure to Environmental Chemicals (CDC 2009) reported a geometric mean of 63.9 pmol acrylamide hemoglobin adduct/g hemoglobin with $50^{th}$, and $95^{th}$ percentile values of 57.0, and 220 pmol acrylamide hemoglobin adduct/g hemoglobin, respectively for the U.S. population of males. The PBPK model-predicted acrylamide intakes associated with these acrylamide hemoglobin adduct levels are 0.003405, 0.00304, and 0.01173 mg/kg/day, respectively. A similar approach for the U.S. population of females results in PBPK model-predicted acrylamide intakes of 0.00313, 0.00285, and 0.00874 mg/kg/day, respectively. A PBPK model-predicted acrylamide hemoglobin adduct level of 787.917 pmol/g can be calculated using the HED of 42 µg/kg/day that serves as the point of departure for the chronic-duration oral MRL for acrylamide. At the chronic-duration oral MRL of 1 µg acrylamide/kg/day, the PBPK model-predicted acrylamide hemoglobin adduct level is 18.75 pmol/g hemoglobin. This value is slightly lower than the mean acrylamide hemoglobin adduct levels of 63.9 and 58.7 pmol/g hemoglobin reported for the U.S. population of males and females, respectively.

# 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) | | 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<br>5 d/wk<br>6 hr/d | | 4.8 | | | American Cyanamid Company<br>1954<br>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

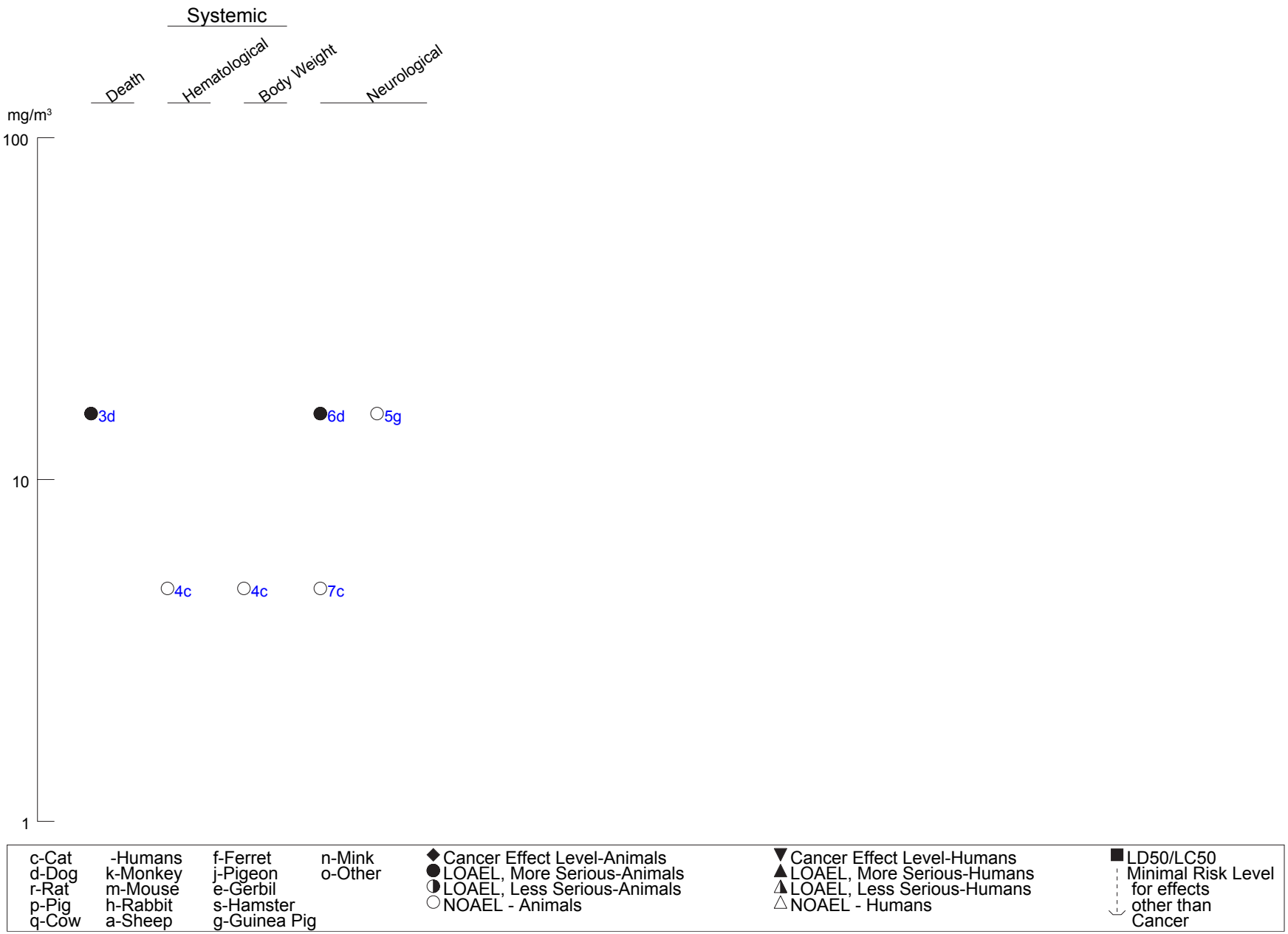
◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ 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]$p<0.01$ ($\chi^2$ 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]$p<0.01$ ($\chi^2$ 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<br>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 | | | | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Less Serious (mg/kg/day) | | Serious (mg/kg/day) | | | |
| 16 | Rat (Fischer- 344) | 14 d (F) | Bd Wt | 22.4 M<br>63.4 F | | | 51.7 M | (28% lower terminal body weight) | NTP 2011<br>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<br>Acrylamide | |
| 18 | Rat (Fischer- 344) | Gd 6-17 (GW) | Bd Wt | 20 F | | | | | Walden et al. 1981<br>Acrylamide | Maternal body weight |
| 19 | Mouse (B6C3F1) | 14 d (W) | Bd Wt | 66.7 M<br>75.8 F | | | 150 | (marked weight loss and moribund sacrifice at treatment day 10) | NTP 2011<br>Acrylamide | |
| 20 | Mouse (B6C3F1) | 14 d (F) | Bd Wt | 72.8 M<br>75.7 F | | | | | NTP 2011<br>Acrylamide | |
| 21 | Mouse ddY | Once (NS) | Bd Wt | 100 M | 150 M | (significantly depressed body weight) | | | Sakamoto et al. 1988<br>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<br>39.4 F | | 76.6 M (hind-leg paralysis in 4/4 males)<br>70 F (hind-leg paralysis in 4/4 females) | NTP 2011 | |
| 31 | Rat (Fischer- 344) | 14 d (F) | | 22.4 M<br>29.4 F | | 51.7 M (hind-leg paralysis in 4/4 males)<br>63.4 F (hind-leg paralysis in 4/4 females) | NTP 2011<br>Acrylamide | |
| 32 | Rat (Fischer- 344) | Once (GW) | | 100 M | | 200 M (decreases in hindlimb grip strength and locomotory performance) | Tilson and Cabe 1979<br>Acrylamide | |
| 33 | Rat (Long- Evans) | 5 d 1 x/d (GW) | | 30 M | | 45 M (clinical signs of neurotoxicity) | Tyl et al. 2000b<br>Acrylamide | |
| 34 | Rat (Fischer- 344) | Gd 6-17 (GW) | | 20 F | | | Walden et al. 1981<br>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) | | [b] 5 M | | 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)<br>24 F (death of 5/10 female rats during exposure week 4) | American Cyanamid Company 1991 | |
| 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<br>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<br>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<br>31.4 F | 70 M (13% lower mean terminal body weight)<br>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<br>5 d/wk<br>1 x/d | | | | 10 (degenerative changes in visual nerve fibers) | Eskin et al. 1985<br>Acrylamide | |
| **76** | Monkey | NS<br>1 x/d | | | | 30 (peripheral neuropathy) | Leswing and Ribelin 1969<br>Acrylamide | |
| **77** | Monkey (NS) | up to 363 d<br>5 d/wk<br>(F) | | 3 F | | 10 F (clinical signs of peripheral neuropathy) | McCollister et al. 1964<br>Acrylamide | Only 1 animal per dose group; no clear signs of toxicity at 3 mg/kg/day |
| **78** | Monkey | 33-47 d<br>5 d/wk<br>1 x/d | | | | 10 F (ataxia, adverse visual effects) | Merigan et al. 1985<br>Acrylamide | |
| **79** | Rat (albino) | 21 d<br>(F) | | | | 47 M (paralysis of hind limbs) | American Cyanamid Company 1953b | |
| **80** | Rat (albino) | NS<br>5 d/wk<br>(G) | | | | 25 M (clinical signs of peripheral neuropathy) | American Cyanamid Company 1959 | |
| **81** | Rat (Sprague-Dawley) | 2 wk premating and<br>Gd 0-19<br>(F) | | 3.82 F | | | American Cyanamid Company 1979<br>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<br>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<br>Acrylamide | |
| 84 | Rat (Wistar) | Up to 21 d<br>1 x/d<br>(G) | | | | 25 M (complete hindlimb paralysis by treatment day 21) | Dixit et al. 1981<br>Acrylamide | |
| 85 | Rat (Wistar) | 21 d lactation period<br>1 x/d<br>(G) | | | | 25 F (progressive clinical signs of neuropathy beginning as early as treatment day 4) | Friedman et al. 1999<br>Acrylamide | electron microscopic examinations were not performed on sciatic nerve |
| 86 | Rat (albino) | 12 doses in 15 d<br>(GW) | | | | 50 (severe weakness) | Fullerton and Barnes 1966<br>Acrylamide | |
| 87 | Rat (albino) | variable<br>(F) | | | | 6 (slight leg weakness after 40 weeks of exposure) | Fullerton and Barnes 1966<br>Acrylamide | |
| 88 | Rat (albino) | 38 d<br>(F) | | | | 25 (severe leg weakness) | Fullerton and Barnes 1966<br>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<br>10 F | | 10 M (clinical and histopathologic evidence of peripheral neuropathy)<br>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<br>6 F | | 22.3 M (hind-leg paralysis in 8/8 males)<br>12.3 F (hind-leg paralysis in 4/8 females) | NTP 2011 | |
| **95** | Rat (Fischer- 344) | 13 wk (F) | | 5.5 M<br>6.6 F | | 14.2 M (hind-leg paralysis)<br>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)<br>14.6 F (increased incidences of foot splay) | Zenick et al. 1986<br>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<br>Acrylamide | |
| 108 | Mouse | 8 wk 2 x/wk (NS) | | | | 36 M (clinical signs of peripheral neuropathy, testicular atrophy) | Hashimoto et al. 1981<br>Acrylamide | |
| 109 | Mouse (ICR) | Up to 38 d (W) | | | | 90.8 M (clinically and histopathologically confirmed peripheral neuropathy) | Ko et al. 1999<br>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<br>12.3 F | | 4.5 M (germinal epithelium degeneration in testes of 5/8 males)<br>26.3 F (8/8 females in anestrus) | NTP 2011 | |
| **123** | Rat (Fischer- 344) | 13 wk (F) | | 1.4 M<br>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<br>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)<br>5 (decreases in implantations and number of live pups) | Tyl et al. 2000a<br>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<br>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<br>35.1 F | | 59.4 M (germinal epithelium degeneration in testes)<br>64 F (8/8 females in anestrus) | NTP 2011 | |
| **133** | Mouse (B6C3F1) | 13 wk (W) | | 32.8 M<br>31.4 F | | 70 M (germinal epithelium degeneration in testes of 6/8 males)<br>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<br>18.7 F | | 13.3 M (decreased number of fetuses/dam) | Sakamoto and Hashimoto 1986<br>Acrylamide | |
| **Developmental** | | | | | | | | |
| **135** | Rat (Sprague-Dawley) | 2 wk premating and Gd 0-19 (F) | | 3.82 | | | American Cyanamid Company 1979<br>Acrylamide | Assessment included mating and pregnancy indices, litter data, and offspring growth and survival |
| **136** | Rat (Fischer- 344) | 16 d (dams) Gd 6-21<br>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<br>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<br>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 | | | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Less Serious (mg/kg/day) | Serious (mg/kg/day) | | | |
| 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<br>4.65 F | (decreased survival)<br>(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<br>3 F[d] | | | Friedman et al. 1995<br>Acrylamide | |
| | | | Cardio | 2 M<br>3 F[d] | | | | |
| | | | Gastro | 2 M<br>3 F[d] | | | | |
| | | | Hemato | 2 M<br>3 F[d] | | | | |
| | | | Musc/skel | 2 M<br>3 F[d] | | | | |
| | | | Hepatic | 2 M<br>3 F[d] | | | | |
| | | | Renal | 2 M<br>3 F[d] | | | | |
| | | | Endocr | 2 M<br>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<br>4.02 F | | | NTP 2011 | |
| | | | Gastro | 2.71 M<br>4.02 F | | | | |
| | | | Hemato | 2.71 M<br>4.02 F | | | | |
| | | | Endocr | 1.84 F | 4.02 F (cytoplasmic vacuolation in adrenal cortex) | | | |
| | | | Ocular | 2.71 M<br>4.02 F | | | | |
| | | | Bd Wt | 1.32 M<br>1.84 F | 2.71 M (14% lower mean body weight than controls at week 104)<br>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<br>2.23 F | 8.93 M (hematopoietic cell proliferation in the spleen)<br>4.65 F (hematopoietic cell proliferation in the spleen) | | | |
| | | | Ocular | 4.11 M<br>2.23 F | 8.93 M (cataracts)<br>4.65 F (cataracts) | | | |
| **Neurological** | | | | | | | | |
| **154** | Rat (Fischer- 344) | 2 yr (W) | | 0.5 M[e] | 2 M (light microscopic evidence of peripheral nerve degeneration) | | Friedman et al. 1995<br>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<br>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<br>1.84 F | | 2.71 M (degenerative effects in retina and sciatic nerve)<br>4.02 F (degenerative effects in sciatic nerve) | NTP 2011 | |
| **157** | Mouse (B6C3F1) | 2 yr (W) | | 8.93 M<br>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<br>Acrylamide | |
| **Reproductive** | | | | | | | | |
| **159** | Rat (Fischer- 344) | 2 yr (W) | | 2 | | | Friedman et al. 1995<br>Acrylamide | Gross and histopathological evaluations of reproductive organs and tissues |
| **160** | Rat (Fischer- 344) | 2 yr (W) | | 2 | | | Johnson et al. 1984, 1986<br>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<br>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<br>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<br>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)<br>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 | | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| | | | | | Less Serious (mg/kg/day) | Serious (mg/kg/day) | | |

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)

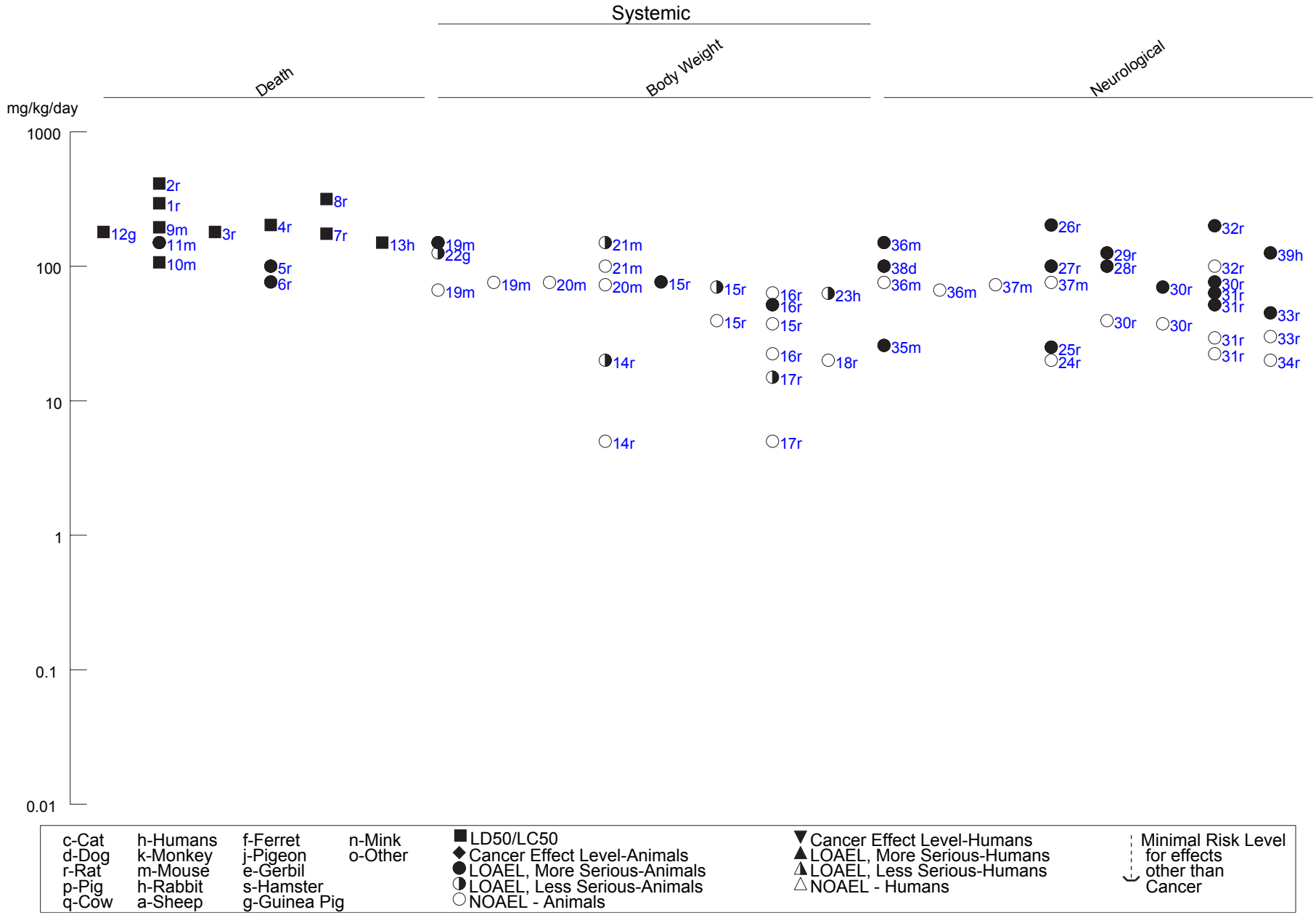
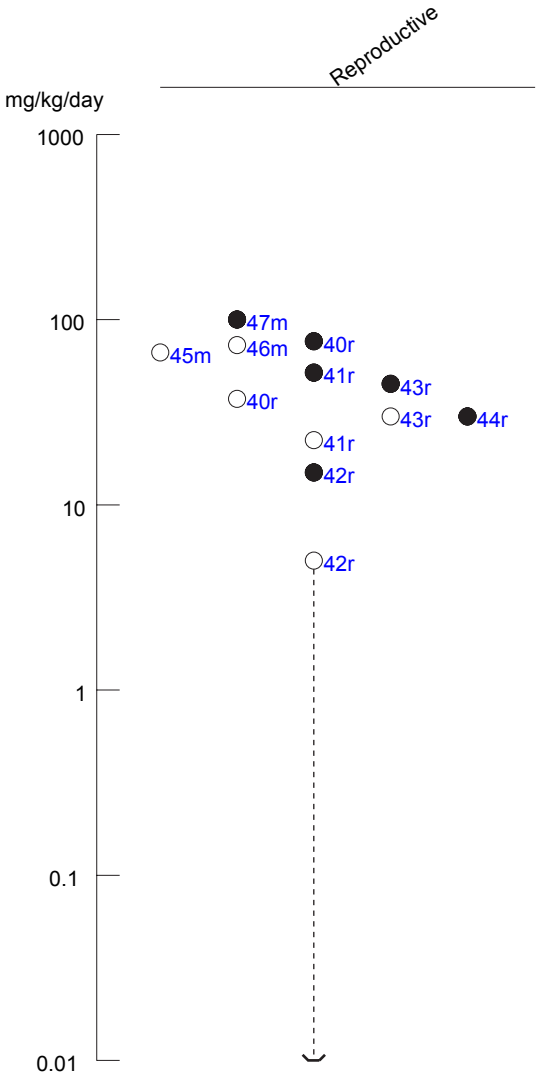


Figure 3-2 Levels of Significant Exposure to Acrylamide - Oral *(Continued)*

Acute (≤14 days)




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)*

Intermediate (15-364 days)

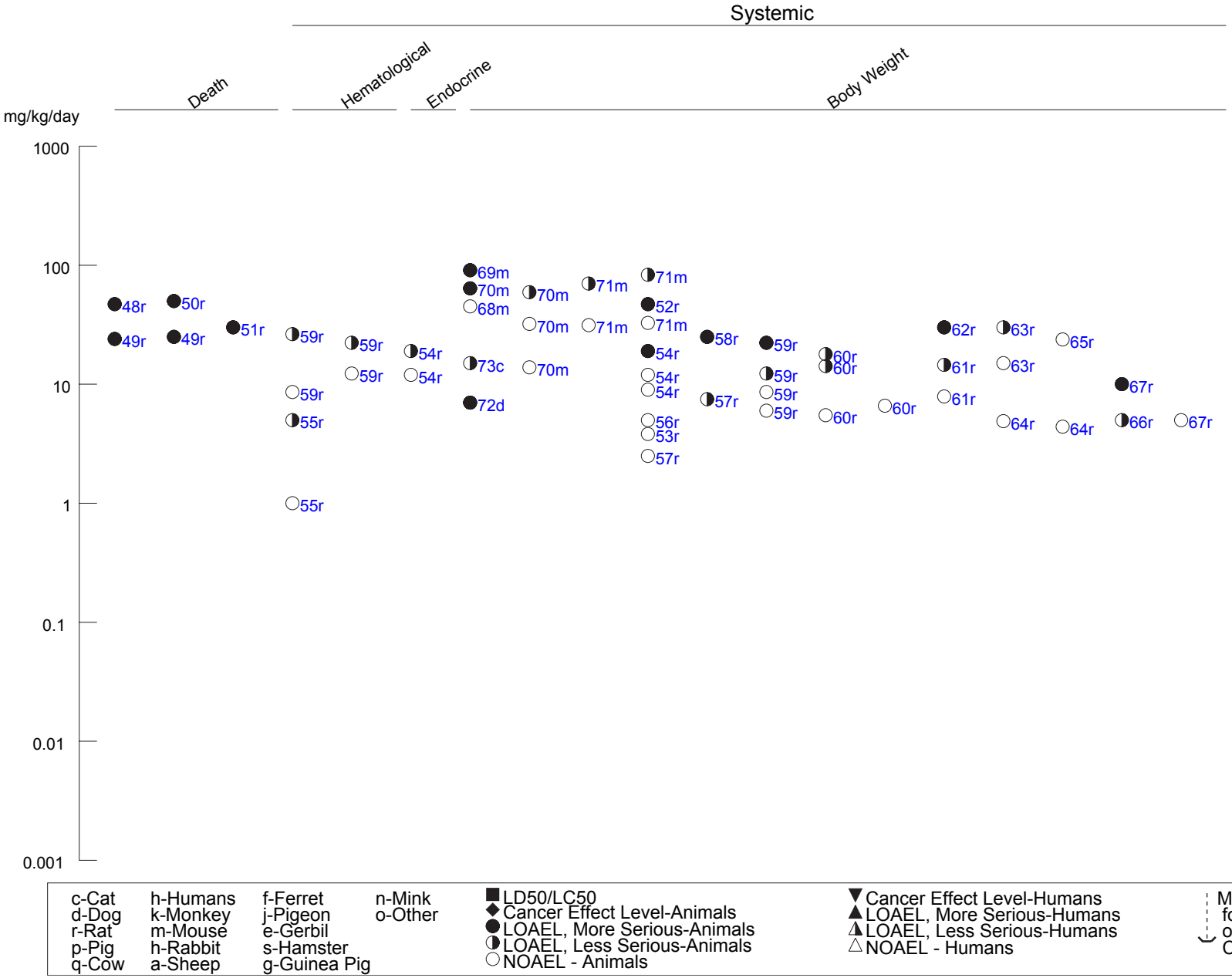
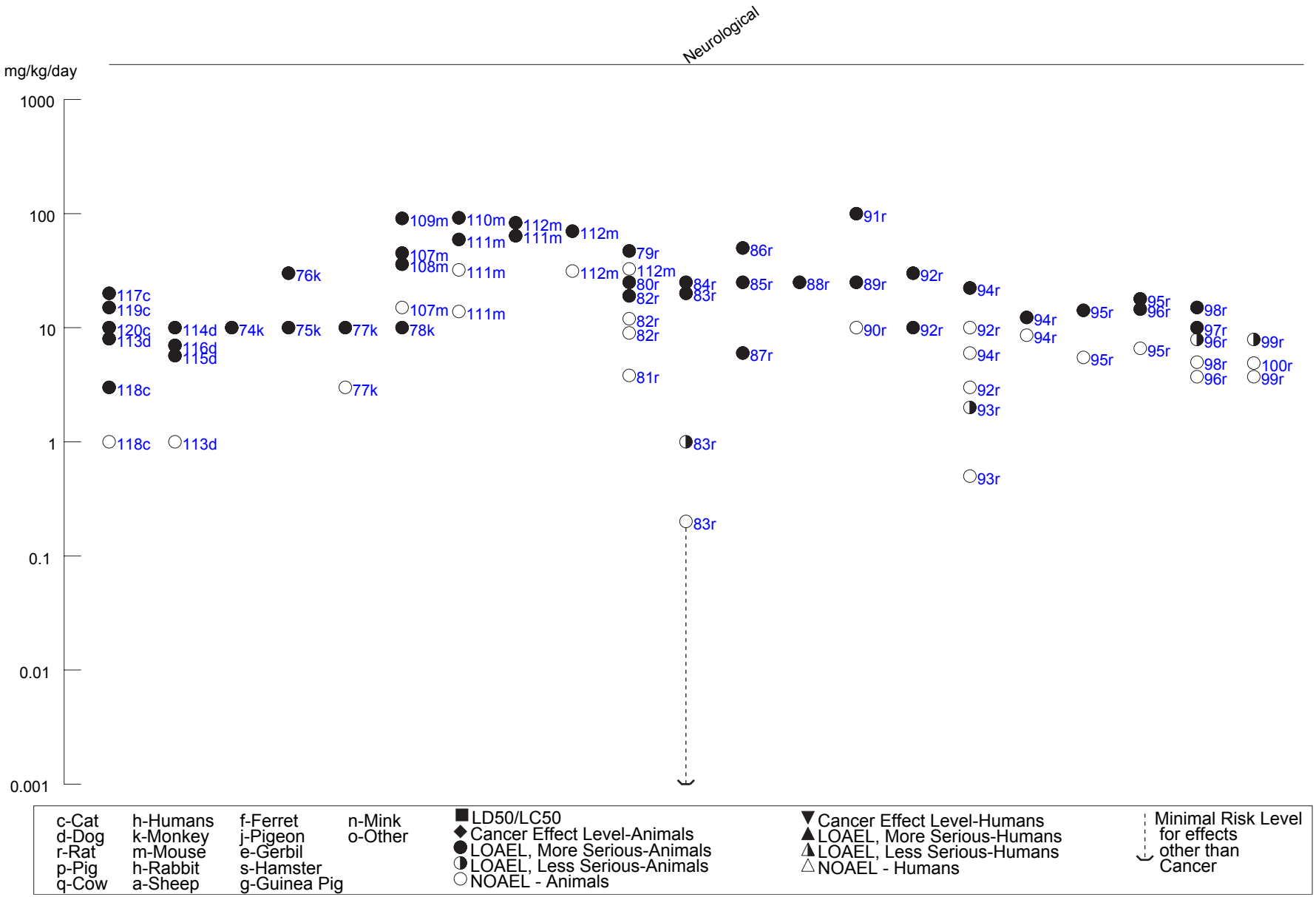


Figure 3-2 Levels of Significant Exposure to Acrylamide - Oral *(Continued)*

Intermediate (15-364 days)



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

Intermediate (15-364 days)

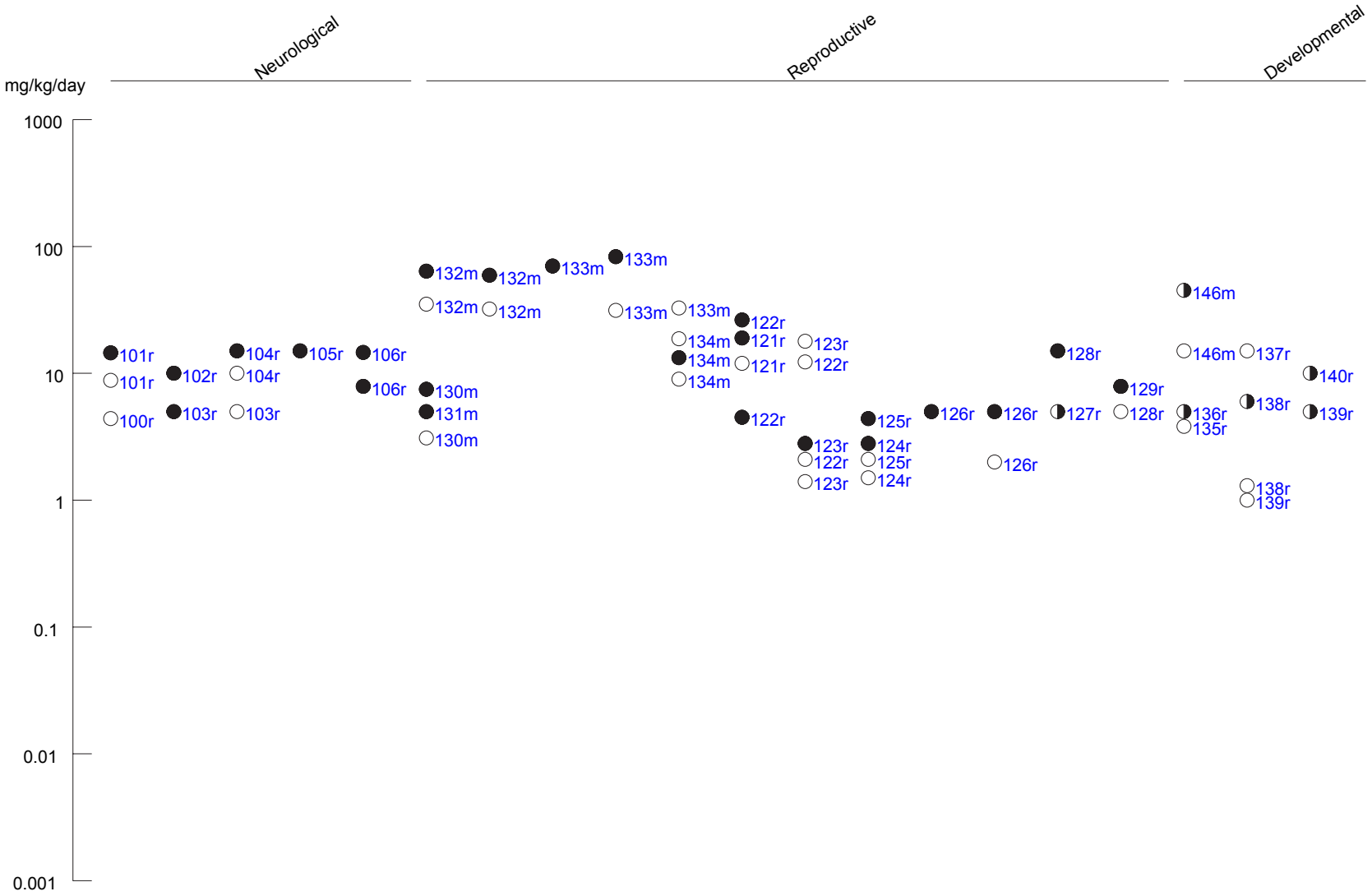
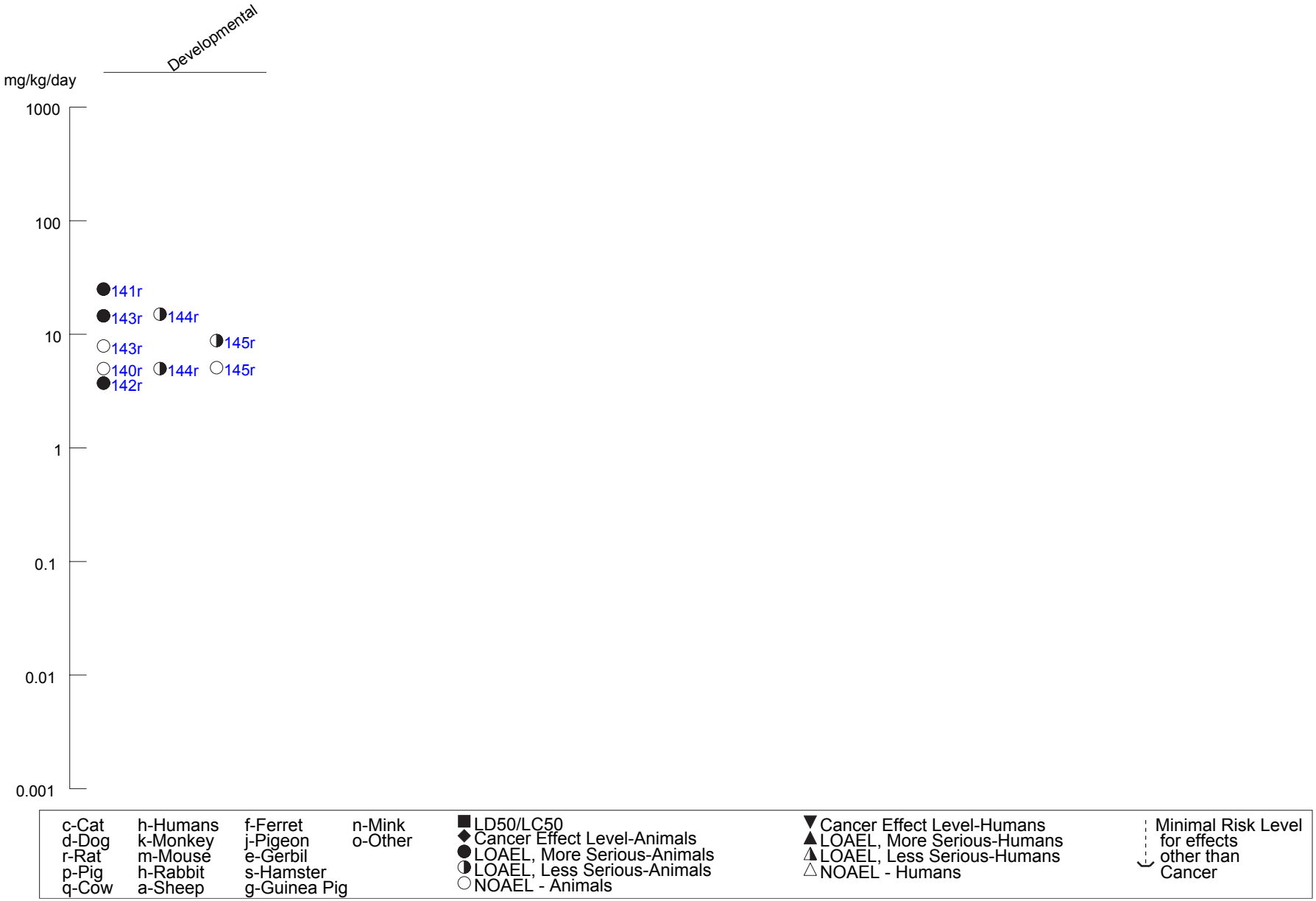


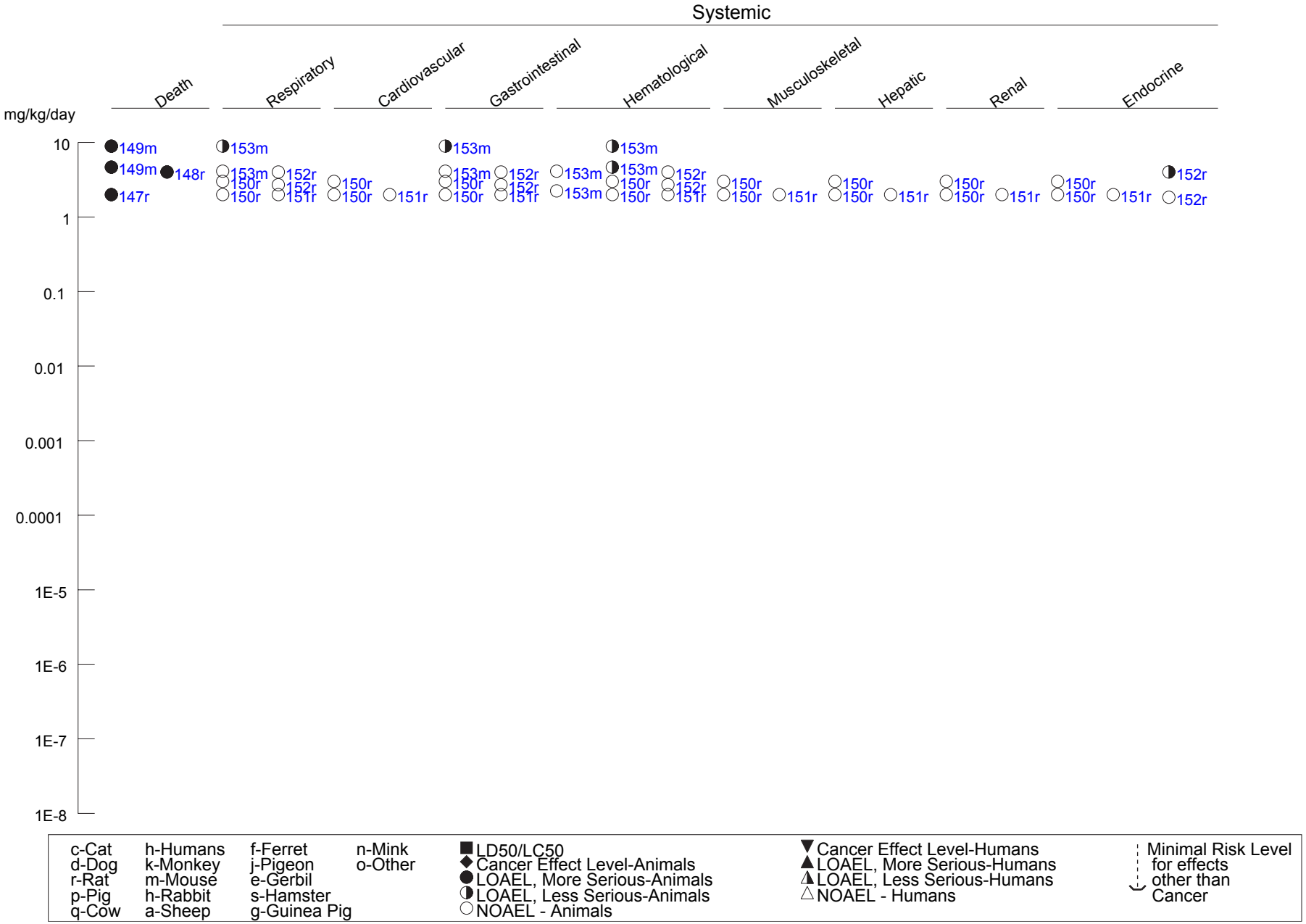
Figure 3-2 Levels of Significant Exposure to Acrylamide - Oral *(Continued)*

Intermediate (15-364 days)



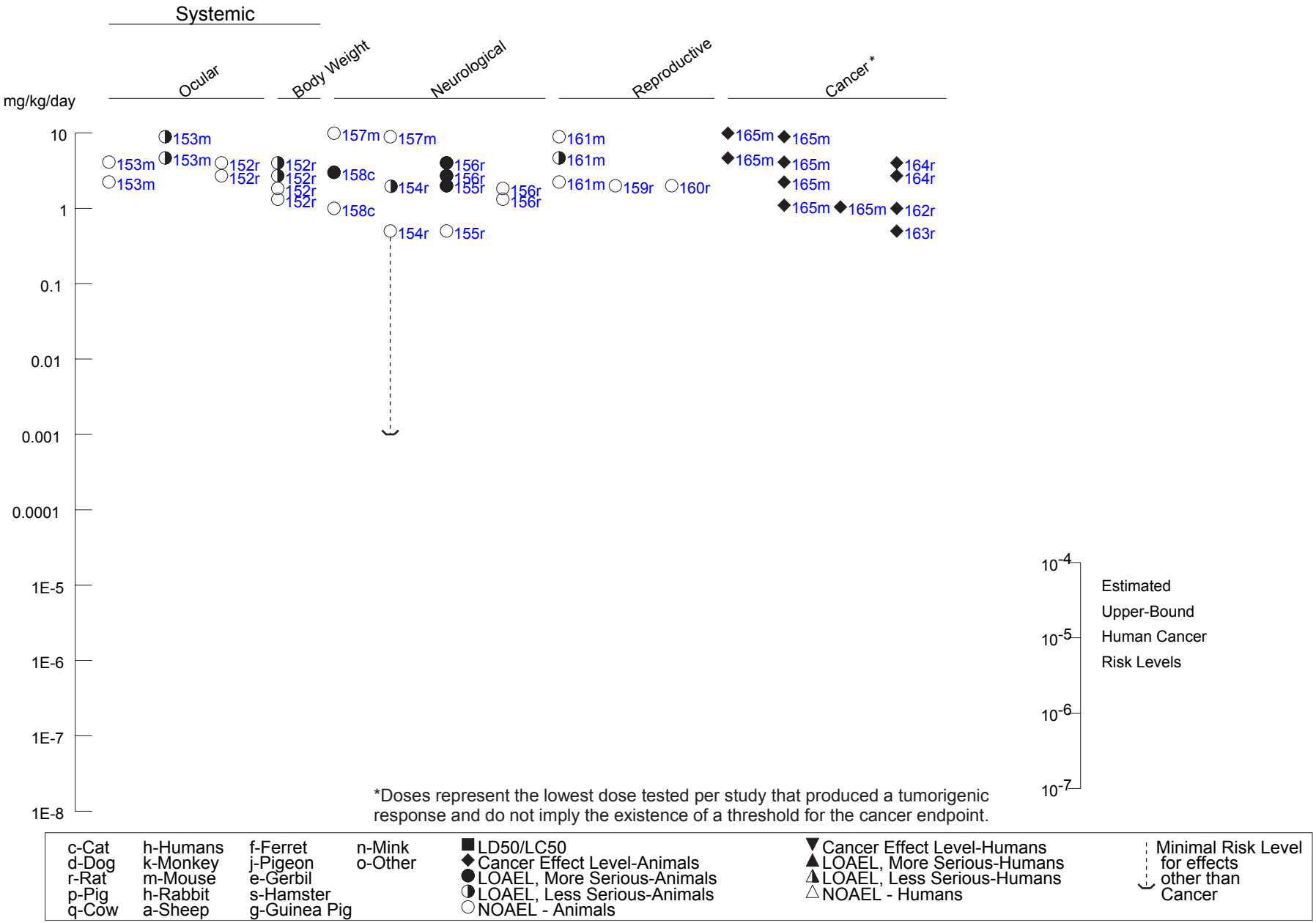
## Figure 3-2 Levels of Significant Exposure to Acrylamide - Oral *(Continued)*

Chronic (≥365 days)



## 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[†] | – | 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 Less Serious | LOAEL Serious | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|
| **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<br>No activation | Mei et al. 1988 |
| Mouse lymphoma L5178Y $TK^{+/-}$, tk and HPRT loci | Gene mutation | – | – | 0.5–7.5 mg/mL<br>+/- S9 activation<br>Mutation only at cytotoxic concentrations | Knaap et al. 1988 |
| Mouse lymphoma L5178Y $TK^{+/-}$, HPRT locus | Gene mutation | + | + | 0.1–0.5 mg/mL<br>Cocultivated activation | Knaap et al. 1988 |
| Chinese hamster V79H3, HPRT locus | Gene mutation | No data | – | 1–7 mM<br>No activation | Tsuda et al. 1993 |
| Human lymphoblastoid cell line (TK6) | Gene mutation | (+) | (+) | 5–15 mM<br>+/- S9 activation | Koyama et al. 2011b |
| Human lymphoblastoid cell line (TK6) | Gene mutation | + | (+) | 5–15 mM<br>+/- 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<br>No activation | Tsuda et al. 1993 |
| Chinese hamster V79 | Chromosomal aberration | + | + | 0.1–3 mg/mL<br>+/- S9 activation | Knaap et al. 1988 |
| Chinese hamster V79 | Chromosomal aberration | No data | – | 0–2,000 µM<br>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<br>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

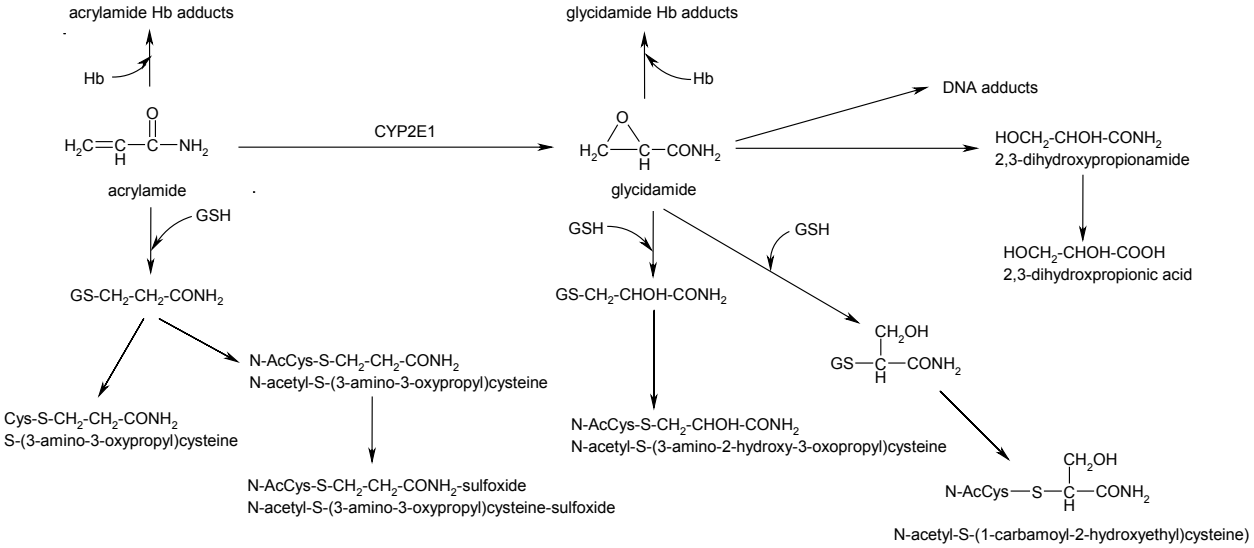
Following 24-hour dermal exposure of male F344 rats to 162 mg/kg [$^{14}$C]-labeled acrylamide, the highest concentration of acrylamide equivalents (excluding skin at the application site) was found in blood cells (71 µg/g), followed by skin at nondosing sites (~28 µg/g); liver, spleen, testes, and kidneys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

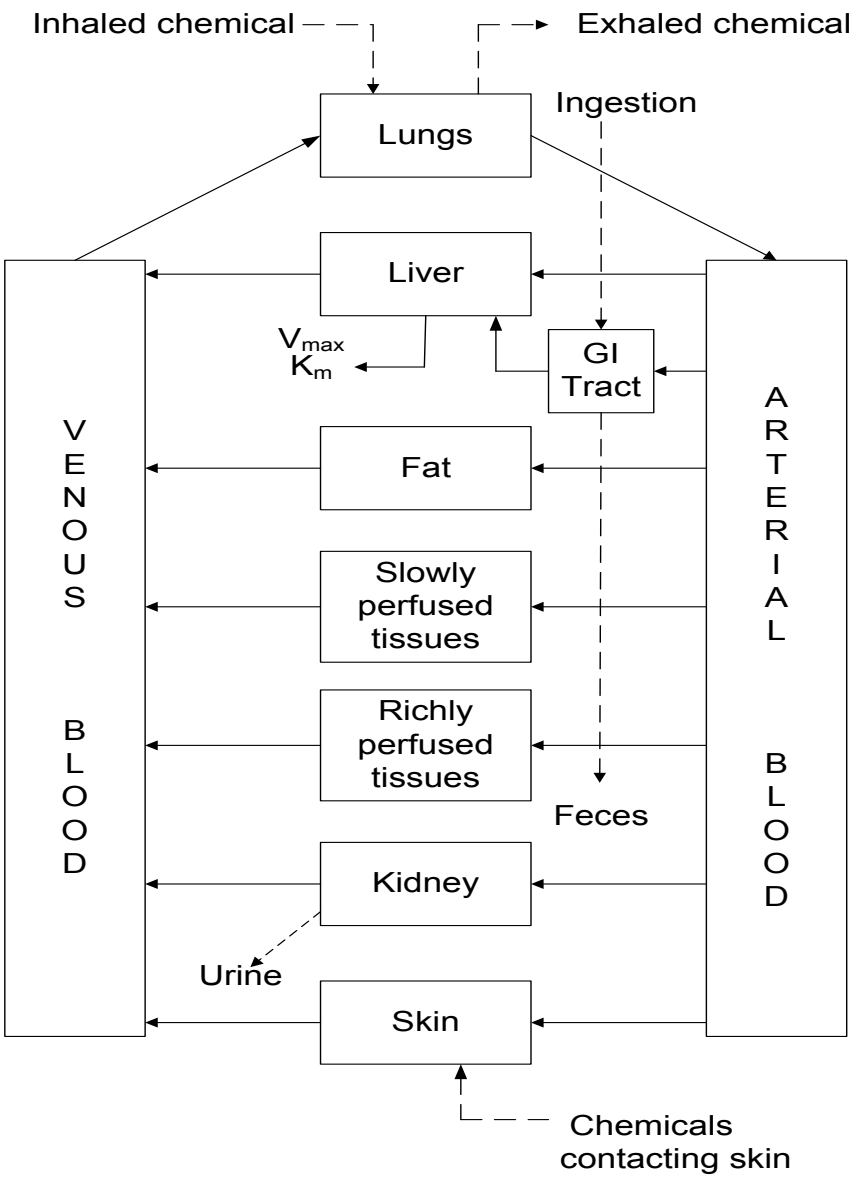
The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many 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

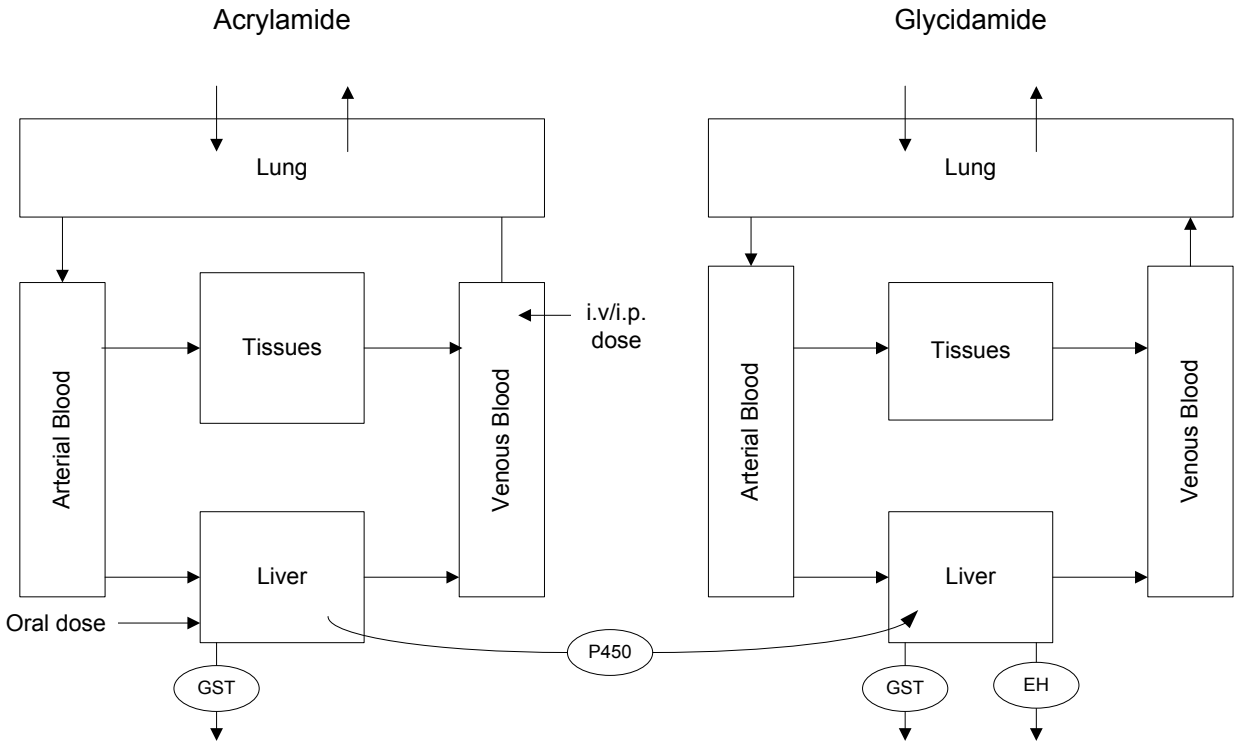
Kirman et al. (2003) developed a PBPK model (Figure 3-5) to predict the behavior of acrylamide and its epoxide metabolite, glycidamide, in the rat for intravenously-, intraperitoneally-, or orally-administered acrylamide. The model includes components for both acrylamide and glycidamide, each consisting 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

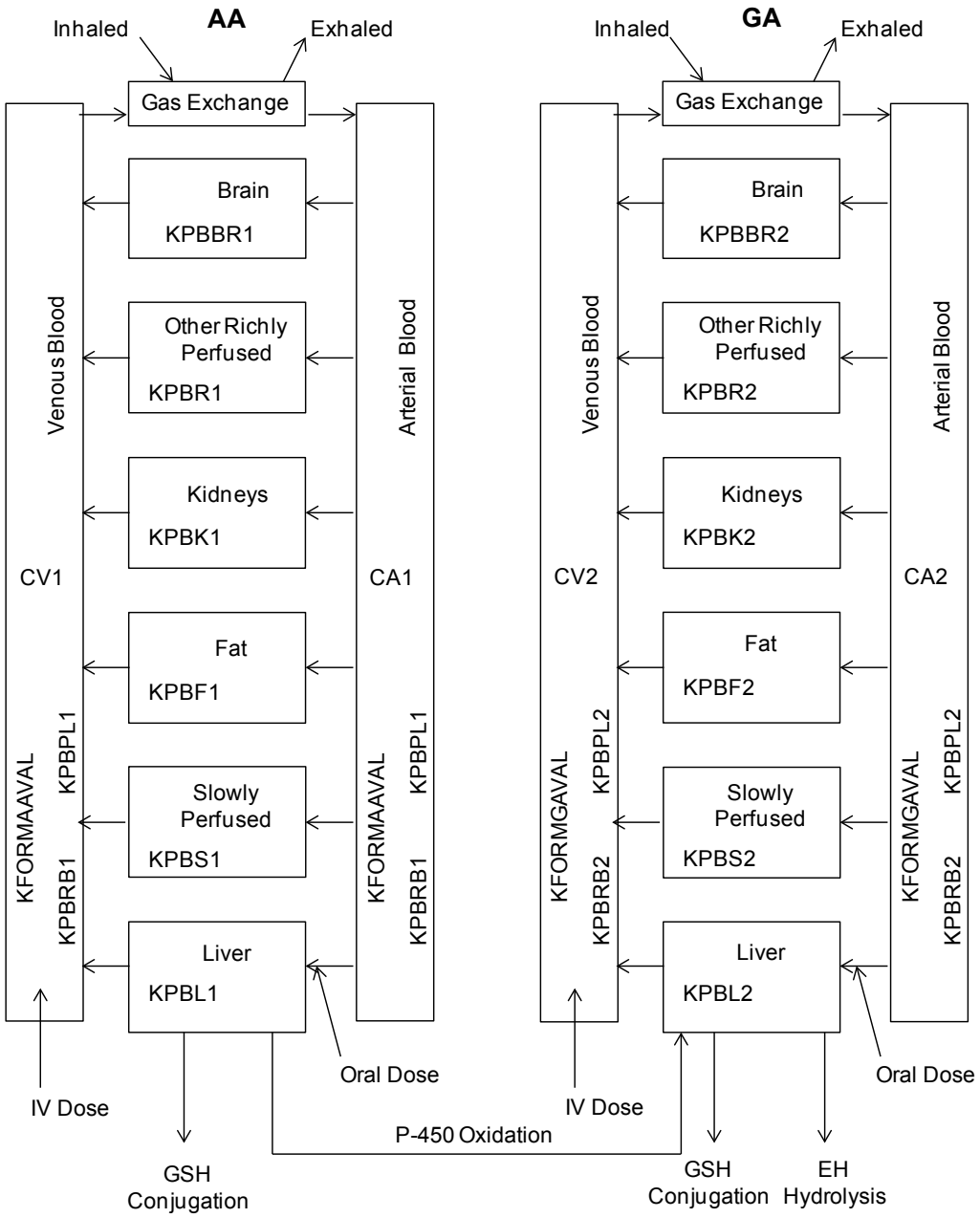
Figure 3-6; parameters are listed in Table 3-12. Like the Kirman et al. (2003) model, the Sweeney et al. (2010) model consists of separate models for acrylamide and glycidamide that are connected at the liver compartments where acrylamide is assumed to undergo oxidation to glycidamide by cytochrome P-450 ($K_m$, $V_{max}$). 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

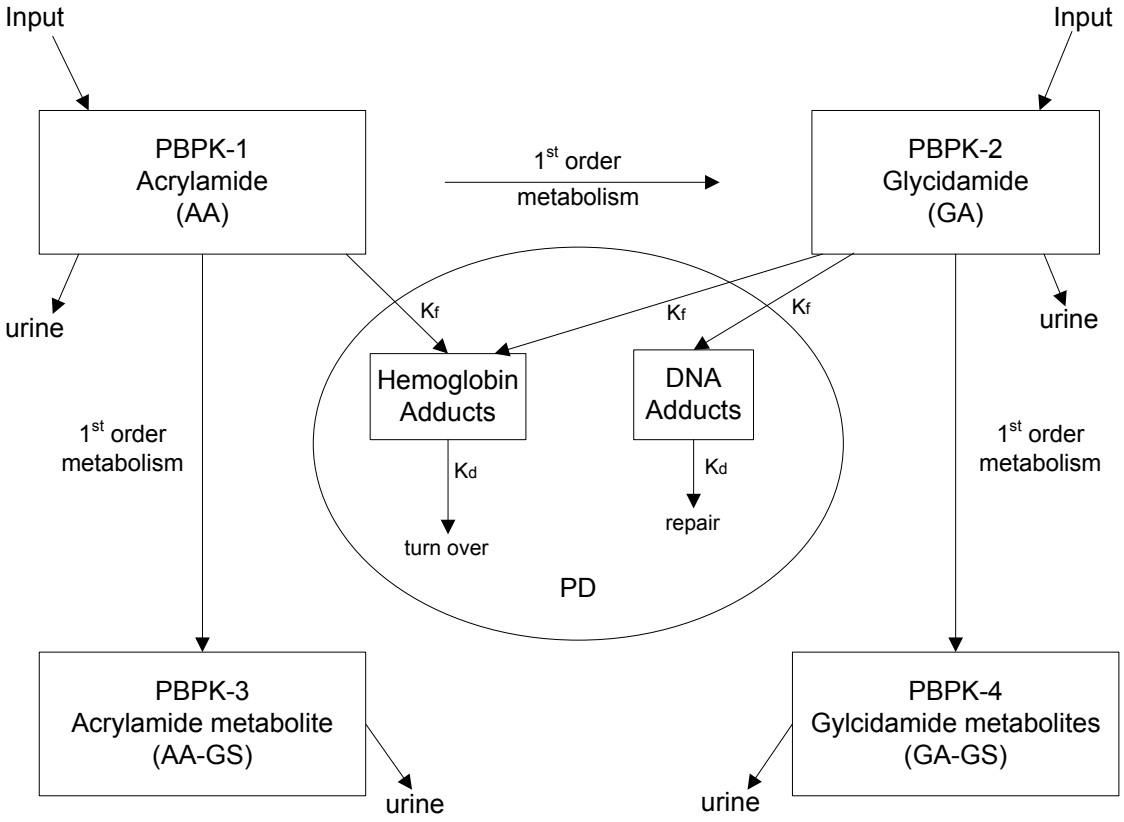
| Parameter (abbreviation) | Units | Male F344 rat | Human[a] | Source/comment |
|---|---|---|---|---|
| Loss rate for bound material in plasma (KPTPl) | $Hour^{-1}$ | 0.012 | | Fit to Miller et al. 1982 |
| Loss of bound material in red blood cells | | | | See Section 4 in Sweeney et al. 2010 |
| Rate 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

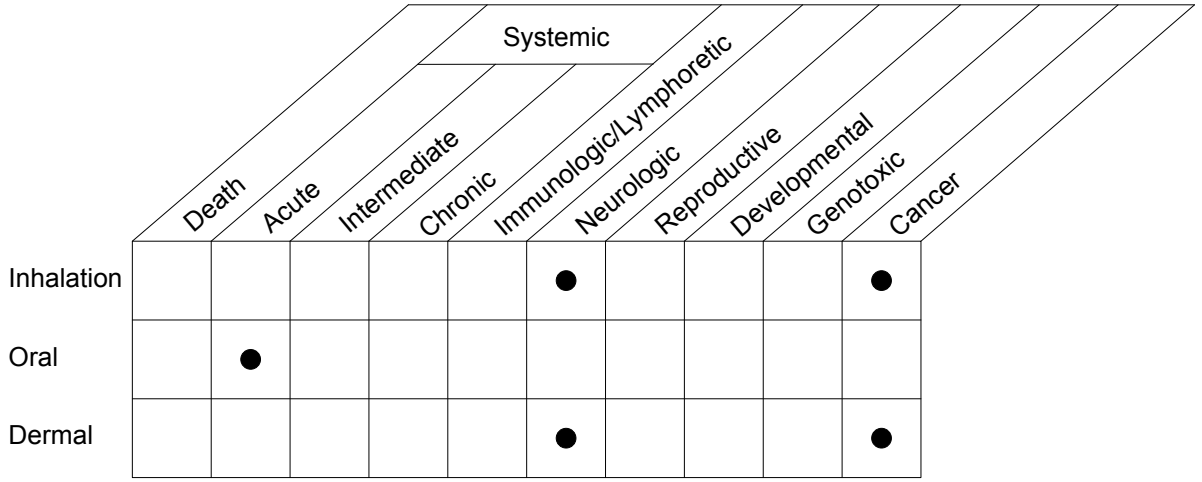
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of acrylamide is available. Where adequate information is not 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.

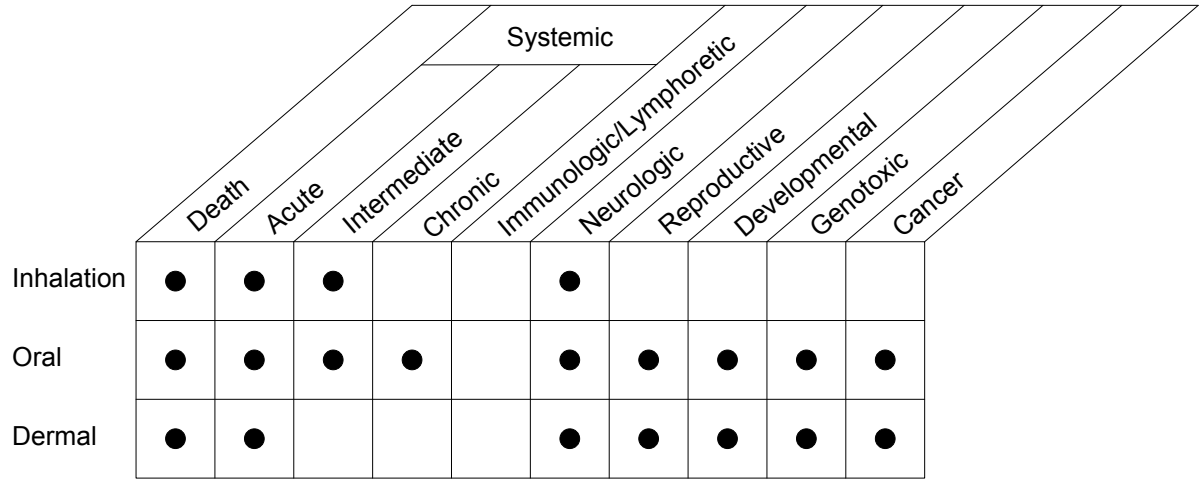
### 3.12.1 Existing Information on Health Effects of Acrylamide

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.

# 4. CHEMICAL AND PHYSICAL INFORMATION

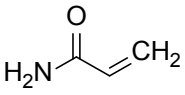
## 4.1 CHEMICAL IDENTITY

Acrylamide is an unsaturated amide that is produced mainly for use as an intermediate in the production of polyacrylamide (Abdelmagic 1982; Haberman 2002). Information regarding the chemical identity of acrylamide is located in Table 4-1.

## 4.2 PHYSICAL AND CHEMICAL PROPERTIES

Acrylamide is a white or colorless, odorless crystalline solid. Information regarding the physical and chemical properties of acrylamide is located in Table 4-2. Acrylamide is stable at room temperature but can violently polymerize at its melting point or under UV light (Lewis 2000, 2007; O'Neil et al. 2006). Acrid fumes, as well as $NO_x$, may be released when it is heated to decomposition (Lewis 2000). Acrylamide is soluble in water, alcohol, and acetone, and is insoluble in benzene and heptanes (Lewis 2007).

## Table 4-1. Chemical Identity of Acrylamide

| Characteristic | Information | Reference |
|---|---|---|
| Chemical name | Acrylamide | |
| Synonym(s) | AAM; acrylagel; acrylic acid amide; acrylic amide; akrylamid (Czech); amresco acryl-40; amid kyseliny akrylove; ethylenecarboxamide; optimum; propenamide; 2-propen-amide; propereamide; propenoic acid amide; vinyl amide | HSDB 2009; Lewis 2000 |
| Registered trade name(s) | No data | |
| Chemical formula | $C_3H_5NO$ | HSDB 2009; WHO 2003 |
| Chemical structure |  | |
| Identification numbers: | | |
| CAS registry | 79-06-1 | |
| NIOSH RTECS | NIOSH/AS3325000 | NIOSH 2005 |
| EPA hazardous waste | U007 | HSDB 2009; Lewis 2000 |
| OHM/TADS | No data | |
| DOT/UN/NA/IMDG shipping | UN 2074; IMO 6.1 | HSDB 2009; Lewis 2000 |
| HSDB | 191 | HSDB 2009 |
| NCI | No data | |
| STCC | 49 091 83<br>49 131 87 | HSDB 2009<br>HSDB 2009 |

CAS = Chemical Abstracts Service; DOT/UN/NA/IMDG = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances; STCC = Standard Transport Commodity Code

## Table 4-2. Physical and Chemical Properties of Acrylamide

| Property | Information | Reference |
|---|---|---|
| Molecular weight | 71.08 | Haberman 2002; HSDB 2009 |
| Color | White<br>Colorless | HSDB 2009; Lewis 2000<br>Lewis 2007; WHO 2003 |
| Physical state | Crystalline solid | HSDB 2009; Lewis 2000<br>Lewis 2007; WHO 2003 |
| Melting point | 84.5 °C | Lewis 2007 |
| Boiling point | 192.6 °C<br>87 °C (2 mm)<br>103 °C (5 mm)<br>125 ºC (25 mm) | Lide 2008<br>O'Neil et al. 2006<br>O'Neil et al. 2006<br>Lewis 2007; O'Neil et al. 2006 |
| Density at 30 °C/4 °C | 1.122 | HSDB 2009; Lewis 2007 |
| Odor | Odorless | Lewis 2007 |
| Odor threshold: | | |
| Water | No data | |
| Air | No data | |
| Solubility: | | |
| Water at 0 °C | No data | |
| at 20 °C | $3.711 \times 10^5$ mg/L | HSDB 2009 |
| at 30 °C | $4.048 \times 10^5$ mg/L | HSDB 2009 |
| Organic solvents | Soluble in water, alcohol, acetone | Lewis 2007 |
| | Insoluble in benzene and heptanes | Lewis 2007 |
| Partition coefficients: | | |
| Log $K_{ow}$ | -0.67 | HSDB 2009 |
| Log $K_{oc}$ | No data | |
| Vapor pressure | | |
| at 25 °C | 0.9 Pa ($7 \times 10^{-3}$ mm Hg) | Haberman 2002; HSDB 2009 |
| at 40 °C | 4.4 Pa ($3.3 \times 10^{-2}$ mm Hg) | Haberman 2002 |
| at 50 °C | 9.3 Pa ($7.0 \times 10^{-2}$ mm Hg) | Haberman 2002 |
| Henry's law constant: | | |
| at 25 °C | $1.7 \times 10^{-9}$ atm-$m^3$/mol (estimated) | HSDB 2009 |
| Autoignition temperature | 424 °C | HSDB 2009 |
| Flash point | 138 °C (closed cup) | HSDB 2009 |
| Flammability limits | No data | |
| Conversion factors (25 °C, 1 atm) | 1 mg/$m^3$=0.34 ppm<br>1 ppm=2.95 mg/$m^3$ | HSDB 2009 |

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# 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

## 5.1 PRODUCTION

No information is available in the TRI database on facilities that manufacture or process acrylamide because this chemical is not required to be reported under Section 313 of the Emergency Planning and Community Right-to-Know Act (Title III of the Superfund Amendments and Reauthorization Act of 1986) (EPA 1998).

Acrylamide can be produced from acrylonitrile treated with sulfuric acid or hydrochloric acid, followed by base neutralization or use of an ion exclusion column to separate the chemical from its sulfate salt (Haberman 2002; HSDB 2009; Lewis 2007; O'Neil et al. 2006). Although historically the primary method of acrylamide production, this method results in sulfate byproducts and significant waste streams, and thus, commercial acrylamide producers no longer use this process (Haberman 2002). A newer process was developed in which a solution of acrylonitrile is passed over a fixed bed copper catalyst at 85 °C, resulting in an acrylamide solution. This method has been modified by various companies since its development. A Raney copper catalyst can also be used in both slurry and fixed-bed reactors (Haberman 2002). American Cyanamid uses a proprietary catalytic direct hydration process to produce acrylamide from acrylonitrile, using a reaction of water of solid surfaces of metal, metallic salts, or metallic oxides, which avoids the formation of unwanted byproducts (HSDB 2009).

Numerous other methods of acrylamide production have been developed. Acryloyl chloride or acrylic anhydride can be reacted with ammonia to yield acrylamide. Microorganisms can also be used to convert acrylonitrile to acrylamide via an enzymatic hydration process (Haberman 2002).

Table 5-1 lists the facilities in each state that manufacture, process, or use acrylamide as well as the intended use and the range of amounts of acrylamide that are stored on site. There are currently 233 facilities that produce, process, or use acrylamide in the United States. The data listed in Table 5-1 are derived from the Toxics Release Inventory (TRI 09 2011). These data should be used with caution, however, since only certain types of facilities are required to report. Therefore, this is not an exhaustive list. Commercial production of acrylamide in 1983 was reported as 86,233 pounds (HSDB 2009). Currently, there are four major producers of acrylamide in the United States, with a combined production capacity of approximately 141,000 metric tons (311 million pounds) (SRI 2008). These producers and their respective plant locations are provided in Table 5-2.

## Table 5-1. Facilities that Produce, Process, or Use Acrylamide

| State[a] | Number of facilities | Minimum amount on site in pounds[b] | Maximum amount on site in pounds[b] | Activities and uses[c] |
|---|---|---|---|---|
| AL | 4 | 10,000 | 9,999,999 | 6, 14 |
| AR | 6 | 0 | 99,999 | 1, 2, 3, 5, 6, 7, 12 |
| CA | 15 | 0 | 9,999,999 | 1, 2, 3, 4, 6, 7, 8, 9, 10 |
| CO | 3 | 0 | 99,999 | 6 |
| CT | 6 | 10,000 | 9,999,999 | 2, 3, 4, 6 |
| DE | 1 | 0 | 0 | 0 |
| FL | 3 | 100,000 | 999,999 | 2, 3, 6 |
| GA | 8 | 0 | 9,999,999 | 1, 4, 6, 7 |
| IL | 13 | 0 | 9,999,999 | 2, 3, 6, 7, 12 |
| IN | 1 | 10,000 | 99,999 | 12 |
| KY | 4 | 10,000 | 999,999 | 6, 12 |
| LA | 16 | 0 | 9,999,999 | 1, 2, 3, 4, 5, 6, 9, 10, 12, 13 |
| MA | 4 | 1,000 | 99,999 | 6, 7 |
| MD | 5 | 0 | 999,999 | 6, 7 |
| MI | 22 | 0 | 49,999,999 | 1, 2, 3, 4, 6, 7, 8, 10, 11, 12 |
| MO | 5 | 100 | 99,999 | 1, 2, 3, 6, 7, 8 |
| NC | 8 | 100 | 999,999 | 6, 7 |
| NE | 4 | 0 | 99,999 | 3, 7, 8, 11, 12 |
| NJ | 9 | 0 | 999,999 | 1, 6, 10, 12, 13 |
| NY | 11 | 100 | 99,999 | 6, 7, 9, 10, 12 |
| OH | 11 | 0 | 999,999 | 1, 6, 7, 12, 13 |
| OR | 1 | 1,000 | 9,999 | 6 |
| PA | 8 | 0 | 499,999,999 | 6 |
| SC | 13 | 0 | 999,999 | 6, 7, 12, 14 |
| TN | 8 | 0 | 9,999,999 | 6, 12 |
| TX | 21 | 0 | 9,999,999 | 1, 2, 3, 5, 6, 7, 12, 13 |
| UT | 1 | 1,000 | 9,999 | 12 |
| VA | 8 | 100,000 | 49,999,999 | 1, 2, 3, 4, 6 |
| WA | 2 | 100,000 | 999,999 | 6, 12 |
| WI | 6 | 0 | 99,999 | 2, 3, 6, 7 |

## Table 5-1. Facilities that Produce, Process, or Use Acrylamide

| State[a] | Number of facilities | Minimum amount on site in pounds[b] | Maximum amount on site in pounds[b] | Activities and uses[c] |
|---|---|---|---|---|
| WV | 5 | 0 | 499,999,999 | 1, 5, 6, 12 |
| WY | 1 | 1,000,000 | 9,999,999 | 9 |

[a]Post office state abbreviations used
[b]Amounts on site reported by facilities in each state
[c]Activities/Uses:

1. Produce
2. Import
3. Onsite use/processing
4. Sale/Distribution
5. Byproduct
6. Impurity
7. Reactant
8. Formulation Component
9. Article Component
10. Repackaging
11. Chemical Processing Aid
12. Manufacturing Aid
13. Ancillary/Other Uses
14. Process Impurity

Source: TRI09 2011 (Data are from 2009)

## Table 5-2. 2008 Acrylamide Production in the United States

| Company | Location | Annual capacity (metric tons[a]) | Remarks |
|---|---|---|---|
| Chemtall, Inc. | Riceboro, Georgia | 65,000 | From purchased acrylonitrile; captive consumption for polyacrylamide |
| Ciba Specialty Chemicals Corporation Water & Paper Treatment Business Segment | Suffolk, Virginia | 15,000 | From purchased acrylonitrile; primarily captive consumption for polyacrylamide |
| Kemira Water Solutions, Inc. | Waggaman, Louisiana | 41,000 | From captive acrylonitrile; captive consumption for polyacrylamide |
| Nalco Company | Garyville, Louisiana | 20,000 | From purchased acrylonitrile; primarily captive consumption for polyacrylamide |
| Total | | 141,000 | |

[a]All production is as solution.

Source: SRI 2008; SRI Consulting estimates as of February 1, 2008

Acrylamide is produced as a byproduct when foods are cooked at high temperatures, such as by frying, roasting, and baking (Muttucumaru et al. 2008). This occurs by the Maillard reaction, where thermal degradation of amino acids occurs in the presence of reducing sugars at temperatures above 120 °C. The major precursors for acrylamide formation by the Maillard reaction are free asparagines and reducing sugars (Arisseto et al. 2007; Mottram 2002; Muttucumaru et al. 2008; Stadler et al. 2002). Acrylamide concentrations in the foods rise with temperature and length of heating, and appear to be affected by water content, food composition, and processing conditions (WHO 2002).

## 5.2 IMPORT/EXPORT

The demand for acrylamide in the United States was reported as 245 and 253 million pounds in 2006 and 2007, respectively. U.S. imports were 61 and 55 million pounds in 2006 and 2007, respectively, while U.S. exports were 57 and 53 million pounds in 2006 and 2007, respectively (CMR 2008).

## 5.3 USE

Commercially-produced acrylamide is used mainly as an intermediate in the production of polyacrylamides (EU 2002; Haberman 2002; O'Neil et al. 2006; WHO 2003). This accounts for 94% of manufactured acrylamide (Haberman 2002). Polyacrylamides are then primarily used as flocculants for clarifying drinking and treating municipal and industrial effluents (Abdelmagid 1982; EPA 2006c; EU 2002; Haberman 2002; WHO 2003). They aid in dewatering sludge from sewage treatment plant effluent as well as industrial waste water from pulp and paper plants (Abdelmagid 1982; Haberman 2002). Polyacrylamides are also found in cosmetics and toiletries and are used to prepare polyacrylamide gels for use in biotechnology laboratories (EU 2002; Lewis 2007). In the oil industry, acrylamide is used as a flow control agent to enhance oil production from wells. Acrylamide and polyacrylamides are also used in the production of dyes and organic chemicals such as N-methylacrylamide, in copolymers for contact lenses, in permanent-press fabrics, for sizing paper and textiles, as a binder and retention aid for pulp and paper production, in the processing of ore, in sugar refining, and as a chemical grouting agent and soil stabilizer for the construction of tunnels, sewers, wells, and reservoirs (Abdelmagid 1982; EPA 2006c; EU 2002; Haberman 2002; Lewis 2007; O'Neil et al. 2006; WHO 2003).

## 5.4 DISPOSAL

Acrylamide is typically produced as an intermediate for the production of polyacrylamides (Haberman 2002; O'Neil et al. 2006), and is therefore consumed in the process. No information was found regarding the disposal of acrylamide.

# 6. POTENTIAL FOR HUMAN EXPOSURE

## 6.1 OVERVIEW

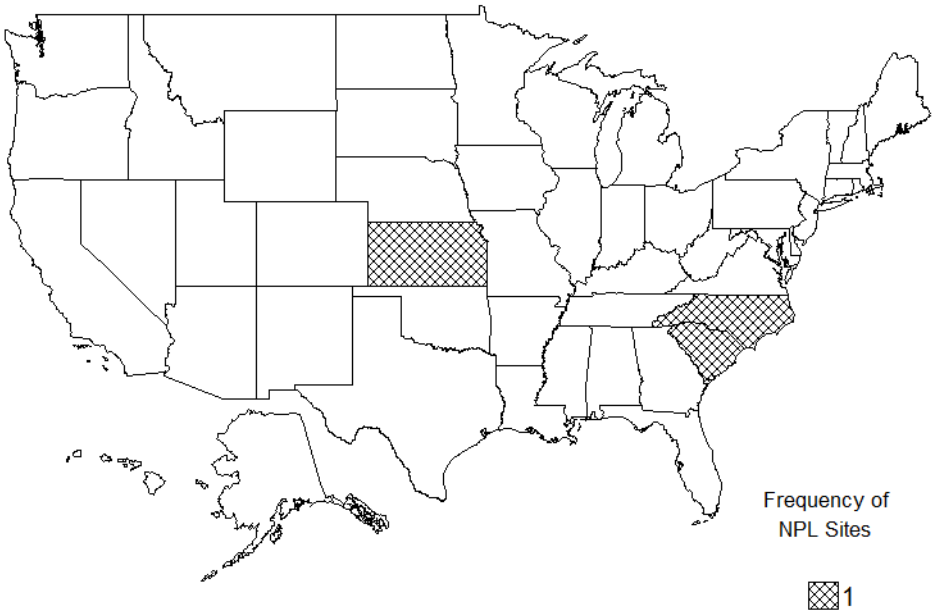
Acrylamide has been identified in at least 3 of the 1,699 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2007). However, the number of sites evaluated for acrylamide is not known. The frequency of these sites can be seen in Figure 6-1.

Acrylamide is an industrial chemical used mainly in the production of polyacrylamides, which are primarily used as flocculants for clarifying drinking and treating municipal and industrial effluents (Abdelmagid 1982; EPA 2006c; EU 2002; Haberman 2002; O'Neil et al. 2006; WHO 2003). Acrylamide may be released to the environment during production and use of polyacrylamides, which are used as clarifiers in water treatment. Residual monomer released from the polyacrylamide coagulants is the main source of acrylamide contamination of drinking water (Abdelmagid 1982; Cavalli et al. 2004; EPA 2006c; WHO 2003), though it can also be released from plastics and dye industries and from acrylamide-containing grouting agents used in reservoirs and wells (Cavalli et al. 2004; EPA 2006c). Acrylamide can be released to the environment during formulation of cosmetics or other consumer products and in the laboratory while gel chromatography is being performed (Boettcher and Angerer 2005; EU 2002). Due to its low vapor pressure and high water solubility, acrylamide is rarely identified in atmospheric samples (WHO 2003).

Acrylamide is expected to be highly mobile in soil and water (EPA 2006c; HSDB 2009; WHO 2003). It is highly susceptible to biodegradation in both soils and surface water (Abdelmagid 1982; EPA 2006c; Haberman 2002; WHO 2003). It is not typically present in the atmosphere (HSDB 2009; Pratt 2000). Acrylamide is not expected to significantly bioconcentrate (EPA 2006c; Haberman 2002; WHO 2003).

Acrylamide is sometimes present in drinking water due to leaching of monomer during treatment processes (Abdelmagid 1982; Cavalli et al. 2004; EPA 2006c; van Dijk-Looijaard and van Genderen 2000; WHO 2003), as well as release from grouting agents in wells and dams and releases from plastics and dye industries (Cavalli et al. 2004). It is rarely found in soil samples (HSDB 2009). Acrylamide is not a common air pollutant, due to its low vapor pressure and high water solubility. It is rarely identified in atmospheric samples (HSDB 2009; Pratt 2000; WHO 2003).

## Figure 6-1. Frequency of NPL Sites with Acrylamide Contamination




Derived from HazDat 2007

In 2002, acrylamide was first identified in samples of food cooked at high temperatures (Tareke et al. 2002). Concentrations of acrylamide in food vary with the type of food and method of cooking, and typically increase with temperature and length of heating (Sorgel et al. 2002; WHO 2002, 2003). Carbohydrate-rich foods typically contain the highest levels of acrylamide (Muttucumaru et al. 2008; Tareke et al. 2002; WHO 2003), whereas protein-based foods contain smaller amounts (Tareke et al. 2002). As acrylamide content in food appears to be affected by temperature, water content, food thickness, and length of heating, various steps can be taken to minimize the exposure of acrylamide from food sources (Sorgel et al. 2002; WHO 2002, 2003).

Acrylamide is a carcinogen with the potential to cause nervous system damage (Arisetto et al. 2007; EPA 2006c; Lewis 2000). Exposure occurs mainly via ingestion, dermal contact, and inhalation routes (Lewis 2000; Sorgel et al. 2002). Ingestion of foodstuffs containing acrylamide appears to be one of the most common methods of exposure for the general public. Average estimated intake of acrylamide from food sources ranged from 0.8 to 6.0 μg/kg bw/day for short-term exposure and 0.3 to 0.8 μg/kg bw/day for long-term exposure (WHO 2002, 2003). Children may be susceptible to food-borne exposure 2–3 times that of adults on a body weight basis (WHO 2002, 2003).

Ingestion of polyacrylamide-treated drinking water containing residual monomer as well as water in contact with acrylamide-containing products, such as grouting agents, can result in exposure to acrylamide (EPA 2006c; EU 2002; WHO 2003). The presence of acrylamide in tobacco smoke can result in inhalation exposure for both adults and children (EU 2002; Moreno Navarro et al. 2007). Dermal exposure can result from contact with cosmetics and toiletries containing polyacrylamides (EU 2002). Once in the body, acrylamide is widely dispersed by body fluids, and can also cross the placental barrier (WHO 2003), resulting in exposure to unborn children. Breast milk of mothers with diets high in acrylamide-containing foods can contain high amounts of acrylamide (Sorgel et al. 2002). Occupational exposure to acrylamide is primarily due to dermal contact when handling bags and drums of the chemical or preparing polyacrylamide gels, followed by inhalation of dust or aerosols (EU 2002; Lewis 2000).

## 6.2 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005a). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ 10 or more full-time employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011,

1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 2005a).

### 6.2.1 Air

Estimated releases of 8,509 pounds (~3.9 metric tons) of acrylamide to the atmosphere from 76 domestic manufacturing and processing facilities in 2009, accounted for about 0.2% of the estimated total environmental releases from facilities required to report to the TRI (TRI09 2011). These releases are summarized in Table 6-1.

Acrylamide may be released to the atmosphere during production of polymers, formulation of cosmetics or other consumer products, and in the laboratory while gel chromatography is being performed (Boettcher and Angerer 2005). Release of tobacco smoke, which was shown to contain acrylamide (Schumacher et al. 1977), may also contribute to airborne acrylamide in confined spaces. However, acrylamide is not anticipated to be a common air contaminant due to its low vapor pressure and high water solubility. Acrylamide is not expected to be removed from soils or water by volatilization (WHO 2003). Limited data indicate that atmospheric acrylamide concentrations are very low, when it is identified at all (Pratt 2000; HSDB 2009).

The total national baseline National Toxics Inventory (NTI) emissions for acrylamide during 1900–1998 were 35.4 tons/year. Total urban emissions were 33.5 tons/year while total rural emissions were 1.9 tons/year (EPA 2000). The National Emissions Inventory database, maintained by the EPA, reports total U.S. acrylamide emissions of 12.12 tons per year in 2005 from various sources, including degreasing, waste disposal, pulp and paper, solvents, oil and gas production, industrial surface coatings, and oil and gasoline production among others (EPA 2009b).

## Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Acrylamide[a]

| | | Reported amounts released in pounds per year[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Total release | | |
| State[c] | RF[d] | Air[e] | Water[f] | UI[g] | Land[h] | Other[i] | On-site[j] | Off-site[k] | On- and off-site |
| AL | 1 | 83 | 0 | 0 | 467 | 0 | 83 | 467 | 550 |
| AR | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 2 |
| CA | 2 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| CO | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CT | 2 | 2 | 3 | 0 | 152 | 0 | 5 | 152 | 157 |
| GA | 5 | 5,117 | 0 | 0 | 1,089 | 0 | 5,117 | 1,089 | 6,206 |
| IL | 7 | 91 | 0 | 0 | 0 | 0 | 91 | 0 | 91 |
| KY | 1 | 458 | 0 | 0 | 0 | 0 | 458 | 0 | 458 |
| LA | 5 | 295 | 86 | 778,620 | 2,735 | 0 | 779,636 | 2,100 | 781,736 |
| MD | 2 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 5 |
| MI | 4 | 183 | 0 | 0 | 0 | 0 | 183 | 0 | 183 |
| NC | 6 | 1,769 | 0 | 0 | 0 | 0 | 1,769 | 0 | 1,769 |
| NE | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| NJ | 2 | 8 | 0 | 0 | 0 | 0 | 8 | 0 | 8 |
| OH | 5 | 1 | 0 | 890,000 | 5 | 12 | 890,001 | 17 | 890,018 |
| PA | 5 | 89 | 5 | 0 | 0 | 987 | 94 | 987 | 1,081 |
| SC | 8 | 281 | 250 | 0 | 0 | 0 | 531 | 0 | 532 |
| TN | 4 | 2 | 0 | 0 | 0 | 200 | 2 | 200 | 202 |
| TX | 8 | 24 | 0 | 2,890,223 | 253 | 250 | 2,890,250 | 500 | 2,890,750 |
| VA | 1 | 65 | 0 | 0 | 180 | 0 | 65 | 180 | 245 |
| WA | 1 | 23 | 0 | 0 | 0 | 0 | 23 | 0 | 23 |
| WI | 3 | 11 | 0 | 0 | 28 | 0 | 11 | 28 | 39 |
| Total | 76 | 8,509 | 344 | 4,558,843 | 4,910 | 1,450 | 4,568,334 | 5,722 | 4,574,057 |

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other onsite landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI09 2011 (Data are from 2009)

### 6.2.2 Water

Estimated releases of 344 pounds (~0.16 metric tons) of acrylamide to surface water from 76 domestic manufacturing and processing facilities in 2009, accounted for about 0.008% of the estimated total environmental releases from facilities required to report to the TRI (TRI09 2011). These releases are summarized in Table 6-1.

Acrylamide in drinking water is typically a result of the release of residual monomer from polyacrylamide coagulants that are used as clarifiers in the treatment of raw water (Abdelmagid 1982; Cavalli et al. 2004; EPA 2006c; WHO 2003). The clarifiers served to coagulate and trap suspended solids such that they may be removed more easily from the water. Acrylamide that does not coagulate is released into the environment as a drinking water contaminant (Abdelmagid 1982; EPA 2006c). Use of polyacrylamides as grouting agents in reservoirs and wells can result in release to drinking water supplies. They can also be released in water from plastics and dye industries (Cavalli et al. 2004; EPA 2006c). When released to land, acrylamide does not bind to soil and will thus move rapidly through the soil column (EPA 2006c), which can result in increased risk of surface or groundwater contamination.

Total release of acrylamide to water between 1987 and 1993 was 36,287 pounds. Major industries found to release acrylamide to water include plastics and resins (19,002 pounds), pulp mills (8,000 pounds), industrial organics (3,107 pounds), and industrial inorganics (2,510 pounds) (EPA 2006c). According to the EPA's Toxic Chemical Release Inventory, release of acrylamide to land and water from 1987 to 1993 was over 40,000 pounds. Acrylamide releases were typically from plastics industries, with the largest releases occurring in Michigan (EPA 2006c).

As required by the 1974 Safe Drinking Water Act, EPA developed a maximum contaminant level (MCL) for acrylamide, which specifies the concentration at which it is not expected to cause health problems. The MCL for acrylamide is zero (EPA 2006c). EPA requires water suppliers to control the amount of acrylamide added to water during the treatment process. This can be accomplished by limiting the amount of acrylamide in the polyacrylamide flocculants or by limiting the dose of flocculants (WHO 2003). Uncoagulated acrylamide in drinking water must be <0.5 µg/L (ppb) (Cavalli et al. 2004; EPA 2006c). This concentration corresponds to a maximum authorized dose of polymer of 1 mg/L (for a monomer content of 0.05%), as this corresponds to 0.5 µg/L of monomer in the water (Cavalli et al. 2004; WHO 2003). In European Union countries, the maximum allowable concentration in drinking water is 0.1 µg/L (Cavalli et al. 2004).

If acrylamide is found to be present in raw water, the concentration can be decreased through ozonation or by treating the water with potassium permanganate. Acrylamide is not removed by conventional water treatment processes (WHO 2003).

### 6.2.3 Soil

Estimated releases of 4,910 pounds (~2.2 metric tons) of acrylamide to soils from 76 domestic manufacturing and processing facilities in 2009, accounted for about 0.1% of the estimated total environmental releases from facilities required to report to the TRI (TRI09 2011). An additional 4,558,843 pounds (~2,783 metric tons), constituting about 99.6% of the total environmental emissions, were released via underground injection (TRI09 2011). These releases are summarized in Table 6-1.

Polyacrylamides releases to land can occur from plastics and dye industries (Cavalli et al. 2004; EPA 2006c). When released to land, acrylamide does not bind to soil and will thus move rapidly through the soil column (EPA 2006c; WHO 2003). Total release of acrylamide to land between 1987 and 1993 was 5,818 pounds. Major industries found to release acrylamide to land include plastics and resins (2,177 pounds), industrial organics (2,200 pounds), and industrial inorganics (500 pounds) (EPA 2006c). According to the EPA's Toxic Chemical Release Inventory, release of acrylamide to land and water from 1987 to 1993 was over 40,000 pounds. Acrylamide releases were typically from plastics industries, with the largest releases occurring in Michigan (EPA 2006c).

## 6.3 ENVIRONMENTAL FATE

### 6.3.1 Transport and Partitioning

Acrylamide is expected to be highly mobile in soil and water. When released to land, acrylamide does not bind to soil and will thus move rapidly through the soil column and into groundwater, where it is also expected to have high mobility (EPA 2006c; HSDB 2009; WHO 2003). Acrylamide has a higher mobility in sandy soils than in clay soils, which can result in increased risk of surface or groundwater contamination (Abdelmagid 1982; EPA 2006c; WHO 2003). Acrylamide is not expected to be removed from soils or water by volatilization (WHO 2003).

Acrylamide is not expected to significantly bioconcentrate in aquatic organisms due to its high water solubility and its ability to be degraded by microorganisms (EPA 2006c; Haberman 2002; WHO 2003).

Acrylamide was determined to have a bioconcentration factor (BCF) of around 1 in both the carcass and viscera (0.86–1.44 and 1.12–1.65, respectively) of rainbow trout exposed to acrylamide concentrations of 0.338 and 0.710 mg/L. This indicates that acrylamide did not bioaccumulate significantly in the trout (Petersen et al. 1985).

### 6.3.2 Transformation and Degradation

Acrylamide is susceptible to biodegradation in both soils and surface water (Abdelmagid 1982; EPA 2006c; Haberman 2002; HSDB 2009; WHO 2003). As it does not bind to soil, acrylamide moves rapidly through the soil column where it is quickly degraded (EPA 2006c; WHO 2003).

In sandy soils, acrylamide has a higher mobility and lower rate of degradation than in clay soils (Abdelmagid 1982; EPA 2006c; WHO 2003). Acrylamide has been shown to biodegrade in effluent from a sludge dewatering process (WHO 2003).

Enzyme-catalyzed hydrolysis is a dominant mechanism for removal of acrylamide from soils (WHO 2003). Abdelmagid (1982) showed that acrylamide is readily hydrolyzed in soils under both aerobic and anaerobic conditions, resulting in the release of $NH_4^+$. The rate of decomposition in soil is influenced by temperature and incubation time. Soil type did not appear to be a factor, as acrylamide decomposed in both sandy and heavy-textured soils (Abdelmagid 1982).

Acrylamide was shown to degrade by an average of 41.5% by BOD in 14 days at 25 °C and 100 mg/L test substance, with 30 mg/L activated sludge (CERI 1999).

#### 6.3.2.1 Air

Limited data indicate that acrylamide concentrations in the atmosphere are very low (HSDB 2009; Pratt 2000). In the atmosphere, acrylamide is susceptible to degradation via photochemically generated hydroxyl radicals and ozone. The rate constant for acrylamide's reaction with hydroxyl radicals has been estimated as $1.1 \times 10^{-11}$ $cm^3$/molecule-second, and its reaction with ozone is estimated as $1.7 \times 10^{-18}$ $cm^3$/molecule-second using a structure estimation method (Meylan and Howard 1993). Using an average atmospheric hydroxyl radical concentration of $1.5 \times 10^6$ molecules/$cm^3$ and ozone concentration of $7 \times 10^{11}$ molecules/$cm^3$, estimated half-lives of approximately 12 hours (hydroxyl radical reaction) and 6.5 days (ozone reaction) can be calculated.

#### 6.3.2.2 Water

Acrylamide is expected to be quickly degraded in water by biological processes (Abdelmagid 1982; EPA 2006c; Haberman 2002; WHO 2003). At an initial concentration of 10 ppm, acrylamide was completely degraded in about 12 days using water obtained from the Hackensack River (Cherry et al. 1956). Acrylamide, at an initial concentration of 8 µg/L, was rapidly degraded following a lag period of approximately 9 days in well-aerated, sunlit river water obtained from the Thames River, England (Croll et al. 1974). Subsequent re-seeding of the water with acrylamide resulted in rapid degradation with little or no lag period.

#### 6.3.2.3 Sediment and Soil

When released to land, acrylamide does not bind to soil and will thus move rapidly through the soil column, where it is expected to be quickly degraded (EPA 2006c; Haberman 2002; WHO 2003). Acrylamide has a higher mobility and lower rate of degradation in sandy soils than in clay soils (Abdelmagid 1982; EPA 2006c; WHO 2003).

Enzyme-catalyzed hydrolysis is a dominant mechanism for removal of acrylamide from soils (WHO 2003). Abdelmagid (1982) showed that acrylamide is readily hydrolyzed in soils, producing $NH_4^+$. The rate of decomposition in soil is influenced by temperature and incubation time. Soil type did not appear to be a factor, as acrylamide decomposed in both sandy and heavy-textured soils. Acrylamide, therefore, does not appear to accumulate in soils (Abdelmagid 1982).

## 6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to acrylamide depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of acrylamide in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on acrylamide levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable. The analytical methods available for monitoring acrylamide in a variety of environmental media are detailed in Chapter 7.

### 6.4.1 Air

Acrylamide is not anticipated to be a common air contaminant due to its low vapor pressure and high water solubility. Limited data indicate that acrylamide concentrations in the atmosphere are very low (HSDB 2009; Pratt 2000). Air samples were collected at 25 sites throughout Minnesota over various periods of time (up to 8 years) from 1991 to 1998. Acrylamide was not identified in any of the samples (Pratt 2000). As of 1978, acrylamide concentrations in the air near six U.S. acrylamide/polyacrylamide producers or users averaged <0.2 μg/m$^3$ in vapor or particulate form. Concentrations ranged from <0.1 to 1.1 μg/m$^3$ (EPA 1978).

The total national baseline NTI emissions for acrylamide during 1900–1998 were 35.4 tons/year. Total urban emissions were 33.5 tons/year, while total rural emissions were 1.9 tons per year (EPA 2000).

### 6.4.2 Water

Acrylamide in drinking water is typically a result of leaching during treatment processes (van Dijk-Looijaard and van Genderen 2000). Acrylamide results from the release of residual monomer from polyacrylamide coagulants that are used as clarifiers in the treatment of raw water (Abdelmagid 1982; Cavalli et al. 2004; EPA 2006c; WHO 2003). Polyacrylamides can enter water supplies due to their use as grouting agents in reservoirs and wells in addition to being released in water from plastics and dye industries (Cavalli et al. 2004; EPA 2006c).

Acrylamide concentrations of <5 μg/L were found in river and tap water. In the sampling area, polyacrylamides were known to be used in treating potable water (WHO 2003). Public drinking water supply wells in West Virginia contained acrylamide concentrations of 0.024–0.041 μg/L (WHO 2003).

One water sample, taken from downstream of the effluent of a producer of polyacrylamide, contained acrylamide at 1,500 μg/L, while samples from other industrial sites (acrylamide and polyacrylamide production and use locations) contained <0.8 μg/L acrylamide (EPA 1978).

Various effluents in the United Kingdom have been found to contain acrylamide, including effluent from a clay pit (16.0 μg/L), a tailings lagoon (39–42 μg/L), coal washing lagoon (1.8 μg/L), colliery/cooking plant, (0.74 μg/L), treated paper mill (0.47–14.4 μg/L), and paper mill process water (45.4 μg/L) (Croll et al. 1974; IPCS 1985). In Devon, England, 17.4 ppb of acrylamide was detected in sewage effluent (Brown and Rhead 1979).

### 6.4.3 Sediment and Soil

Soil samples obtained near six U.S. acrylamide and/or polyacrylamide producers contained <0.02 μg/g of acrylamide. Concentrations ranged from <0.02 to <0.08 μg/g (EPA 1978).

### 6.4.4 Other Environmental Media

Acrylamide was detected in waste materials and containers at 3 of the 1,699 hazardous waste sites that have been proposed for inclusion on the EPA NPL (HazDat 2007). Acrylamide was also identified in tobacco smoke samples, obtained from nonfiltered cigarettes smoked under typically conditions. The presence of acrylamide was identified in the smoke condensate by infrared (IR), mass spectrometry (MS), and nuclear magnetic resonance (NMR) (Schumacher et al. 1977).

In 2002, acrylamide was first shown to be produced when foods are cooked at high temperatures (Tareke et al. 2002). Concentrations of acrylamide in food vary with the type of food and method of processing and cooking. Acrylamide concentrations in food typically increase with temperature and length of heating (Sorgel et al. 2002; WHO 2002, 2003). Starchy foods, such as potato-based products, typically contain the highest levels of acrylamide (Muttucumaru et al. 2008; Tareke et al. 2002; WHO 2003), whereas protein-based foods contain smaller amounts (Tareke et al. 2002).

Tareke et al. (2002) analyzed acrylamide content of various heated foods. Acrylamide concentrations were determined by gas chromatography (GC)-MS and liquid chromatography (LC)-MS/MS. In heated protein-rich foods, acrylamide concentrations of 5–50 μg/kg were found. Carbohydrate-rich foods, such as potato, beetroot, potato products, and crispbread, contained much higher concentrations, ranging from 150 to 4,000 μg/kg. The median acrylamide concentration in fried foods, including beef, chicken, soymeal, grated potatoes, boiled mashed potatoes, and grated beetroot were 17, 28, 16, 447, 172, and 850 μg/kg, respectively. In microwave-heated grated potatoes, the median acrylamide concentration was found to be 551 μg/kg, while microwave-heated cod was less than the detection limit (<5 μg/kg). In restaurant-prepared or purchased foods, the median acrylamide concentrations for hamburger, French fries, potato crisps, and three types of crispbread were 18, 424, 1,739, and 208, respectively. Unheated controls and boiled foods contained very little acrylamide, with all results being less than the detection limits for the methods (<5 μg/kg by GC-MS and <10 μg/kg by LC-MS/MS) (Tareke et al. 2002).

Acrylamide concentrations were analyzed in food samples in Norway, Sweden, Switzerland, the United Kingdom, and the United States in various studies (Table 6-2). Almost all items analyzed contained some level of acrylamide. Of the food tested, chips and crisps contained the highest average concentrations, ranging from not detectable to 3.5 mg/kg (WHO 2002).

Acrylamide concentrations were determined for various foods prepared using home cooking methods, including the use of a household oven and microwave oven. The data are presented in Tables 6-3 and 6-4. The authors concluded that longer cooking times and higher temperatures appear to cause increased acrylamide concentrations in food (Sorgel et al. 2002).

The acrylamide content of various carbohydrate-rich Brazilian foods was determined by LC-MS/MS. Samples were obtained from grocery stores, restaurants, and fast food restaurants in Campinas, Sao Paulo, Brazil between September 2004 and April 2006. The foods sampled included French fries, potato chips, bread, crispbread, crackers, breakfast cereals, coffee, beer, and other high carbohydrate foods typically processed at high temperatures. Of 111 samples from 19 product categories, acrylamide concentrations ranged from <20 to 2,528 mg/kg. Considerable differences in concentration existed between individual foods within the same product class. Potato chips, processed at high temperatures, and instant coffee had the highest concentrations of acrylamide, while cassava- and maize-based foods, bread, and beer were found to have the lowest levels. The detection limit and limit of quantification were 10 and 20 mg/kg, respectively (Arisseto et al. 2007).

An ongoing U.S. Food and Drug Administration (FDA) survey collects acrylamide data on U.S. food products under the Total Diet Study (TDS). Data on approximately 280 core foods (or TDS foods) were collected to determine the nutrient and contaminant levels in the foods from each of the geographic regions in the United States (West, North Central, South, and Northeast). Food samples are collected from grocery stores and fast food restaurants from three cities in each region, prepared for consumption, and then analyzed (FDA 2009). The summary results for the 2003–2006 TDS data on acrylamide are presented in Table 6-5. Foods within certain food groups (i.e., grains/starches/baked goods) have been found to have very high levels of acrylamide. It should be noted that the high levels within the vegetable group were primarily due to potato chips and French fries.

## Table 6-2. Acrylamide Levels in Different Food and Food Product Groups from Norway, Sweden, Switzerland, the United Kingdom, and the United States

| Food/product group | Acrylamide levels (µg/kg)[a] | | | |
|---|---|---|---|---|
| | Mean[b] | Median[2] | Minimum-Maximum | Number of Samples |
| Crisps, potato/sweet potato[c] | 1,312 | 1,343 | 170–2,287 | 38 |
| Chips, potato[d] | 537 | 330 | <50–3,500 | 39 |
| Batter based products | 36 | 36 | <30–42 | 2 |
| Bakery products | 112 | <50 | <50–450 | 19 |
| Biscuits, crackers, toast, bread crisps | 423 | 142 | <30–3,200 | 58 |
| Breakfast cereals | 298 | 150 | <30–1346 | 29 |
| Crisps, corn | 218 | 167 | 34–416 | 7 |
| Bread, soft | 50 | 30 | <30–162 | 41 |
| Fish and seafood products, crumbled, battered | 35 | 35 | 30–39 | 4 |
| Poultry or game, crumbed, battered | 52 | 52 | 39–64 | 2 |
| Instant malt drinks | 50 | 50 | <50–70 | 3 |
| Chocolate powder | 75 | 75 | <50–100 | 2 |
| Coffee powder | 200 | 200 | 170–230 | 3 |
| Beer | <30 | <30 | <30 | 1 |

[a]The limits of detection and quantification varied among laboratories; values reported as less than a value are below the limit reported by the laboratory.
[b]Mean and median values were calculated where individual data were available; sample sizes were extremely small, particularly for some food categories; where the mean and median are different, it reflects the skewed distribution of the underlying data that were collected in different countries and may represent different food items within the larger category.
[c]Products that are thinly sliced and fried (such as potato chips).
[d]Products that are more thickly sliced (such as French fries).

Source: WHO 2002

## Table 6-3. Acrylamide in Potato Products

| Sample | Size (mm) | Cooking temperature (°C) | Cooking time (minutes) | Acrylamide concentration (µg/kg) |
|---|---|---|---|---|
| Potato chips | 3 | 180 | 2 | 2,557.9 |
| | 3 | 140 | 2 | 36.3 |
| | 3 | 180 | 4 | 7,678.3 |
| | 3 | 140 | 4 | 53.3 |
| | 1 | 180 | 1 | 121.1 |
| | 1 | 140 | 2 | 20.0 |
| | 1 | 180 | 3 | 9,670.2 |
| | 1 | 140 | 4 | 35.3 |
| French fries | 5 | 180 | 2 | 1,716.9 |
| | 5 | 160 | 2 | 53.8 |
| | 5 | 140 | 2 | 11 |
| | 5 | 180 | 4 | 2,687.0 |
| | 5 | 160 | 4 | 1,049.5 |
| | 5 | 140 | 4 | 77.0 |
| | 10 | 180 | 2 | 674.2 |
| | 10 | 160 | 2 | <8 |
| | 10 | 140 | 2 | <8 |
| | 10 | 180 | 4 | 1,084.3 |
| | 10 | 160 | 4 | 87.3 |
| | 10 | 140 | 4 | 10.0 |
| | 13 | 180 | 4 | 476.2 |
| | 13 | 140 | 2 | <8 |
| | 13 | 180 | 6 | 880.0 |
| | 13 | 140 | 4 | <8 |

Source: Sorgel et al. 2002

## Table 6-4. Acrylamide Content of Food

| Sample | Description | Cooking method | Cooking time (minutes) | Acrylamide concentration (µg/kg) |
|---|---|---|---|---|
| Walnuts | | Roasted | | <8 |
| Pine seeds | | Roasted | | 16.4 |
| Candied chestnuts | | Baked | | 8.3 |
| Crème carmel | | Boiled | | <8 |
| Whiskey | | | | <8 |
| Liver of beef | | Baked | | <8 |
| Breaded fish | | Baked | | <8 |
| Goose skin | | Oven | | <8 |
| Goose meat | | Oven | | <8 |
| Gingerbread | Lightly baked | 200 °C | | 12.1 |
| Gingerbread | Dark baked | 200 °C | | 113.0 |
| Gingerbread | | | | 180.7 |
| Gingerbread balls | | | | 639.5 |
| Spiced cookies | | | | 143.6 |
| Popcorn | | Microwave, 800 W | 1 | 29.7 |
| Popcorn | | Microwave, 600 W | 2 | 50.0 |
| Popcorn | | Microwave, 800 W | 2 | 132.8 |
| Popcorn | | Microwave, 600 W | 3 | 250.3 |
| Popcorn | | Microwave, 600 W | 4 | 307.1 |
| Croquettes | | 180 °C | 2 | 354.7 |
| Croquettes | | 180 °C | 4 | 453.4 |
| Hash browns | | 180 °C | 2 | 446.1 |
| Hash browns | | 180 °C | 4 | 751.7 |
| Fried potatoes | | | | 543.1 |
| Croutons | | Baked | | 27.9 |
| Bread crust | | | | 370.6 |
| Toast | | Toasted | | 54.1 |

Source: Sorgel et al. 2002

## Table 6-5. Acrylamide Levels in Food Products Sampled for the 2003–2006 Total Diet Studies (TDS) Summary

| TDS food category | Acrylamide range (ppb) for 2003 | Acrylamide range (ppb) for 2004 | Acrylamide range (ppb) for 2005 | Acrylamide range (ppb) for 2006 |
|---|---|---|---|---|
| Dairy | ND–16 | ND | ND–16 | ND–18 |
| Eggs | ND | No data | ND | No data |
| Baby food | ND–267 | ND–407 | ND–442 | ND–381 |
| Meat/poultry/fish | ND–26 | ND–27 | ND–30 | ND–19 |
| Legumes | ND–93 | ND–93 | ND–84 | ND–54 |
| Grains/starches/ baked goods | ND–647 | ND–946 | ND–616 | ND–470 |
| Fruits | ND–202 | 109–355[a] | ND–250 | 326[a] |
| Vegetables | ND–536[b] | ND-829[b] | ND–529 | ND–393 |
| Mixtures (e.g., casseroles, sandwiches, soups, pizza) | ND–187 | ND–210 | ND–93 | ND–55 |
| Candy/sweets/ Sugars/syrups | ND–29 | ND–42 | 13–50 | 17–32 |
| Fats/oils | ND | No data | No data | No data |
| Beverages | ND–12 | ND–21 | ND–28 | ND |

[a]Only bottle prune juice was included in this category for 2004 and 2006.
[b]The highest levels in the vegetable category are for potato chips and French fries; the majority of the vegetables had low or non-detectable levels.

ND = Not detected

Source: U.S. Food and Drug Administration 2006

Temperature, water content, food thickness, and length of heating appear to be important factors affecting the amount of acrylamide that is formed while cooking foods (Sorgel et al. 2002; WHO 2002, 2003). Reversing these factors could help minimize acrylamide formation (e.g., heating to <180 °C, making products very thick, etc.) (Sorgel et al. 2002). In many countries, food manufacturers have been requested to take steps to decrease the formation of acrylamide in their products, which has become an important area of research (Amrein et al. 2007).

With potato products, controlling reducing sugars, processing temperature, and moisture content can help limit formation of acrylamide (Amrein et al. 2007). It is thought that the amount of asparagine is also an important factor in acrylamide formation in potatoes (Muttucumaru et al. 2008). With bakery products, the amount of free asparagines as well as the type of baking agent can affect acrylamide concentration. Replacing the baking agent $NH_4HCO_3$, for example, with $NaHCO_3$ in sweet bakery products can help reduce acrylamide content. High concentrations of acrylamide can form during heating of olives and dried fruit (Amrein et al. 2007). In almonds, roasting time and temperature, water content, and asparagine levels (which can range from 500 to 2,760 mg/kg in almonds) are thought to affect the amount of acrylamide formed during roasting (Amrein et al. 2007; Zhang et al. 2011). Additional research is needed to determine the acrylamide reduction strategies for each type of food.

## 6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

Acrylamide is a probable or confirmed carcinogen with the potential to cause nervous system damage, weakness, and incoordination of the legs following short-term exposure to high levels. Long-term effects of acrylamide include nervous system damage, paralysis, and cancer (Arisetto et al. 2007; EPA 2006c; Lewis 2000). Exposure can occur via ingestion, dermal contact, inhalation, and intraperitoneal routes (Lewis 2000; Sorgel et al. 2002). Once in the body, acrylamide is widely dispersed by body fluids. It can also cross the placental barrier (WHO 2003).

Exposure to high levels of acrylamide can result from ingestion of various foodstuffs. Acrylamide is produced when certain foods are cooked at high temperatures (Tareke et al. 2002). Concentrations of acrylamide in food vary with the type of food and method of processing and cooking, and typically increase with temperature and length of heating (Sorgel et al. 2002; WHO 2002, 2003). Foodstuffs, particularly those rich in carbohydrates, may develop high levels of acrylamide when cooked at high temperatures (Moreno Navarro et al. 2007). As shown in Table 6-3, acrylamide concentrations in potato products such as potato chips and French fries are related to size and cooking temperature and duration.

Potato chips made from 1 mm thick potato slices cooked at 180 °C for 3 minutes contain an estimated 9,670 µg acrylamide/kg potato or the equivalent of 541 µg acrylamide in a 2 ounce bag of potato chips. For an adult (70 kg) or child (15 kg), acrylamide doses from consumption of such a 2-ounce bag of potato chips are estimated at 7.7 and 36 µg/kg, respectively, and are in the range of the acute-duration oral MRL of 10 mg/kg/day for acrylamide. For potato chips made from 1 mm thick potato slices, but cooked at a lower temperature (140 °C) for 4 minutes, estimated adult and child doses are 0.028 and 0.13 µg/kg, respectively, and are somewhat lower than the ATSDR intermediate- and chronic-duration oral MRL of 1 µg/kg for acrylamide. Potato products, including chips and other potatoes cooked at high temperatures, may comprise a large percentage of the total acrylamide intake from food. However, foods with lower acrylamide content but eaten at a higher frequency, such as bread, may contribute significantly to the total exposure from foods (WHO 2002).

Food was found to contribute significantly to the overall exposure of the general population to acrylamide. Short-term dietary intake of acrylamide was estimated using Monte Carlo techniques based on data from The Netherlands, United States, and Sweden. The short-term exposure estimates ranged from 0.8 to 6.0 µg/kg-bw/day for the average consumer to the 98$^{th}$ percentile consumer (WHO 2002). Average estimated long-term intake of acrylamide from food sources was determined based on data from Australia, Norway, The Netherlands, Sweden, and the United States, as well as data from the International Agency for Research on Cancer (IARC). Long-term intake estimates ranged from 0.3 to 0.8 µg/kg-bw/day (WHO 2002, 2003). Tareke et al. (2002) concluded that the acrylamide levels found in heated foods could result in a daily acrylamide intake of a few tens of micrograms.

Due to the presence of acrylamide in tobacco smoke (EU 2002; Schumacher et al. 1977), the general population can be exposed to acrylamide via inhalation (Moreno Navarro et al. 2007). This may include second-hand smoke, and is thought to be a significant source of exposure for the general population (WHO 2002). Due to its low vapor pressure and high water solubility, acrylamide is not anticipated to be a common air contaminant (WHO 2003), and thus, inhalation exposure is likely limited to tobacco smoke exposure.

Nonfood exposure to acrylamide is thought to be low for nonsmokers. Exposure can result from ingestion of polyacrylamide-treated drinking water containing residual monomer (EPA 2006c; EU 2002; WHO 2003), and from water in contact with acrylamide-containing products (e.g., grouting agents in dams) (Sorgel et al. 2002, van Dijk-Looijaard and van Genderen 2000). The MCL for acrylamide in drinking water, which specifies the concentration at which it is not expected to cause health problems, is

zero (EPA 2006c). Dermal exposure can result from contact with cosmetics and toiletries containing polyacrylamides (EU 2002).

Acrylamide and its metabolite, glycidamide, can react with biomolecules such as hemoglobin. Concentrations of adducts in the blood relevant to dietary exposure can be determined, and thus, the adducts can be used as biomarkers to determine a time-averaged estimate of acrylamide exposure. Urinary biomarker use may also be possible (WHO 2002). The Fourth National Report on Human Exposures to Environmental Chemicals (CDC 2009) reported the levels of acrylamide and glycidamide hemoglobin adducts from the National Health and Nutrition Examination Survey (NHANES) 2003–2004. The acrylamide and glycidamide hemoglobin adduct levels for a variety of age groups and ethnicities are presented in Tables 6-6 and 6-7, respectively. The geometric mean acrylamide and glycidamide hemoglobin adduct concentrations were 61.2 and 59.3 pmol/g hemoglobin, respectively.

Occupational exposure occurs mainly through dermal contact of acrylamide from solution, followed by inhalation of dry monomer or aerosols of solution during manufacture of acrylamide and polyacrylamide as well as during acrylamide grouting or preparation of polyacrylamide gels in the laboratory. Ingestion is a minimal concern in the workplace (EU 2002; Lewis 2000). A unique danger of acrylamide is its ability to be absorbed through unbroken skin (Lewis 2000). In the workplace, handling of dry acrylamide, such as emptying bags and drums, results in an inhalation and dermal hazard from dust (Haberman 2002).

Smoking and alcohol consumption, as well as race/ethnicity, may impact acrylamide toxicity. Therefore, results from studies of acrylamide toxicity performed in different geographical areas may indicate variation in levels of concern for acrylamide toxicity.

## 6.6 EXPOSURES OF CHILDREN

This section focuses on exposures from conception to maturity at 18 years in humans. Differences from adults in susceptibility to hazardous substances are discussed in Section 3.7, Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk

## Table 6-6. Geometric Mean and Selected Percentile of Acrylamide Hemoglobin Adduct Concentrations (pmol/g hemoglobin) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES)[a]

| | Survey years | Geometric mean (95% CI) | | Selected percentiles (95% CI) | | | | | | | | Sample size |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 50th | | 75th | | 90th | | 95th | | |
| **Total** | 03–04 | **61.2** | (58.1–64.4) | **54.8** | (52.8–57.7) | **79.1** | (73.5–85.6) | **141** | (124–155) | **192** | (168–217) | 7,101 |
| **Age group** | | | | | | | | | | | | |
| 3–5 years | 03–04 | **59.4** | (53.6–65.7) | **58.6** | (51.7–64.9) | **75.7** | (63.4–83.6) | **90.6** | (81.9–105) | **108** | (86.2–118) | 350 |
| 6–11 years | 03–04 | **58.6** | (56.1–61.2) | **57.3** | (55.2–59.7) | **71.0** | (67.4–76.3) | **86.8** | (81.2–91.4) | **98.8** | (91.0–104) | 769 |
| 12–19 years | 03–04 | **57.4** | (54.4–60.5) | **54.5** | (52.1–57.4) | **70.7** | (65.6–75.7) | **100** | (89.2–114) | **132** | (115–151) | 1,889 |
| 20–59 years | 03–04 | **66.2** | (62.2–70.6) | **57.9** | (54.6–61.1) | **96.1** | (83.6–108) | **163** | (147–191) | **223** | (194–243) | 2,570 |
| ≥60 years | 03–04 | **50.1** | (47.9–52.3) | **46.5** | (44.0–49.2) | **61.0** | (57.6–66.0) | **96.1** | (88.0–108) | **141** | (120–152) | 1,523 |
| **Gender** | | | | | | | | | | | | |
| Males | 03–04 | **63.9** | (60.2–67.9) | **57.0** | (53.7–60.1) | **85.5** | (79.2–93.7) | **152** | (139–175) | **220** | (189–237) | 3,509 |
| Females | 03–04 | **58.7** | (55.9–61.5) | **53.4** | (51.8–55.9) | **73.9** | (69.5–80.6) | **126** | (111–142) | **164** | (147–191) | 3,592 |
| **Race/ethnicity** | | | | | | | | | | | | |
| Mexican Americans | 03–04 | **61.7** | (58.7–64.9) | **57.4** | (54.4–60.4) | **73.0** | (69.2–77.3) | **101** | (95.0–115) | **149** | (125–179) | 1,792 |
| Non-Hispanic blacks | 03–04 | **63.8** | (57.3–71.1) | **57.1** | (52.1–64.1) | **86.5** | (74.6–104) | **156** | (120–203) | **218** | (172–271) | 1,874 |
| Non-Hispanic whites | 03–04 | **62.4** | (59.0–66.0) | **55.3** | (53.0–58.6) | **82.2** | (75.4–89.1) | **146** | (129–163) | **197** | (172–223) | 2,994 |

[a]Limit of detection is 3.0 pmol/g hemoglobin.

CI = confidence interval

Source: CDC 2009

## Table 6-7. Geometric Mean and Selected Percentile of Glycidamide Hemoglobin Adduct Concentrations (pmol/g hemoglobin) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES)[a]

| | Survey years | Geometric mean (95% CI) | Selected percentiles (95% CI) | | | | Sample size |
|---|---|---|---|---|---|---|---|
| | | | 50th | 75th | 90th | 95th | |
| **Total** | 03–04 | **59.3** (56.7–62.1) | **59.9** (57.6–62.5) | **85.9** (81.6–90.5) | **130** (120–141) | **167** (153–181) | 7,278 |
| **Age group** | | | | | | | |
| 3–5 years | 03–04 | **71.6** (66.9–76.7) | **71.1** (66.9–78.9) | **94.7** (87.3–101) | **118** (103–126) | **126** (119–135) | 411 |
| 6–11 years | 03–04 | **74.1** (70.3–78.2) | **75.0** (70.9–77.9) | **95.6** (90.4–103) | **121** (112–134) | **141** (126–157) | 784 |
| 12–19 years | 03–04 | **55.4** (51.1–60.1) | **59.2** (56.1–62.1) | **79.2** (72.7–86.7) | **113** (94.9–138) | **146** (123–169) | 1,931 |
| 20–59 years | 03–04 | **62.5** (59.4–65.8) | **60.9** (58.7–64.4) | **90.7** (84.4–98.2) | **143** (130–159) | **187** (169–204) | 2,623 |
| ≥60 years | 03–04 | **45.5** (42.8–48.3) | **46.8** (44.8–49.3) | **65.2** (63.5–66.9) | **96.4** (90.0–103) | **129** (111–141) | 1,529 |
| **Gender** | | | | | | | |
| Males | 03–04 | **59.5** (56.9–62.3) | **59.4** (56.8–61.8) | **87.1** (82.5–92.3) | **136** (123–148) | **174** (157–197) | 3,604 |
| Females | 03–04 | **59.1** (56.0–62.5) | **60.4** (57.5–64.0) | **85.0** (80.2–90.0) | **125** (116–135) | **159** (143–175) | 3,674 |
| **Race/ethnicity** | | | | | | | |
| Mexican Americans | 03–04 | **64.7** (61.2–68.4) | **65.4** (61.1–70.1) | **87.4** (81.5–94.4) | **118** (110–129) | **152** (135–170) | 1,841 |
| Non-Hispanic blacks | 03–04 | **53.8** (51.1–56.7) | **56.0** (52.4–59.7) | **83.0** (75.2–91.5) | **121** (108–140) | **159** (129–204) | 1,954 |
| Non-Hispanic whites | 03–04 | **61.1** (57.6–64.9) | **60.7** (57.9–64.2) | **87.5** (83.0–93.5) | **136** (124–149) | **172** (157–194) | 3,044 |

[a]Limit of detection is 4.0 pmol/g hemoglobin.

CI = confidence interval

Source: CDC 2009

or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

Acrylamide exposure to children can occur via ingestion and inhalation. Food was found to contribute significantly to the overall exposure of the general population to acrylamide. The short-term exposure estimates ranged from 0.8 to 6.0 µg/kg bw/day, while the estimated long-term intake of acrylamide from food sources ranged from 0.3 to 0.8 µg/kg bw/day for adults. Exposure in children is expected to be 2–3 times that of adults on a body weight basis (WHO 2002, 2003). In a study of 110 children between the ages of 5 and 6, the median urinary levels were found to be 36.0 µg AAMA/L and 13.4 µg GAMA/L (where AAMA are mercapturic acids of acrylamide and GAMA are mercapturic acids of glycidamide). The median acrylamide exposure was determined to be 0.54 µg/kg bw/day, mainly resulting from dietary sources (Heudorf et al. 2009). Children can also be exposed to acrylamide via inhalation of second-hand smoke (Moreno Navarro et al. 2007; WHO 2002).

In the body, acrylamide is widely dispersed by body fluids, and can also cross the placental barrier, exposing the fetus to high levels of acrylamide (Sorgel et al. 2002, WHO 2003). Fetuses and newborns do not have a fully developed blood-brain barrier, and thus, women who consume large amounts of acrylamide-containing foods risk significant acrylamide transfer to the fetus. Postnatally, acrylamide can be transferred via breast milk. According to Sorgel et al. (2002), when mothers consume foods with high acrylamide concentrations, daily consumption of 500 mL of breast milk can result in up to 10 µg of acrylamide transferred to the baby. Women who eat lesser amounts of acrylamide-containing foods, such as potato chips, may still transfer 2 µg of acrylamide to the baby. For these two scenarios, the doses for a 3 kg infant would be 3.3 and 0.66 µg/kg, respectively (Sorgel et al. 2002).

## 6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

In addition to the individuals who are occupationally exposed to acrylamide (see Section 6.5), there are several groups within the general population that may receive potentially high exposures (higher than background levels) to acrylamide. These populations include individuals living in proximity to sites where acrylamide is produced or used in manufacturing or sites where acrylamide is disposed, and includes individuals living near the three NPL hazardous waste sites where acrylamide has been detected in some environmental media (HazDat 2007). Smokers and those breathing second-hand smoke are

subject to high acrylamide exposures (Moreno Navarro et al. 2007). Children of mothers whose diets are high in acrylamide-containing foods can be exposed to high amounts of acrylamide through breast milk (Sorgel et al. 2002).

## 6.8 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 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.

### 6.8.1 Identification of Data Needs

**Physical and Chemical Properties**. The physical and chemical properties of acrylamide are well understood and have been discussed in Chapter 4 (Table 4-2). There are no data needs.

**Production, Import/Export, Use, Release, and Disposal.** According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit substance release and off-site transfer information to the EPA. The TRI, which contains this information for 2009, became available in February of 2011. This database is updated yearly and should provide a list of industrial production facilities and emissions.

Methods of manufacturing, production volumes, and uses of acrylamide are available and have been discussed in Chapter 5. Demand and import/export volumes are also available. No data needs are identified.

**Environmental Fate**. The environmental fate of acrylamide is reasonably well understood. If released to the environment, acrylamide has a low potential to volatilize to air from water or soil surfaces; however, its low soil adsorption coefficient suggests that it has potential to leach into groundwater. Acrylamide appears to degrade fairly rapidly by biological processes in both soil (Abdelmagid 1982) and water (Cherry et al. 1956; Croll et al. 1974). If released to air, acrylamide is expected to degrade through reaction with photochemically generated hydroxyl radicals and ozone molecules. No data needs are identified.

**Bioavailability from Environmental Media.** No data exist regarding acrylamide's bioavailability from environmental media such as soil or drinking water. Acrylamide is produced when certain foods are cooked at high temperatures (Tareke et al. 2002). Foodstuffs, particularly those rich in carbohydrates, may develop high levels of acrylamide when cooked at high temperatures (Moreno Navarro et al. 2007).

**Food Chain Bioaccumulation.** Acrylamide is not expected to significantly bioconcentrate due to its high water solubility and its ability to be degraded by microorganisms (EPA 2006c; Haberman 2002; WHO 2003). Therefore, bioaccumulation through the food chain is expected to be low. No data needs are identified.

**Exposure Levels in Environmental Media.** Reliable monitoring data for the levels of acrylamide in contaminated media at hazardous waste sites are needed so that the information obtained on levels of acrylamide in the environment can be used in combination with the known body burden of acrylamide to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

**Exposure Levels in Humans.** Acrylamide was found to be present in various foods at high concentrations (Tareke et al. 2002). In order to better characterize the degree of exposure from food sources, further information is necessary to determine the background levels of acrylamide in both the environment and in humans. Additional information concerning methods to decrease acrylamide content in foods, and thus decrease exposure, is needed.

This information is necessary for assessing the need to conduct health studies on these populations.

**Exposures of Children.** Children are exposed to acrylamide by the same routes as adults. Food was found to contribute to high levels of exposure in children, possibly 2–3 times that of an adult (WHO

2002, 2003). Inhalation of second-hand smoke can also result in exposure (Moreno Navarro et al. 2007; WHO 2002). Acrylamide has been detected in breast milk, which can result in significant exposure to infants (Sorgel et al. 2002). Additional data would be useful to determine the exposures of children to acrylamide via these methods.

Child health data needs relating to susceptibility are discussed in Section 3.12.2, Identification of Data Needs: Children's Susceptibility.

**Exposure Registries.** No exposure registries for acrylamide were located. This substance is not currently one of the compounds for which a sub-registry has been established in the National Exposure Registry. The substance will be considered in the future when chemical selection is made for sub-registries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to exposure to this substance.

### 6.8.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2009) database provides additional information obtainable from a few ongoing studies that may fill data needs identified in Section 6.8.1. Titles for these studies are listed in Table 6-8.

## Table 6-8. Ongoing Research Regarding the Environmental Fate and Exposure to Acrylamide

| Investigator | Affiliation | Description | Sponsor |
|---|---|---|---|
| Lehotay SJ, Handel A | Drexel University | Development of new methods to detect and control acrylamide formation in deep-fat fried foods | USDA |
| Chen H | University of Delaware | Improvement of thermal and alternative processes for foods | USDA |
| Calder BL, Perkins LB, Bushway AA, et al. | University of Maine | Improving the post-harvest quality of fresh-cut and processed Maine potatoes | USDA |
| Daley LS | Oregon State University | Regulation of photosynthetic processes | USDA |

USDA = U.S. Department of Agriculture

Source: FEDRIP 2009

# 7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring acrylamide, its metabolites, and other biomarkers of exposure and effect to acrylamide. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

## 7.1 BIOLOGICAL MATERIALS

Methods for the detection of acrylamide in biological materials are summarized in Table 7-1. Acrylamide can be detected in biological samples using gas chromatography (GC) with electron-capture detection. Acrylamide is converted to its 2,3-dibromopropionamide derivation in aqueous solution, plasma, or tissue homogenates by ionic bromination. The limits of detection for this method correspond to $9.5 \times 10^{-12}$ g of acrylamide on the column or $8.4 \times 10^{-9}$ g in the final biological extract of 0.5 mL. Acrylamide recovery by this method exceeds 80% at nanogram levels (HSDB 2009).

A liquid chromatography/tandem mass spectrometry (LC-MS/MS) assay was developed to examine unchanged acrylamide concentrations in bodily fluids, specifically urine, breast milk, and placental perfusion medium. A bioanalytical method was developed to analyze acrylamide in bodily fluids using LC-MS/MS following liquid/liquid extraction of acrylamide and evaporation under a stream of $N_2$ at 35 °C. A reversed-phase column eluted with an isocratic solvent system consisting of water, acetic acid, and an organic modifier was used for chromatographic separation. Method detection limits were 1 ng/mL for urine, 2 ng/mL for placenta perfusate, and 5 ng/mL for breast milk (Sorgel et al. 2002).

GC-MS methods have been used to determine the adducts of acrylamide and its metabolites (such as glycidamide) with hemoglobin in blood samples. These methods are sensitive enough to measure adducts at blood levels relevant to potential dietary acrylamide exposure, which allows for use of the adducts as

## Table 7-1. Analytical Methods for Determining Acrylamide in Biological Samples

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Urine | Liquid/liquid extraction, evaporation under $N_2$ stream at 35 °C | LC-MS/MS | 1 ng/mL | | Sorgel et al. 2002 |
| Breast milk | Liquid/liquid extraction, evaporation under $N_2$ stream at 35 °C | LC-MS/MS | 5 ng/mL | | Sorgel et al. 2002 |
| Placental perfusion medium | Liquid/liquid extraction, evaporation under $N_2$ stream at 35 °C | LC-MS/MS | 2 ng/mL | | Sorgel et al. 2002 |
| Biological samples | Ionic bromination of acrylamide in sample | GC with electron-capture device | Corresponds to $9.5x10^{-12}$ g acrylamide (column), $8.4x10^{-9}$ g (extract) | | HSDB 2009 |

GC = gas chromatography; LC = liquid chromatography; MS = mass spectrometry

biomarkers of exposure (WHO 2002, 2003). Recent methods of detection for hemoglobin adducts of acrylamide and/or glycidamide utilize LC-MS/MS, High-Performance Liquid Chromatography (HPLC)/MS/MS, or GC-Negative Chemical Ionization (NCI)/MS/MS) (Fennell et al. 2003, Schettgen et al. 2010; Urban et al. 2006; Vesper et al. 2006, 2007, 2010; Von Stedingk et al. 2010; Wirfält et al. 2008).

Acrylamide and glycidamide were measured in serum and tissues of mice following dosing by intravenous, gavage, and dietary routes at 0.1 mg/kg acrylamide and by intravenous and gavage routes at equimolar amounts of glycidamide. LC-electrospray (ES)/MS/MS was utilized to measure acrylamide and glycidamide in serum and tissues with recoveries of 70% for acrylamide and 63% for glycidamide. The limit of detection was below 0.1 pmol/mg tissue for both acrylamide and glycidamide (Doerge et al. 2005b). Doerge et al. (2005a) detected DNA adducts derived from administration of acrylamide and glycidamide to mice and rats. A single intraperitoneal injection containing an aqueous solution of 100 µL of 50 mg/kg acrylamide or 61 mg/kg glycamide was given to the mice. Rats were treated with a single intraperitoneal injection of the same concentration using a volume of 100 µL per 100 g body weight. Six hours after dosing, the tissues were removed following gassing by carbon dioxide. Tissues were frozen and stored at -80 °C. DNA adducts were analyzed using a Quattro Ultima triple stage guadrupole mass spectrometer with an electrospray source, with an ion source temperature of 120 °C, desolvation gas temperature of 400 °C, and constant cone voltage of 35 V. DNA was isolated using a Blood and Cell Culture Maxi kit and adducts were quantified using LC-/MS/MS. The limit of quantification ranged from 1 to 1.5 adducts in $10^8$ nucleotides and the limit of detection was 0.5 adducts in $10^8$ nucleotides (Doerge et al. 2005a).

## 7.2 ENVIRONMENTAL SAMPLES

Methods for the detection of acrylamide in environmental samples are summarized in Table 7-2. Acrylamide detection in water samples can be achieved using direct injection and a reversed-phase high performance liquid chromatography (HPLC)-ultraviolet (UV) absorption procedure, which has a limit of detection of 5 µg/L (Cavalli et al. 2004). An HPLC method can be used to determine the amount of acrylamide monomer in natural and polluted aqueous environments. Acrylamide undergoes bromination and the resulting dibromopropionamide is assayed. The detection limit for the method was found to be 0.2 µg/L in river, sea, and estuarine waters as well as potable waters, sewage, and china clay works effluents (HSDB 2009).

## Table 7-2. Analytical Methods for Determining Acrylamide in Environmental Samples

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Water | Direct injection | Reversed-phase HPLC-UV absorption | 5 µg/L | | Cavalli et al. 2004 |
| Natural and polluted water | Bromination of acrylamide in sample | HPLC | 0.20 µg/L | | HSDB 2009 |
| Organic-free reagent water | | HPLC | 10 µg/L | | HSDB 2009 |
| Aqueous matrices | | GC | 0.032 µg/L | | HSDB 2009 |
| Drinking water | Direct injection of 500 µL | Ion-exclusion chromatographic separation/MS | 0.20 ppb | | Cavalli et al. 2004 |
| Airborne dust, vapors | | Differential pulse polarographic method | | | HSDB 2009 |
| Food | Water extract food, brominates sample | GC-MS | 5–10 µg/kg | | WHO 2002 |
| Food | Bromination of sample | GC-MS | 5 µg/kg | | Tareke et al. 2002 |
| Food | Water extract food | LC-MS/MS | 20–50 µg/kg | | WHO 2002 |
| Food | | LC-MS/MS | 10 µg/kg | | Tareke et al. 2002 |
| Food | | LC-MS/MS | 10 µg/kg | 100–115% | Arisseto et al. 2007 |

GC = gas chromatography; HPLC = high performance liquid chromatography; LC = liquid chromatography; MS = mass spectrometry; SIM = selected ion monitoring; UV = ultraviolet

Solid phase extraction, extraction with activated carbon filter, and GC-MS analyses can also be used (Cavalli et al. 2004). A water sample is brominated to form 2,3-dibromopropionamide, which is then analyzed by GC-MS using methacrylamide as an internal standard (Ahn et al. 2002). Additional methods include polarography and electron capture GC (WHO 2003). EPA-OSW 8316 method determines acrylamide concentrations by HPLC in organic-free reagent water, with a limit of detection of 10 µg/L. The EPA-OSW 8032A method analyzes acrylamide in aqueous matrices using GC, with a detection limit of 0.032 µg/L (HSDB 2009).

Cavalli et al. (2004) utilized a method for detecting acrylamide in drinking water using a combination of ion-exclusion chromatographic separation and MS detection. Drinking water samples are injected directly into the microbore ICE-AS1 column and are then detected in the selected-ion monitoring mode by a single quadrupole system with electrospray ionization. The detection limit for the method was determined to be 0.20 ppb with an injection volume of 500 µL (Cavalli et al. 2004).

Airborne acrylamide vapors and dust can be determined in air by a differential pulse polarographic method (HSDB 2009). Two additional methods of detection in air were found (called Method 21 and OSHA PV2004), though only very limited details were provided (HSDB 2009).

Various methods are available to determine the acrylamide content of food. The most common methods utilize either GC-MS or LC-MS/MS (Ahn et al. 2002; Arisseto et al. 2007). Water is often used as the extraction solvent. Solid-phase extraction (SPE) is typically use to prepare samples. SPE phases that have been used for this method include graphitized carbon black, mixed mode anion and cation exchange, and polymeric materials (Arisseto et al. 2007)

The GC-MS method of determining acrylamide content in food is well established. A water extract of the food is brominated to form 2,3-dibromopropionamide, a derivative of acrylamide with enhanced GC properties. The derivative is then analyzed by GC-MS using methacrylamide as an internal standard (Ahn et al. 2002; WHO 2002). Detection limits for the GC-MS method are typically in the 5–10 µg/kg range (WHO 2002). Tareke et al. (2002) analyzed food samples for acrylamide using an improved GC-MS method involving bromination. A detection limit of 5 µg/kg was achieved.

The LC-MS/MS method was developed over concerns that artifacts were forming during the bromination procedure used with the GC-MS method. LC-MS/MS allows for direct acrylamide analysis without the need for a derivative (WHO 2002). In this method, a water extract of food is tested by LC-MS/MS using

deuterated acrylamide as an internal standard (Ahn et al. 2002). Acrylamide is then identified by its retention time and the relative ion intensities. WHO (2002) reports that the detection limits of this method are typically 20–50 µg/kg, although lower detection limits have since been obtained for this method.

Tareke et al. (2002) also utilized the LC-MS/MS method to determine underivitized acrylamide concentrations. This method had a detection limit of 10 µg/kg. Sorgel et al. (2002) used a modified LC-MS/MS method, similar to that developed for analysis of acrylamide in bodily fluids, to determine acrylamide content in food. Brazilian foods were analyzed by LC-MS/MS to determine acrylamide content (Arisseto et al. 2007). Detection limits of 10 µg/kg were achieved, along with a limit of quantification of 20 µg/kg and mean recoveries ranging from 100 to 115%.

## 7.3 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 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.

### 7.3.1 Identification of Data Needs

#### Methods for Determining Biomarkers of Exposure and Effect.

***Exposure.*** Exposure to acrylamide can be determined directly by LC-MS/MS for biological samples, including urine, breast milk, and placental perfusion medium with detection limits of 1, 5, and 2 ng/mL, respectively (Sorgel et al. 2002). GC-MS methods are also used to detect the adducts of acrylamide and its metabolites in blood and tissue samples (Fennell et al. 2003, HSDB 2009; Schettgen et al. 2010; Urban

et al. 2006; Vesper et al. 2006, 2007, 2010; Von Stedingk et al. 2010; WHO 2002, 2003; Wirfält et al. 2008). Selected methods are described in Table 7-1. Additional methods for the detection of acrylamide and its metabolites in biological samples would be helpful.

***Effect.*** Information on biomarkers of effect for acrylamide would be useful.

**Methods for Determining Parent Compounds and Degradation Products in Environmental Media.** There are several variations on methods for identifying acrylamide in water, air, and food. The methods are summarized in Table 7-2. The methods for water, primarily involving the use of HPLC and GC are fairly sensitive (e.g., ~0.03-10 µg/L for water (Cavalli et al. 2004; HSDB 2009). In air, a differential pulse polarographic method was identified, although limited details were provided (HSDB 2009). Additional information concerning the detection of acrylamide in air, including detection limits, would be useful. Various methods are available to determine acrylamide content of food using primarily GC-MS or LC-MS/MS (Ahn et al. 2002; Arisseto et al. 2007). GC-MS involved bromination, while LC-MS/MS can determine acrylamide content directly. Detection limits for GC-MS methods typically range from 5 to 10 µg/kg, while limits of detection for the LC-MS/MS method are typically in the range of 10–50 µg/kg (Tareke et al. 2002; WHO 2002). More sensitive methods for direct determination of acrylamide in food via the LC-MS/MS method would use useful.

### 7.3.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2009) database contains a study sponsored by the U.S. Department of Agriculture in which new methods are being developed for detecting and controlling acrylamide formation in deep-fat fried foods.

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# 8. REGULATIONS, ADVISORIES, AND GUIDELINES

MRLs are substance specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites.

ATSDR has derived an acute-duration oral MRL of 0.01 mg/kg/day for acrylamide based on results of fertility testing of male Long-Evans hooded rats administered acrylamide by gavage for 5 days prior to 1-week mating sessions with untreated female rats (Sublet et al. 1989). As discussed in detail in Appendix A, the PBPK model of Sweeney et al. (2010) was used to estimate rat internal dose metrics for blood acrylamide and glycidamide for each of the administered acrylamide dose levels in the principal study. BMD analysis was used to predict the fraction of nonpregnant female rats for each of the PBPK-predicted male rat internal dose metrics for blood acrylamide and glycidamide. The PBPK model was then used to predict the HED associated with the best-fitting BMD result. The resulting HED of 0.31 mg/kg/day was divided by an uncertainty factor of 30 (3 for interspecies extrapolation using a PBPK model and 10 for human variability). See Appendix A for a more detailed description of the acute-duration oral MRL derivation for acrylamide.

ATSDR has derived an intermediate-duration oral MRL of 0.001 mg/kg/day for acrylamide based on a NOAEL of 0.2 mg/kg/day and a LOAEL of 1 mg/kg/day for ultrastructural changes in peripheral nerve fibers in male F344 rats receiving acrylamide from the drinking water for up to 93 days (Burek et al. 1980). As discussed in detail in Appendix A, the PBPK model of Sweeney et al. (2010) was used to estimate rat internal dose metrics for blood acrylamide and glycidamide at the NOAEL of 0.2 mg/kg/day and for estimating the corresponding HED. The resulting HED of 0.038 mg/kg/day was divided by an uncertainty factor of 30 (3 for interspecies extrapolation using a PBPK model and 10 for human variability). See Appendix A for a more detailed description of the intermediate-duration oral MRL derivation for acrylamide.

ATSDR has derived a chronic-duration oral MRL of 0.001 mg/kg/day for acrylamide based on degenerative changes in sciatic nerves from male F344 rats receiving acrylamide from the drinking water for up to 2 years, as detected by light microscopy (Friedman et al. 1995). As discussed in detail in Appendix A, the PBPK model of Sweeney et al. (2010) was used to estimate rat internal dose metrics for blood acrylamide and glycidamide for each of the administered acrylamide dose levels in the principal study (Friedman et al. 1995). BMD analysis was used to predict degenerative peripheral nerve incidence

for each of the PBPK-predicted male rat internal dose metrics for blood acrylamide and glycidamide. The PBPK model was then used to predict the HED associated with the best-fitting BMD result. The resulting HED of 0.042 mg/kg/day was divided by an uncertainty factor of 30 (3 for interspecies extrapolation using PBPK modeling and 10 for human variability). See Appendix A for a more detailed description of the chronic-duration oral MRL derivation for acrylamide.

EPA has established an oral reference dose (RfD) of 0.002 mg/kg/day for acrylamide (EPA 2010; IRIS 2012) based on a $BMDL_{05}$ of 0.27 mg/kg/day for degenerative nerve changes in male F344 rats exposed to acrylamide in the drinking water for up to 2 years (Johnson et al. 1986). The rat $BMDL_{05}$ was converted to a human equivalent dose ($HED_{BMDL05}$) of 0.053 mg/kg/day using acrylamide AUC and glycidamide AUC to estimate the internal dose in rats, extrapolate that dose to the internal dose in humans, and then to estimate the daily human intake of acrylamide needed to produce the human internal dose comparable to that of the rat at the rat $_{BMDL05}$. The $HED_{BMDL05}$ of 0.053 mg/kg/day was divided by an uncertainty factor of 30 (3 to account for interspecies toxicodynamic differences and 10 for human variability).

EPA has established an inhalation reference concentration (RfC) of 0.006 $mg/m^3$ for acrylamide (EPA 2010; IRIS 2012) based on route-to-route extrapolation using the $BMDL_{05}$ of 0.27 mg/kg/day for degenerative nerve changes in male F344 rats exposed to acrylamide in the drinking water for up to 2 years (Johnson et al. 1986) as the point of departure. The rat $BMDL_{05}$ was converted to a human equivalent dose ($HED_{BMDL05}$) of 0.053 mg/kg/day using acrylamide AUC and glycidamide AUC to estimate the internal dose in rats, extrapolate that dose to the internal dose in humans, and then to estimate the daily human intake of acrylamide needed to produce the human internal dose comparable to that of the rat at the rat $BMDL_{05}$. The $HED_{BMDL05}$ of 0.053 mg/kg/day was converted to a human equivalent concentration ($HEC_{BMDL05}$) of 0.18 $mg/m^3$ based on a 70-kg person who breathes 20 $m^3$ of air daily. The $HEC_{BMDL05}$ of 0.18 $mg/m^3$ was divided by an uncertainty factor of 30 (3 to account for interspecies toxicodynamic differences and 10 for human variability).

The International Agency for Research on Cancer (IARC) has classified acrylamide as a Group 2A carcinogen (probably carcinogenic to humans) (IARC 2011). The National Toxicology Program (NTP) has determined that acrylamide is reasonably anticipated to be a human carcinogen (NTP 2011a). EPA has characterized acrylamide as "likely to be carcinogenic to humans" (EPA 2010; IRIS 2012). The American Conference of Governmental Industrial Hygienists (ACGIH) has classified acrylamide as an A3 carcinogen (confirmed animal carcinogen with unknown relevance to humans) (ACGIH 2011).

OSHA has required employers of workers who are occupationally exposed to acrylamide to institute engineering controls and work practices to reduce and maintain employee exposure at or below permissible exposure limits (PELs). The employer must use engineering and work practice controls to reduce exposures to not exceed 0.3 $mg/m^3$ for acrylamide at any time (OSHA 2010).

EPA has designated acrylamide as a hazardous air pollutant (HAP) under the Clean Air Act (CAA) (EPA 2011a). Acrylamide is on the list of chemicals appearing in "Toxic Chemicals Subject to Section 313 of the Emergency Planning and Community Right-to-Know Act of 1986" and has been assigned a reportable quantity (RQ) limit of 5,000 pounds (EPA 2011b). Acrylamide is also considered to be an extremely hazardous substance (EPA 2011c). The RQ represents the amount of a designated hazardous substance which, when released to the environment, must be reported to the appropriate authority.

The international and national regulations, advisories, and guidelines regarding acrylamide in air, water, and other media are summarized in Table 8-1.

## Table 8-1. Regulations and Guidelines Applicable to Acrylamide

| Agency | Description | Information | Reference |
|---|---|---|---|
| INTERNATIONAL | | | |
| Guidelines: | | | |
| IARC | Carcinogenicity classification | Group 2A[a] | IARC 2011 |
| WHO | Air quality guidelines | No | WHO 2000 |
| | Drinking water quality guidelines | 0.0005 mg/L[b] | WHO 2008 |
| NATIONAL | | | |
| Regulations and Guidelines: | | | |
| a. Air | | | |
| ACGIH | TLV (8-hour TWA)[c,d] | 0.03 mg/m$^3$ | ACGIH 2011 |
| | TLV-basis (critical effect) | Central nervous system impairment | |
| AIHA | ERPG values | No | AIHA 2009 |
| EPA | Hazardous air pollutant | Yes | EPA 2009a<br>42 USC 7412 |
| | Second AEGL Chemical Priority List | Yes[e] | EPA 2008 |
| NIOSH | REL (10-hour TWA)[f] | 0.03 mg/m$^3$ | NIOSH 2011 |
| | IDLH | 60 mg/m$^3$ | |
| | Potential occupational carcinogen | Yes | |
| | Target organs | Eyes, skin, central nervous system, peripheral nervous system, and reproductive system | |
| OSHA | PEL (8-hour TWA) for general industry[f] | 0.3 mg/m$^3$ | OSHA 2010<br>29 CFR 1910.1000, Table Z-1 |
| b. Water | | | |
| EPA | Drinking water standards and health advisories | | EPA 2011a |
| | 1-day health advisory for a 10-kg child | 1.5 mg/L | |
| | 10-day health advisory for a 10-kg child | 0.3 mg/L | |
| | RfD | 0.002 mg/kg/day | |
| | DWEL | 0.07 mg/L | |
| | Lifetime | No data | |
| | $10^{-4}$ Cancer risk | No data[g] | |

## Table 8-1. Regulations and Guidelines Applicable to Acrylamide

| Agency | Description | Information | Reference |
|---|---|---|---|
| NATIONAL *(cont.)* | | | |
| EPA | National primary drinking water standards | | EPA 2003 |
| | Treatment technique | Yes[h] | |
| | Potential health effects from exposure above the MCL | Nervous system or blood problems | |
| | Common sources of acrylamide in drinking water | Added to water during sewage/waste water treatment; increased risk of cancer treatment | |
| | Public health goal | Zero | |
| | National recommended water quality criteria | No | EPA 2006 |
| c. Food | | | |
| FDA | EAFUS[i] | No | FDA 2008 |
| d. Other | | | |
| ACGIH | Carcinogenicity classification | A3[j] | ACGIH 2011 |
| EPA | Carcinogenicity classification | Likely to be carcinogenic to humans[k] | EPA 2010; IRIS 2012 |
| | RfC | 0.006 $mg/m^3$ | |
| | RfD | 0.002 mg/kg/day | |
| | Inhalation unit risk | $1x10^{-4}$ per $\mu g/m^3$ | |
| | Oral slope factor | 0.5 per mg/kg/day | |
| | Superfund, emergency planning, and community right-to-know | | |
| | Designated CERCLA hazardous substance | Yes[l] | EPA 2011b 40 CFR 302.4 |
| | Reportable quantity | 5,000 pounds | |
| | Effective date of toxic chemical release reporting | 01/01/1987 | EPA 2011d 40 CFR 372.65 |
| | Extremely hazardous substances and its threshold planning quantity | 1,000/10,000 pounds | EPA 2011c 40 CFR 355, Appendix A |
| | TSCA health and safety data reporting | | EPA 2011e 40 CFR 716.120 |
| | Effective date | 10/04/1982 | |
| | Sunset date | 10/04/1992 | |

## Table 8-1. Regulations and Guidelines Applicable to Acrylamide

| Agency | Description | Information | Reference |
|---|---|---|---|
| NATIONAL *(cont.)* | | | |
| NTP | Carcinogenicity classification | Reasonably anticipated to be a human carcinogen | NTP 2011a |

[a]Group 2A: probably carcinogenic to humans
[b]For substances that are considered to be carcinogenic, the guideline value is the concentration in drinking-water associated with an upper-bound excess lifetime cancer risk of $10^{-5}$ (one additional cancer per 100,000 of the population ingesting drinking-water containing the substance at the guideline value for 70 years) (WHO 2006).
[c]TWA: inhalable fraction and vapor. Material exerts sufficient vapor pressure such that it may be present in both particle and vapor phases.
[d]Skin: refers to the potential significant contribution to the overall exposure by the cutaneous route.
[e]Acryalmide is included on the list of 371 priority chemicals that are acutely toxic and represent the selection of chemicals for AEGL development by the NAC/AEGL committee during the next several years.
[f]Carcinogen; skin designation
[g]Likely to be carcinogenic to humans
[h]Each water system must certify, in writing, to the state (using third-party or manufacturers certification) that when it uses acrylamide to treat water, the combination (or product) of dose and monomer level does not exceed 0.05% of acrylamide dosed at 1 mg/L (or equivalent).
[i]The EAFUS list of substances contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS.
[j]A3: confirmed animal carcinogen with unknown relevance to humans.
[k]Based on sufficient evidence of carcinogenicity in animals.
[l]Designated CERCLA hazardous substance pursuant to Section 112 of the Clean Air Act and Section 3001 of the Resource Conservation and Recovery Act.

ACGIH = American Conference of Governmental Industrial Hygienists; AEGL = acute exposure guideline levels; AIHA = American Industrial Hygiene Association; CERCLA = Comprehensive Environmental Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; DWEL = drinking water equivalent level; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; ERPG = emergency response planning guidelines; FDA = Food and Drug Administration; GRAS = Generally Recognized As Safe; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; MCL = maximum contaminant level; NAC = National Advisory Committee; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; TLV = threshold limit values; TSCA = Toxic Substances Control Act; TWA = time-weighted average; USC = United States Code; WHO = World Health Organization

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9. REFERENCES

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# 10. GLOSSARY

**Absorption—**The taking up of liquids by solids, or of gases by solids or liquids.

**Acute Exposure—**Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

**Adsorption—**The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

**Adsorption Coefficient ($K_{oc}$)—**The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

**Adsorption Ratio (Kd)—**The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

**Benchmark Dose (BMD)—**Usually defined as the lower confidence limit on the dose that produces a specified magnitude of changes in a specified adverse response. For example, a $BMD_{10}$ would be the dose at the 95% lower confidence limit on a 10% response, and the benchmark response (BMR) would be 10%. The BMD is determined by modeling the dose response curve in the region of the dose response relationship where biologically observable data are feasible.

**Benchmark Dose Model—**A statistical dose-response model applied to either experimental toxicological or epidemiological data to calculate a BMD.

**Bioconcentration Factor (BCF)—**The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

**Biomarkers—**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.

**Cancer Effect Level (CEL)—**The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

**Carcinogen—**A chemical capable of inducing cancer.

**Case-Control Study—**A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-controlled study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without outcome.

**Case Report—**Describes a single individual with a particular disease or exposure. These may suggest some potential topics for scientific research, but are not actual research studies.

**Case Series—**Describes the experience of a small number of individuals with the same disease or exposure. These may suggest potential topics for scientific research, but are not actual research studies.

**Ceiling Value—**A concentration of a substance that should not be exceeded, even instantaneously.

**Chronic Exposure—**Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

**Cohort Study—**A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome. At least one exposed group is compared to one unexposed group.

**Cross-sectional Study—**A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at one point in time.

**Data Needs—**Substance-specific informational needs that if met would reduce the uncertainties of human health assessment.

**Developmental Toxicity—**The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

**Dose-Response Relationship—**The quantitative relationship between the amount of exposure to a toxicant and the incidence of the adverse effects.

**Embryotoxicity and Fetotoxicity—**Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurs. The terms, as used here, include malformations and variations, altered growth, and *in utero* death.

**Environmental Protection Agency (EPA) Health Advisory—**An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

**Epidemiology—**Refers to the investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

**Genotoxicity—**A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

**Half-life—**A measure of rate for the time required to eliminate one half of a quantity of a chemical from the body or environmental media.

**Immediately Dangerous to Life or Health (IDLH)—**The maximum environmental concentration of a contaminant from which one could escape within 30 minutes without any escape-impairing symptoms or irreversible health effects.

**Immunologic Toxicity—**The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

**Immunological Effects—**Functional changes in the immune response.

**Incidence—**The ratio of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

**Intermediate Exposure—**Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

**Internal Dose—**The amount of a substance available for interaction by organs, tissues, or cells without respect to specific absorption barriers or exchange boundaries.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

**Lethal Concentration$_{(LO)}$ ($LC_{LO}$)—**The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

**Lethal Concentration$_{(50)}$ ($LC_{50}$)—**A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

**Lethal Dose$_{(LO)}$ ($LD_{Lo}$)—**The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

**Lethal Dose$_{(50)}$ ($LD_{50}$)—**The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

**Lethal Time$_{(50)}$ ($LT_{50}$)—**A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

**Lowest-Observed-Adverse-Effect Level (LOAEL)—**The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

**Lymphoreticular Effects—**Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

**Malformations—**Permanent structural changes that may adversely affect survival, development, or function.

**Minimal Risk Level (MRL)—**An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

**Modifying Factor (MF)—**A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

**Morbidity—**State of being diseased; morbidity rate is the incidence or prevalence of disease in a specific population.

**Mortality—**Death; mortality rate is a measure of the number of deaths in a population during a specified interval of time.

**Mutagen—**A substance that causes mutations. A mutation is a change in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

**Necropsy—**The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

**Neurotoxicity—**The occurrence of adverse effects on the nervous system following exposure to a chemical.

**No-Observed-Adverse-Effect Level (NOAEL)—**The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

**Octanol-Water Partition Coefficient ($K_{ow}$)—**The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

**Odds Ratio (OR)—**A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An OR of greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

**Organophosphate or Organophosphorus Compound—**A phosphorus-containing organic compound and especially a pesticide that acts by inhibiting cholinesterase.

**Permissible Exposure Limit (PEL)—**An Occupational Safety and Health Administration (OSHA) allowable exposure level in workplace air averaged over an 8-hour shift of a 40-hour workweek.

**Pesticide—**General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests.

**Pharmacokinetics—**The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

**Pharmacokinetic Model—**A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

**Physiologically Based Pharmacodynamic (PBPD) Model—**A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic end

points. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

**Physiologically Based Pharmacokinetic (PBPK) Model—**Comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information: tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as air/blood partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

**Prevalence—**The number of cases of a disease or condition in a population at one point in time.

**Prospective Study—**A type of cohort study in which the pertinent observations are made on events occurring after the start of the study. A group is followed over time.

**$q_1$*—**The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The $q_1$* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually µg/L for water, mg/kg/day for food, and µg/$m^3$ for air).

**Recommended Exposure Limit (REL)—**A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

**Reference Concentration (RfC)—**An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation reference concentration is for continuous inhalation exposures and is appropriately expressed in units of mg/$m^3$ or ppm.

**Reference Dose (RfD)—**An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the no-observed-adverse-effect level (NOAEL, from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

**Reportable Quantity (RQ)—**The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

**Reproductive Toxicity—**The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

**Retrospective Study—**A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

**Risk—**The possibility or chance that some adverse effect will result from a given exposure to a chemical.

**Risk Factor—**An aspect of personal behavior or lifestyle, an environmental exposure, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

**Risk Ratio—**The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

**Short-Term Exposure Limit (STEL)—**The American Conference of Governmental Industrial Hygienists (ACGIH) maximum concentration to which workers can be exposed for up to 15 minutes continually. No more than four excursions are allowed per day, and there must be at least 60 minutes between exposure periods. The daily Threshold Limit Value-Time Weighted Average (TLV-TWA) may not be exceeded.

**Standardized Mortality Ratio (SMR)—**A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

**Target Organ Toxicity—**This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

**Teratogen—**A chemical that causes structural defects that affect the development of an organism.

**Threshold Limit Value (TLV)—**An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a Time Weighted Average (TWA), as a Short-Term Exposure Limit (STEL), or as a ceiling limit (CL).

**Time-Weighted Average (TWA)—**An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

**Toxic Dose$_{(50)}$ (TD$_{50}$)—**A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

**Toxicokinetic—**The absorption, distribution, and elimination of toxic compounds in the living organism.

**Uncertainty Factor (UF)—**A factor used in operationally deriving the Minimal Risk Level (MRL) or Reference Dose (RfD) or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis, 3 being the approximate logarithmic average of 10 and 1.

**Xenobiotic—**Any chemical that is foreign to the biological system.

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# APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that

are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Human Health Sciences, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-57, Atlanta, Georgia 30333.

Acute-, intermediate-, and chronic-duration oral MRLs were derived for acrylamide using the PBPK model described by Sweeney et al. (2010). The PBPK model was used to estimate rat internal dose metrics for blood acrylamide and glycidamide (a readily-formed metabolite of acrylamide in aqueous environment) for each of the administered acrylamide dose levels in the principal study and for estimating HEDs. Code for acrylamide and glycidamide rat and human PBPK models was provided by L.M. Sweeney along with data used to evaluate model performance and documentation of parameter values reported in Sweeney et al. (2010). Refer to Section 3.4.5 for a detailed description of the PBPK model of Sweeney et al. (2010).

The internal dose metric selected for interspecies dosimetry extrapolation was the time-weighted average (TWA) concentration of either acrylamide or glycidamide. A time-integrated dose metric (e.g., TWA) was selected based, in part, on mechanistic considerations. The extent to which acrylamide and glycidamide contribute to toxicity observed following ingestion exposures to acrylamide is not known with certainty. There is evidence to suggest that epoxide metabolites of acrylamide contribute to germ cell mutations in male mice (Ghanayem et al. 2005a). Given the uncertainty in the relative importance of

acrylamide and glycidamide in producing toxicity, the conservative assumption was made that toxic response would be related to a time-integrated function of blood acrylamide concentration (e.g., TWA). This assumption resulted in the lowest HED and MRL based on male rat reproductive effects.

There is evidence to suggest that 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 acrylamide (25 or 50 mg/kg/day) or glycidamide (50 or 100 mg/kg/day) administered via intraperitoneal injection for 8 days, only acrylamide elicited poor performance on the hindlimb splay test (Costa et al. 1995). Given the uncertainty in the relative importance of acrylamide and glycidamide in producing toxicity, the conservative assumption was made that toxic response would be related to a time-integrated function of blood acrylamide concentration (e.g., TWA). This assumption resulted in the lowest HEDs and MRLs based on neurotoxicity in rats.

TWA concentrations of acrylamide and glycidamide in mixed venous blood were calculated as follows:

$$TWA = \left( \int_{t=0}^{t=i} C_{Blood} \right) \div t_i$$

where $C_{Blood}$ is the mixed venous blood concentration of acrylamide or glycidamide (mM) and $t_i$ is the exposure time (hours). Note, TWA as calculated above, is a time-integrated blood concentration, is conceptually equivalent to the area under the blood-time curve (AUC), and can be converted to units of AUC by multiplying the TWA by the time integration interval of interest. For example, if the TWA is multiplied by 24 hours, the resulting value is the AUC for 24 hours (mM 24 hours).

The general procedure for dosimetry extrapolation was as follows:

1. The rat model was used to simulate external rat dosages and to predict the corresponding internal dose metric, TWA concentrations of acrylamide and glycidamide in mixed venous blood.

2. In PBPK model simulations of the rat, acute-duration gavage doses (mg/kg/day) were assumed to be delivered as a single bolus administration each day at the exposure duration and frequency used in the acute-duration study (e.g., 5 consecutive days for the Sublet et al. 1989 study). Intermediate- and chronic-duration doses were assumed to be delivered during a daily 12-hour period (e.g., the daily food and water consumption period) for the entire exposure duration and frequency of the study (e.g., daily for 90 days for the Burek et al. [1980] study). Rat body weights were the TWA for the study.

3. The rat PBPK model simulations resulted in selection of a point of departure (POD) for dosimetry extrapolation (e.g., BMDL or NOAEL for TWA concentrations of acrylamide and glycidamide in mixed venous blood).

4. The human PBPK model was used to simulate the daily dosage (mg/kg/day) of acrylamide that corresponded to the POD. The human model was iterated for a series of ingestion doses until the selected value for the POD was predicted; the corresponding ingestion intake was the HED. A body weight of 70 kg was assumed for humans. Daily doses (mg/kg/day) in humans were assumed to be delivered in 12 consecutive hourly doses, separated by 12-hour intervals for the duration of the rat study. The intermediate-duration MRL is intended to be protective of human exposures up to 365 days, and the chronic-duration MRL is intended to be protective for human exposures exceeding 365 days; therefore, the HEDs for both intermediate- and chronic-duration MRLs were simulated as 365-day exposures. The elimination kinetics of acrylamide and glycidamide are relatively fast, resulting in a steady state within 1–2 days of repeated exposure; therefore, as the duration of the simulated exposure extends beyond several days, the TWA of acrylamide or glycidamide in blood approaches a constant value (see Figures A-1 and A-2).

**Figure A-1. Simulation of Blood Acrylamide (AA) and Glycidamide (GA) Concentrations in a Rat Exposed to 1 mg Acrylamide/kg/day (Simulations Were for 12 Consecutive Hours of Exposure per Day)**

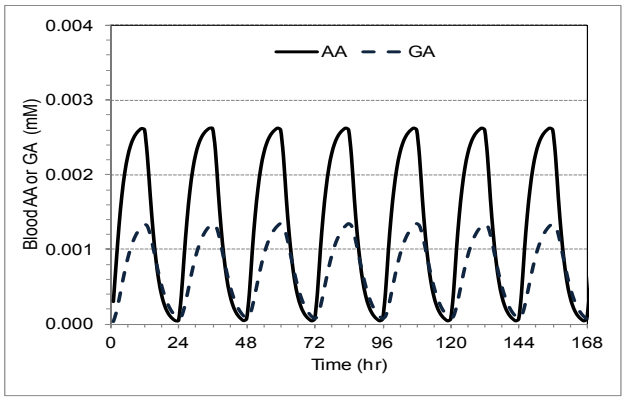
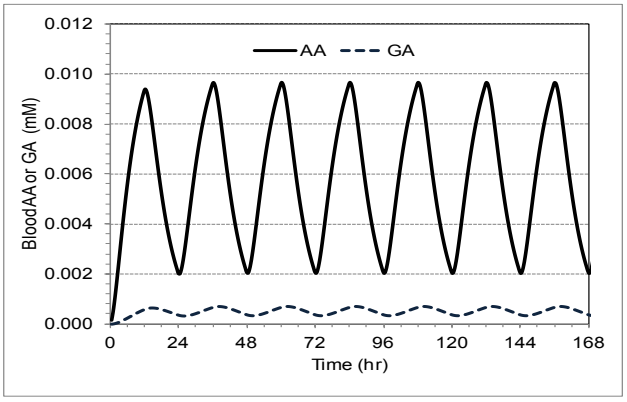


**Figure A-2. Simulation of Blood Acrylamide (AA) and Glycidamide (GA) Concentrations in an Adult Human Exposed to 1 mg Acrylamide/kg/day (Simulations were for 12 Consecutive Hours of Exposure per Day)**




The rationale for assuming a 12-hour dosing period in PBPK model simulations was the expectation that ingestion would occur only during a 12-hour (food and water) consumption period of the day. Other ingestion profiles could apply to specific individuals and populations. However, at the dose range of interest (<1 mg/kg/day), the model is essentially linear, which means that blood AUC has a very low dependence on dosing interval at a constant daily dose. For example, for an external dose of 1 mg/kg/day, decreasing the dosing interval from 12 hours (daily dose administered over a 12-hour period) to 24 hours results in a change in the TWA for acrylamide or glycidamide of <1%.

The PBPK models predict HEDs that are higher than equivalent rat doses when TWA for blood acrylamide is the internal dose metric and HEDs that are lower than equivalent rat doses when TWA for blood glycidamide is the internal dose metric (Figures A-3 and A-4). The HED:rat dose ratio is not a constant function of dose. At a dose of 1 mg/kg/day, the ratio is approximately 0.23 based on TWA for acrylamide in blood and 1.3 based on TWA for glycidamide in blood.

**Figure A-3. Relationship Between Oral Dose of Acrylamide and Time-Weighted Average for Acrylamide in Blood in a Rat and Human (Simulations were for 12 Consecutive Hours of Exposure per Day for 365 Days)**

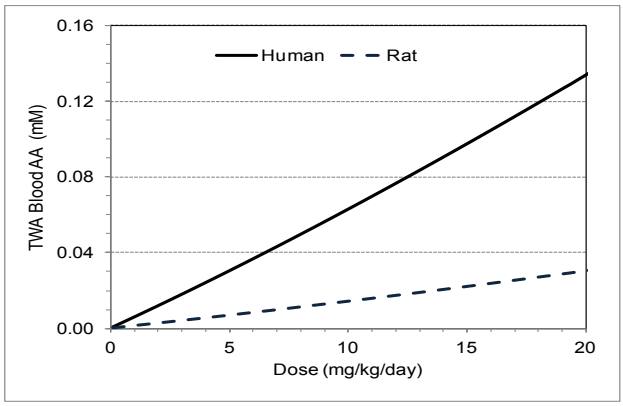
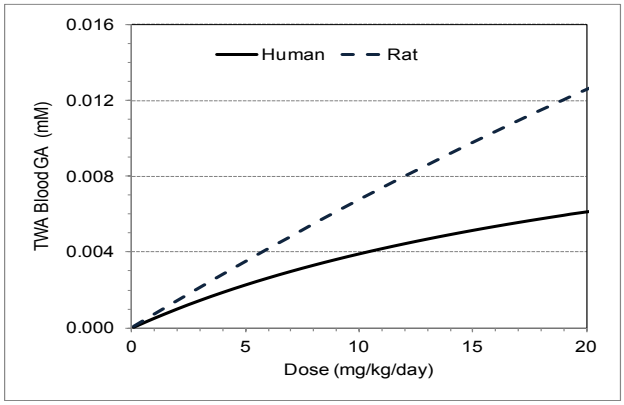



**Figure A-4. Relationship Between Oral Dose of Acrylamide and Time-Weighted Average for Glycidamide in Blood in a Rat and Human (Simulations were for 12 Consecutive Hours of Exposure per Day for 365 Days)**



APPENDIX A

# MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Acrylamide
CAS Numbers: 79-06-1
Date: May 2012
Profile Status: Post-Public Comment Draft 3
Route: [ ] Inhalation [x] Oral
Duration: [x] Acute [ ] Intermediate [ ] Chronic
Graph Key: 42
Species: Rat

Minimal Risk Level: 0.01 [x] mg/kg/day [ ] ppm

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

Experimental design: Groups of male Long-Evans hooded rats (15/group) were administered acrylamide (in distilled water) by oral gavage for 5 days at doses of 0, 30, 45, or 60 mg/kg/day (experiment 1) and 0, 5, or 15 mg/kg/day (experiment 2). Males were then mated on weeks 1, 2, 3, 4, 7, and 10 to proestrus females (weeks 1–4 only for experiment 2). Mating was confirmed by vaginal lavage. Females were sacrificed on gestation day 15 and numbers of corpora lutea, implantation sites, and fetuses were determined and estimates of pre- and postimplantation losses were calculated.

Effect noted in study and corresponding doses: Effects of acrylamide exposure on mating, fertility, and pre-and postimplantation losses are summarized in Table A-1. There were no significant treatment-related effects on mating index (number of sperm positive/number mated) at any dose level and no significant effects on any measures of reproductive success at the 5 mg/kg/day dose level. Assessments of reproductive indices at week 1 revealed significantly depressed fertility rates and elevated preimplantation losses at doses ≥15 mg/kg/day. Increased postimplantation loss was seen at weeks 2 and 3 at doses ≥15 mg/kg/day. In sperm samples collected from the 45 mg/kg/day group, the percentage of motile sperm was modestly (but significantly) decreased (58 vs. 73% in controls) at week 3, but not at weeks 2 or 4. This study identified a NOAEL of 5 mg/kg/day and a LOAEL of 15 mg/kg/day for significantly decreased fertility and increased preimplantation loss during week 1 posttreatment and significantly increased postimplantation loss during weeks 2 and 3 posttreatment.

## Table A-1. Effects of Acrylamide on Selected Reproductive Indices Following Gavage Dosing of Male Long-Evans Rats for 5 Days

| Treatment (mg/kg) | Post-treatment mating week | Mating index (percent)[a] | Fertility index (percent)[b] | Preimplantation loss index (percent)[c] | Postimplantation loss index (percent)[d] |
|---|---|---|---|---|---|
| Control[e] | 1 | 25/30 (83) | 22/25 (88) | 19.57 | 4.20 |
| | 2 | 26/30 (87) | 25/26 (96) | 11.54 | 5.70 |
| | 3 | 29/30 (97) | 28/29 (97) | 11.46 | 6.80 |
| | 4 | 27/30 (90) | 26/27 (96) | 9.48 | 5.00 |
| 5 | 1 | 15/15 (100) | 12/15 (80) | 24.29 | 8.30 |
| | 2 | 14/15 (93) | 11/14 (79) | 25.40 | 10.80 |
| | 3 | 15/15 (100) | 14/15 (93) | 10.77 | 5.40 |
| | 4 | 13/15 (87) | 11/13 (85) | 17.08 | 7.10 |
| 15 | 1 | 13/15 (87) | 6/13 (46)[f] | 74.69[f] | 10.30 |
| | 2 | 11/15 (73) | 11/11 (100) | 8.5 | 15.40[f] |
| | 3 | 13/15 (87) | 12/13 (92) | 13.42 | 15.70[f] |
| | 4 | 13/15 (87) | 12/13 (92) | 10.36 | 5.50 |
| 30 | 1 | 12/15 (80) | 2/12 (17)[f] | 97.83[f] | 0.00 |
| | 2 | 15/15 (100) | 15/15 (100) | 15.90 | 25.40[f] |
| | 3 | 15/15 (100) | 14/15 (93) | 14.51 | 35.00[f] |
| | 4 | 15/15 (100) | 15/15 (100) | 4.90 | 17.50 |
| 45 | 1 | 13/15 (87) | 2/13 (15)[f] | 85.89[f] | 26.77 |
| | 2 | 15/15 (100) | 12/15 (80) | 33.04[f] | 60.77[f] |
| | 3 | 15/15 (100) | 10/15 (67)[f] | 59.13[f] | 55.50[f] |
| | 4 | 15/15 (100) | 15/15 (100) | 11.60 | 7.67 |
| 60 | 1 | 15/15 (100) | 1/15 (7)[f] | 98.33[f] | 33.33 |
| | 2 | 15/15 (100) | 8/15 (53)[f] | 73.39[f] | 77.87[f] |
| | 3 | 15/15 (100) | 1/15 (7)[f] | 98.80[f] | 50.00[f] |
| | 4 | 15/15 (100) | 9/15 (60)[f] | 70.84[f] | 17.11[f] |

[a]Mating index = number sperm/positive females/number mated.
[b]Fertility index = number pregnant/number sperm-positive females.
[c]Preimplantation loss = (number of corpora lutea/female – number of implant sites/female)/(number of corpora lutea/female) x100.
[d]Postimplantation loss = (number of implant sites/female – number of fetuses/females)/(number of implant sites/female) x100.
[e]Control data from "high" and "low" dose dominant lethal studies are combined for tabular presentation. However statistical differences associated with acrylamide treatment are based upon comparison with appropriate control group.
[f]$p \leq 0.05$.

Source: Sublet et al. 1989

Dose and end point used for MRL derivation: The MRL is based on a $BMDL_{10}$ of 0.00177669 mM (PBPK model-predicted rat blood TWA acrylamide dose) for decreased fertility in rats (as assessed by number of nonpregnant rats/number of sperm-positive females) following oral administration of acrylamide to male Long-Evans hooded rats for 5 days and subsequent mating with untreated female rats.

[ ] NOAEL [ ] LOAEL [x] $BMDL_{10}$

PBPK modeling was used to estimate rat internal dose metrics for blood acrylamide and glycidamide (a readily-formed metabolite of acrylamide in aqueous environment) for each of the administered acrylamide dose levels in the principal study and for estimating HEDs. Code for acrylamide and glycidamide rat and human PBPK models was provided by L.M. Sweeney along with data used to evaluate model performance and documentation of parameter values (Sweeney et al. 2010). Refer to Section 3.4.5 for a detailed description of the PBPK model of Sweeney et al. (2010).

For the 5-day gavage study of Sublet et al. (1989), the model was used for interspecies extrapolation of rat internal dosimetry to humans using the following procedure:

1. The rat model was used to simulate external rat dosages and to predict the corresponding internal dose metric, TWA concentrations of acrylamide and glycidamide in mixed venous blood, where TWA is calculated as follows:

$$TWA = \left( \int_{t=0}^{t=i} C_{Blood} \right) \div t_i$$

   where $C_{Blood}$ is the mixed venous blood concentration of acrylamide or glycidamide (mM) and $t_i$ is the exposure time (hours).

2. The gavage doses (mg/kg/day) were assumed to be delivered as a single bolus each day, at the exposure frequency (5 consecutive days) used in the study.

3. Rat internal doses (blood TWA) were estimated for the 5-day exposure duration (120 hours).

4. Rat body weight used in the simulations was 0.362 kg, which was the EPA (1988) reported mean body weight for 112-day-old male Long-Evans rats in a table (Table 3-4, page 3-72) used by EPA (1988) to generate a growth curve for male Long-Evans rats (Figure 3-26, page 3-88). The reported age of the male Long-Evans rats used in the 5-day gavage study of Sublet et al. (1989) was 90–100 days; additional body weight data were not included in the study report.

5. BMD modeling was performed to estimate the internal dose of the 95% lower confidence limit (BMDL) on the BMD for TWA acrylamide or glycidamide blood concentration in the rat.

6. The human model was used to predict the daily dosage (mg/kg/day) corresponding to the BMDL.

7. A body weight of 70 kg was assumed for humans.

8. Daily doses (mg/kg/day) in humans were assumed to be delivered in 12 consecutive hourly doses, separated by 12-hour intervals, on 5 consecutive days (the duration of the rat study).

9. Human external doses corresponding to the BMDLs were estimated for the 5-day exposure duration.

The PBPK model-predicted rat blood TWA acrylamide and glycidamide doses for each of administered dose levels used in the principal study (Sublet et al. 1989) are presented in Table A-2 along with the corresponding fraction of nonpregnant female rats (number of nonpregnant/number of sperm-positive females) at week 1 posttreatment, the time period shown to be most sensitive to male reproductive effects as demonstrated in Table A-1.

**Table A-2. PBPK Model-Predicted Rat TWA Acrylamide and Glycidamide Doses to the Blood and Fraction of Nonpregnant Female Rats after 1 Week of Mating to Male Rats that had been Administered Acrylamide by Gavage for 5 Days Prior to Mating (Sublet et al. 1989)**

| Administered dose (mg/kg/day) | Rat blood TWA acrylamide dose (mM) | Rat blood TWA glycidamide dose (mM) | Fertility index[a] | Fraction of nonpregnant females[b] |
|---|---|---|---|---|
| 0 | 0 | 0 | 22/25 | 3/25 |
| 5 | 0.00707 | 0.00335 | 12/15 | 3/15 |
| 15 | 0.0240 | 0.00881 | 6/13 | 7/13 |
| 30 | 0.0552 | 0.0148 | 2/12 | 10/12 |
| 45 | 0.0919 | 0.0193 | 2/13 | 11/13 |
| 60 | 0.133 | 0.0229 | 1/15 | 14/15 |

[a]Fertility index = number pregnant/number sperm-positive females.
[b]Number nonpregnant/number of sperm-positive females.

PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

The acute-duration oral MRL for acrylamide was derived using a BMD modeling approach. All dichotomous models in the EPA Benchmark Dose Software (Version 2.1.2) were fit to the incidence data for number of nonpregnant females/number of sperm-positive females using PBPK model-predicted rat TWA acrylamide dose to the blood as the dose metric; the models were also fit to the incidence data using PBPK model-predicted rat TWA glycidamide dose to the blood as the dose metric. A BMR of 10% extra risk was selected because the group sizes in this study (15 male rats/dose) did not support the application of a more sensitive BMR. Model results for the fraction of nonpregnant females are shown in Table A-3 for the rat blood acrylamide dose metric and Table A-4 for the rat blood glycidamide dose metric.

## Table A-3. Benchmark Results for PBPK Model-Predicted Rat Blood TWA Acrylamide and Fraction of Nonpregnant Female Rats after 1 Week of Mating to Male Rats that had been Administered Acrylamide by Gavage for 5 Days Prior to Mating (Sublet et al. 1989)

| Model | $\chi^2$ Goodness of fit p-value[a] | Scaled residuals[b] Dose below BMD | Dose above BMD | Overall largest | AIC | Rat blood TWA acrylamide dose (mM)[c] $BMD_{10}$ | $BMDL_{10}$ |
|---|---|---|---|---|---|---|---|
| Gamma[d,e], Multistage[e,f], Weibull[d,e] | 0.88 | 0.05 | -0.41 | 0.72 | 85.86 | 0.0047264 | 0.00340605 |
| Logistic | 0.25 | -0.40 | 1.15 | 1.16 | 89.81 | 0.011528 | 0.00850744 |
| ***LogLogistic[g,h]*** | ***0.94*** | ***0.07*** | ***-0.19*** | ***-0.46*** | ***87.04*** | ***0.00621986*** | ***0.00177669*** |
| LogProbit[g] | 0.96 | 0.03 | 0.23 | -0.59 | 85.26 | 0.00782648 | 0.00536075 |
| Probit | 0.18 | -0.44 | 1.21 | 1.40 | 90.69 | 0.0119204 | 0.00920888 |
| | | | | | | $BMD_{05}$ | $BMDL_{05}$ |
| ***LogLogistic[g,h]*** | ***0.94*** | ***0.07*** | ***-0.19*** | ***-0.46*** | ***87.04*** | ***0.00381519*** | ***0.000841591*** |

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Rat blood acrylamide doses predicted by the PBPK model of Sweeney et al. (2010) based on oral doses estimated by Sublet et al. (1989).
[d]Power restricted to ≥1.
[e]Gamma, multistage, and Weibull models took the form of a 1-degree multistage model and provided identical fit to the data.
[f]Betas restricted to ≥0.
[g]Slope restricted to ≥1.
[h]Selected model. All models provided adequate fit to the data. BMDLs for models providing adequate fit were not sufficiently close (differed by >2–3-fold), so the model with the lowest BMDL was selected (LogLogistic).

AIC = Akaike's Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: e.g., $_{10}$ = exposure concentration associated with 10% extra risk); PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

## Table A-4. Benchmark Results for PBPK Model-Predicted Rat Blood TWA Glycidamide and Fraction of Nonpregnant Female Rats after 1 Week of Mating to Male Rats that had been Administered Acrylamide by Gavage for 5 Days Prior to Mating (Sublet et al. 1989)

| | $X^2$ | Scaled residuals[b] | | | | Rat blood TWA glycidamide dose (mM)[c] | |
|---|---|---|---|---|---|---|---|
| Model | Goodness of fit p-value[a] | Dose below BMD | Dose above BMD | Overall largest | AIC | $BMD_{10}$ | $BMDL_{10}$ |
| Gamma[d] | 0.94 | 0.06 | -0.17 | 0.44 | 87.04 | 0.0030958 | 0.000975396 |
| ***Logistic[e]*** | ***0.92*** | ***-0.14*** | ***-0.28*** | ***-0.56*** | ***85.53*** | ***0.00296497*** | ***0.00220167*** |
| LogLogistic[f] | 0.96 | 0.05 | -0.10 | -0.41 | 86.92 | 0.00363193 | 0.00143998 |
| LogProbit[f] | 0.96 | 0.05 | -0.14 | -0.39 | 86.94 | 0.00359742 | 0.00168997 |
| Multistage (1-degree)[g] | 0.71 | 0.37 | -1.12 | -1.12 | 86.96 | 0.00114806 | 0.000853692 |
| Multistage (≥2-degree)[g] | 0.86 | 0.12 | -0.41 | 0.58 | 87.38 | 0.00235936 | 0.000945326 |
| Probit | 0.89 | -0.14 | -0.32 | 0.68 | 85.75 | 0.00283547 | 0.00218776 |
| Weibull[d] | 0.91 | 0.10 | -0.30 | 0.51 | 87.18 | 0.00271645 | 0.000962598 |
| | | | | | | $BMD_{05}$ | $BMDL_{05}$ |
| ***Logistic[e]*** | ***0.92*** | ***-0.14*** | ***-0.28*** | ***-0.56*** | ***85.53*** | ***0.00151342*** | ***0.000860953*** |

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Rat blood glycidamide doses predicted by the PBPK model of Sweeney et al. (2010) based on oral doses estimated by Sublet et al. (1989).
[d]Power restricted to ≥1.
[e]Selected model. All models provided adequate fit to the data. BMDLs for models providing adequate fit were sufficiently close (differed by >2–3-fold), so the model with the lowest AIC was selected (Logistic).
[f]Slope restricted to ≥1.
[g]Betas restricted to ≥0.

AIC = Akaike's Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: e.g., $_{10}$ = exposure concentration associated with 10% extra risk); PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

Adequate model fit was judged by three criteria: $X^2$ goodness-of-fit p-value (p>0.1), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. As judged by $X^2$ goodness-of-fit statistic, all of the models provided adequate fit to the data. Comparing across models, the best fit to the data is generally indicated by the model with the lowest Akaike's Information Criteria (AIC) when BMDLs for all models providing adequate fit to the data differ from one another by <2–3-fold; otherwise, the model with the lowest BMDL is selected as the best-fitting model if it is not considered to be an outlier. For the modeled data using rat blood TWA acrylamide as the dose metric, the best-fitting model (log-logistic; lowest $BMDL_{10}$) provided a $BMD_{10}$ of 0.00621986 mM and a $BMDL_{10}$ of 0.00177669 mM (Table A-3). BMD and BMDL values for a BMR of 5% extra risk from the best-fitting model are included in Table A-3 for comparison. For the modeled data using rat blood TWA glycidamide as the dose metric, the best-fitting model (logistic; lowest AIC) provided a $BMD_{10}$ of 0.00296497 mM and a $BMDL_{10}$ of 0.00220167 mM (Table A-4). BMD and BMDL values for a BMR of 5% extra risk from the best-fitting model are included in Table A-4 for comparison.

Figure A-5 shows the plotted results for fraction of nonpregnant females during week 1 posttreatment from the best-fitting model (log-logistic) based on PBPK model-predicted rat blood TWA acrylamide

dose metric and a BMR of 10% extra risk. Figure A-6 shows the plotted results for fraction of nonpregnant females during week 1 posttreatment from the best-fitting model (logistic) based on PBPK model-predicted rat blood TWA glycidamide dose metric and a BMR of 10% extra risk.

**Figure A-5. Fit of Log-Logistic Model to Fertility Data (Fraction of Nonpregnant Female Rats) at Week 1 Following 5 Days of Gavage Administration of Acrylamide to Male Rats Using Time-Weighted Average Acrylamide Blood Concentration (mM) as the Dose Metric and a Benchmark Response of 10% Extra Risk**

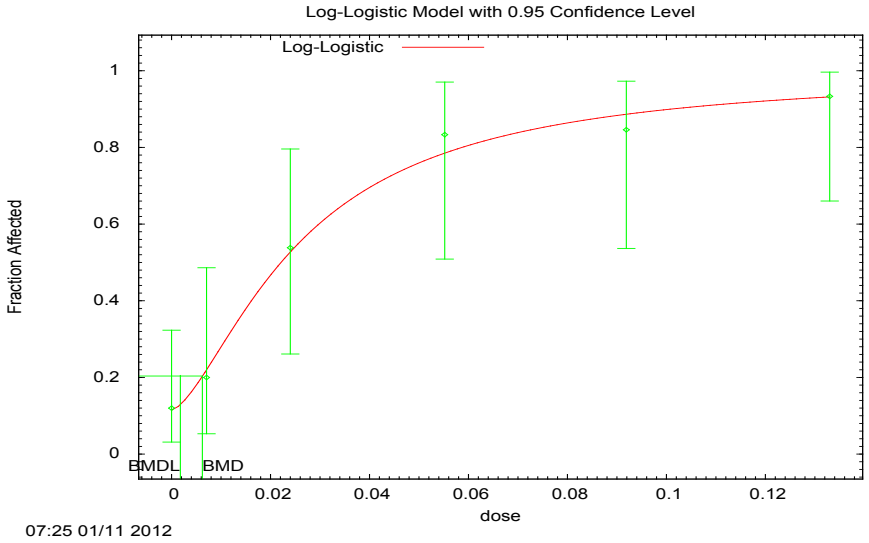
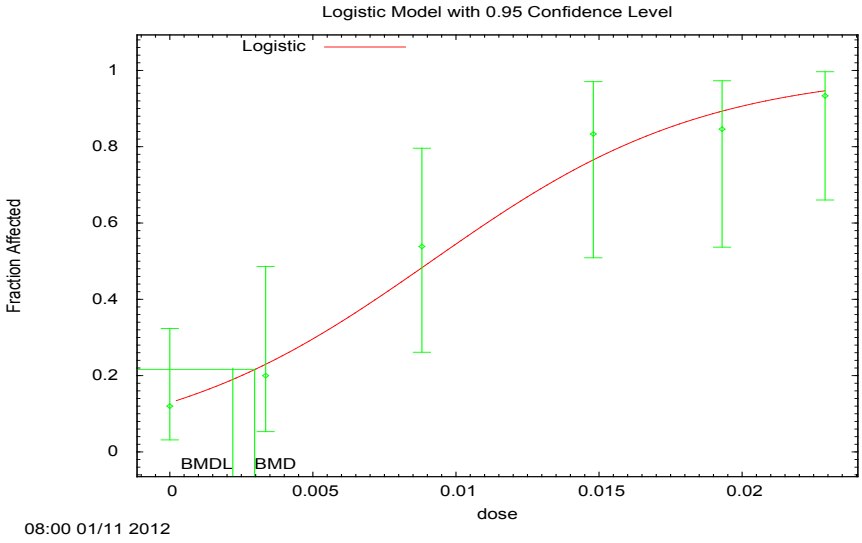


**Figure A-6. Fit of Logistic Model to Fertility Data (Fraction of Nonpregnant Female Rats) at Week 1 Following 5 Days of Gavage Administration of Acrylamide to Male Rats Using Time-Weighted Average Glycidamide Blood Concentration (mM) as the Dose Metric and a Benchmark Response of 10% Extra Risk**




Based on the rat blood TWA acrylamide $BMDL_{10}$ of 0.00177669 mM, the PBPK model-predicted HED is 0.31 mg acrylamide/kg/day and the rat equivalent dose is 1.33 mg acrylamide/kg/day. Based on the rat blood TWA glycidamide $BMDL_{10}$ of 0.00220167 mM, the PBPK model-predicted HED is 5.25 mg acrylamide/kg/day and the rat equivalent dose is 3.20 mg acrylamide/kg/day. Both acrylamide and glycidamide are widely distributed by the blood and both are reactive. However, based on uncertainty regarding the proximal toxicant(s) responsible for acrylamide-induced reproductive toxicity in the male rat, a conservative public health approach was taken and the lowest HED of 0.31 mg acrylamide/kg/day was selected as the POD for deriving an acute-duration oral MRL for acrylamide. A total uncertainty factor of 30 (3 for interspecies extrapolation using a PBPK model and 10 for human variability) was applied to the HED of 0.31 mg/kg/day, resulting in an acute-duration oral MRL of 0.01 mg/kg/day for acrylamide. An uncertainty factor of 10 for human variability is justified based on findings that key metabolic enzymes for acrylamide are CYP2E1, GST, and EH for which human polymorphisms are known (Huang et al. 2011a) and wide variation in human CYP2E1 expression as reviewed by Bolt et al. (2003).

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[x] 3 for extrapolation from animals to humans with dosimetric adjustment
[x] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information that lend support to this MRL: As demonstrated in Table 3-4 (Levels of Significant Exposure to Acrylamide – Oral), male-mediated implantation loss and decreased mean body weight gain represent the most sensitive effects of acute-duration oral exposure to acrylamide. The lowest dose level reported to induce significantly decreased male-mediated implantation loss was 15 mg/kg/day in male Long-Evans rats administered acrylamide by gavage for 5 days followed by mating sessions with unexposed female rats (Sublet et al. 1989); this study identified a NOAEL of 5 mg/kg/day. A similarly-designed study (Tyl et al. 2000b) confirmed the findings of acrylamide-induced increased implantation loss, although, based on pairwise comparisons with controls, statistical significance was achieved only at the highest dose level (60 mg/kg/day). Tyl et al. (2000b) also noted significantly decreased body weight gain (>40% lower than controls) during the 5 days of dosing, a finding not included in the study report of Sublet et al. (1989). Based on the reproducible results for male-mediated implantation loss in the studies of Sublet et al. (1989) and Tyl et al. (2000b) and the lack of supporting information regarding the body weight effects, the male-mediated decreased fertility was selected as the critical effect for deriving an acute-duration oral MRL for acrylamide. The study of Sublet et al. (1989) identified the lowest LOAEL for the critical effect and was therefore selected as the principal study.

Results of several other studies corroborate the findings of male-mediated decreases in fertility and increases in implantation losses following oral exposure to acrylamide (Chapin et al. 1995; Sakamoto and Hashimoto 1986; Smith et al. 1986; Tyl et al. 2000a; Zenick et al. 1986).

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APPENDIX A

# MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Acrylamide
CAS Numbers: 79-06-1
Date: May 2012
Profile Status: Post-Public Comment Draft 3
Route: [ ] Inhalation [x] Oral
Duration: [ ] Acute [x] Intermediate [ ] Chronic
Graph Key: 83
Species: Rat

Minimal Risk Level: 0.001 [x] mg/kg/day [ ] ppm

Reference: Burek JD, Albee RR, Beyer JE, et al. 1980. Subchronic toxicity of acrylamide administered to rats in the drinking water followed by up to 144 days of recovery. J Environ Pathol Toxicol 4(5-6):157-182.

Experimental design: Groups of 6-week-old male (23–29/group) and female (10/group) F344 rats were administered acrylamide in the drinking water for up to 93 days at concentrations designed to result in acrylamide intakes of 0, 0.05, 0.2, 1, 5, or 20 mg/kg/day. Ten rats/sex/group were assigned to the basic 90-day study and were observed for body weight and water consumption (recorded weekly) throughout the treatment period. Following 7 and 33 days of treatment, three control and three high-dose male rats were sacrificed for interim electron microscopic examination of the sciatic nerve. Ten male (nine in the high-dose group, due to one death prior to treatment termination) and all female rats from each treatment group were subjected to gross and histopathologic examination of all major organs and tissues at the end of the treatment period, at which time, three other male rats from each group were processed for electron microscopic examination of the sciatic nerve. The remaining rats (all males) in each group were observed for signs of recovery from treatment-related effects for up to 144 days following cessation of treatment. Three rats/group were subjected to electron microscopic examination of the sciatic nerve on recovery days 25, 111, and 144. Body weights were recorded for two rats/dose level prior to sacrifice on recovery day 111. At the end of the 144-day recovery period, the remaining four rats of each dose level were weighed and sacrificed for gross and histopathologic examination of all major organs and tissues.

All rats were observed daily (during the 5-day work week) for general health and clinical signs. Hindlimb foot splay was measured weekly in four control and four high-dose (20 mg/kg/day) male and female rats until the onset of neuropathy was detected, after which neuropathy in the high-dose group was monitored by clinical signs. After neuropathy was detected in high-dose rats, male and female rats in the 5 mg/kg/day dose groups were also subjected to weekly testing of foot splay (rats in the lower treatment groups were not tested due to the lack of response at 5 mg/kg/day). Blood samples collected from seven rats/sex in the control and high-dose groups on treatment day 76 and from all rats alive on day 60 of the recovery period were examined for packed cell volume, total erythrocyte count, total and differential leukocyte counts, and hemoglobin concentration. The study design included urinary sampling from 10 control and 10 high-dose rats per sex on treatment day 76 and at the end of the treatment period. Blood serum was collected from the 10 rats/sex/dose that were sacrificed at the end of treatment and from the 4 male rats/group that were maintained throughout the 144-day recovery period. Blood urea nitrogen, alkaline phosphatase, serum glutamic pyruvic transaminase, and serum cholinesterase activity were determined.

Light microscopic examinations were performed on brain, spinal cord, and peripheral nerves (including brachial plexus, sciatic, and femoral nerves) from selected male and female rats of each dose group. Nervous tissues were fixed in glutaraldehyde-paraformaldehyde and stained with hematoxylin eosin.

Additional sections of brain, spinal cord, and peripheral nerves were subjected to the luxol fast blue-periodic acid Schiff (LFB/PAS) reaction for myelin staining and to Bodian's stain to elucidate more subtle axonal changes. Myelin and axonal degeneration was classified as severe (degeneration in approximately 50% of the observed fibers), moderate (degeneration in 20–50% of observed fibers), slight (degeneration in <20% of observed fibers), very slight (effects restricted to focal or multifocal changes in individual nerves), or equivocal (nerves could not be graded as clearly normal). Only sciatic nerve tissues from male rats were examined by electron microscopy. Three blocks of sciatic nerve fibers, two longitudinal and one transverse, were selected per rat for thin sectioning and ultrastructural analysis. Ultrastructural alterations were counted by examining a maximum of 50 fields per block, a field defined as a section through any Schwann cell. This resulted in an examined maximum of 150 fields/rat or 450 fields/treatment group of three rats.

Hematology, urinary and clinical chemistry parameters, body weights, organ-to-body weight ratio data, foot spread results, and water consumption were statistically analyzed by one-way analysis of variance followed by Dunnett's test. The level of significance chosen was $p<0.05$. The study report did not, however, include individual or averaged incidences or extent of changes in these parameters, so an independent analysis of the results of body and organ weights, water consumption, foot splay, hematology, urinalysis, or serum chemistry was not possible.

Effect noted in study and corresponding doses: Significantly lower body weights were reported in the high-dose male and female rats relative to controls: 8% lower (males and females) on treatment days 13 and 20, and 21 and 24% lower (males and females, respectively) on treatment day 91. No significant body weight effect was seen in rats of lower dose groups. High-dose rats also exhibited treatment-related effects on organ weights including significantly decreased absolute liver, kidney, and thymus weights in males (also testicular) and females, significantly decreased absolute brain and heart weights in females (trend for decreased weights in males), increased relative brain, heart, liver, and kidney weights in males and females, and decreased relative thymus (females only) and testicular weight in males. Absolute and relative liver weight was increased in 5 mg/kg/day males. Marginally statistically significant increases in relative heart weight in 0.05 and 0.2 mg/kg/day females were not considered to be of toxicological significance due to the lack of a dose response. High-dose female rats exhibited significantly decreased water consumption (15–39% decreased) between treatment days 20 and 90. Although decreased water consumption was noted in high-dose males, the decrease reached the level of statistical significance in only 4 of the 13 intervals recorded. The few instances of significantly increased water consumption in low-dose rats did not follow a consistent pattern or trend, and may be of no toxicological significance. By day 144 of the posttreatment recovery period, the high-dose group had recovered with higher (but not statistically significant) body weights than controls, significantly higher absolute liver and kidney weights, and as significantly higher relative brain and liver weights.

Significantly increased instances of hindlimb foot splay were observed in high-dose male and female rats on treatment day 22 (incidences were not reported), which became more pronounced on treatment day 29. Foot splay testing was terminated with this treatment group (to prevent injury), but clinical signs of neuropathy (including curling of the toes, rear limb splay, incoordination, and posterior weakness) progressed in severity throughout the remainder of the treatment period. Beginning on treatment day 29, rats of the 5 mg/kg/day dose level were tested, but foot splay was not detected at this treatment level in either males or females. No other treatment-related clinical effects were observed in the 5 mg/kg/day males or females or any of the lower dose groups. By day 7 of the posttreatment recovery period, the high-dose groups showed clear signs of improvements continuing to day 111 with only slight posterior weakness and curling of the toes. By day 144, these high-dose rats appeared clinically similar to the controls.

At the end of the treatment period, serum cholinesterase activity was increased and alkaline phosphatase activity was statistically significantly increased in high-dose females. Significant decreases in packed cell volume, total erythrocyte count, and hemoglobin concentrations in high-dose males and females and 5 mg/kg/day females were noted. Results of urinalysis did not reveal any acrylamide-induced abnormalities. By day 144 posttreatment, the high-dose group (sex not specified) had statistically significant decreased serum cholinesterase levels and no significant differences in other clinical chemistry parameters.

Upon necropsy, gross observations of rats following the 92- or 93-day treatment period revealed treatment-related alterations only in the high-dose group, including perineal soiling, decreased adipose tissue, decreased liver size, darkened kidneys, foci or mottled appearance of lungs, decreased size or flaccid testicles, decreased size of male accessory genitalia, decreased uterus size, altered appearance of peripheral nerves, atrophy of skeletal muscle in the posterior portion of the body, bladder distention, and diffuse mural thickening of the stomach. The authors did not include incidence data regarding gross examination data, however. Histopathologic examination of high-dose rats revealed effects such as atrophy of skeletal muscle (2/10 males, 8/10 females), slightly increased hematogenous pigment in the spleen (4/9 males), ulcerative gastritis or hyperkeratosis in the nonglandular stomach (4/10 males), atrophy of mesenteric fat (8/10 females), vacuolization of the smooth muscle in the bladder wall (1/10 males, 2/9 females), inflammation in the lungs (3/10 males, 5/10 females), and testicular effects that included atrophy (10/10), mineralization in seminiferous tubules (5/10), and increased cellular debris and/or decreased spermatogenic segments in the tubular lamina of the epididymides (9/10). The statistical significance of these findings could not be assessed because incidence data for controls were not reported. By day 144 posttreatment, only the high-dose rats had persistent gross pathological effects, primarily dark testicles and slightly distended bladders. The testicular histological lesions consisted of focal or multifocal atrophy to individual seminiferous tubules, some with mineral and cellular debris, and indication of partial reversibility of the testicular atrophy. Light microscopic examination of the sciatic nerve sections (stained with hematoxylin and eosin) revealed severe degeneration in the high-dose groups that was characterized by demyelinization (LFB/PAS-treated sections) and axonal degeneration (Bodian's-treated sections) in 10/10 females and similar but less severe effects in males (degeneration moderate in 5/10 and severe in the other 5). These lesions were also seen in other peripheral nerve sections (brachial plexus and femoral nerve) but varied in severity from equivocal to severe (incidences not reported). The authors noted equivocal to very slight degenerative changes in peripheral nerves of 5 mg/kg/day males (9/10) and females (6/10) but found no light microscopic evidence of peripheral nerve lesions in 0.05, 0.2, or 1 mg/kg/day treatment groups. Very slight to slight degenerative changes (demyelinization, swollen astrocytes and axons) were seen in spinal cord sections of high-dose male (5/10) and female (9/10) rats. No treatment-related lesions were observed at any dose level within brain sections examined by light microscopy. After 144 days of posttreatment recovery, no nerve tissue alterations were observed in any of the 5 mg/kg/day or lower dose groups. In the high-dose group, alterations ranged from very slight to slight in the sciatic nerve and no alterations were noted in sections of the brachial nerve. The authors stated that if the recovery period had been extended beyond 144 days, the remaining tissue changes would likely have completely reversed.

Ultrastructural (electron microscope) examinations of sciatic nerve preparations from three male rats/group included the examination of fields (defined as a section through any Schwann cell) for signs of axolemma invaginations, axonal invaginations with cell organelles and/or dense bodies, and Schwann cells without axons and/or with degenerating myelin. After 7 days of treatment, no significant differences were seen between control and high-dose rats (other treatment groups were not subjected to 7-day interim sacrifice). After 33 days of treatment, high-dose male rats exhibited increased prevalence of fields showing axolemma invaginations with cell organelles and/or dense bodies and fields exhibiting Schwann cells without axons and/or with degenerating myelin (other groups were not subjected to 33-day interim sacrifice). Following 90 days of treatment, severe axonal degeneration and axonal loss were seen in high-

dose rats. Approximately 55% of the fields examined exhibited alterations in myelinated nerves or Schwann cells (compared with 12 and 21% after treatment days 7 and 33, respectively). Similar, but less severe, ultrastructural alterations in approximately 34% of the fields examined were seen in the 5 mg/kg/day dose group. At the 1 mg/kg/day dose level, approximately 24% of the fields examined showed axolemma invaginations with cell organelles and/or dense bodies, but not more severe signs of ultrastructural alterations. The alterations in the sciatic nerve fields examined in the control, 0.05, and 0.2 mg/kg-day groups were roughly comparable (15, 9, and 12%, respectively), suggesting that there were no adverse effects at the 0.05 and 0.2 mg/kg/day doses. Importantly, the increase in lesions observed via electron microscopy in the 1 and 5 mg/kg/day groups appeared to have completely reversed by days 25 and 111 posttreatment, respectively. The observed lesions in the high-dose group were partially or completely reversed by day 144 posttreatment.

In summary, the study of Burek et al. (1980) identified a NOAEL of 0.2 mg/kg/day and a LOAEL of 1 mg/kg/day, based on ultrastructural degeneration (axolemma invaginations with cell organelles and/or dense bodies) in the sciatic nerve of male rats (as detected by electron microscopic examinations, which were limited to males). The increased frequency was characterized by the study authors as "slight" for the LOAEL at 1 mg/kg/day, and the lesions were reversible (back to control levels) by day 25 posttreatment in all 1 mg/kg/day treated rats. At the resolution of the light microscope, the 5 mg/kg/day dose was the lowest dose resulting in degenerative effects in the sciatic nerve of male and female rats.

Dose and end point used for MRL derivation: 0.2 mg/kg/day

[x] NOAEL [ ] LOAEL

A NOAEL/LOAEL approach was selected because results of the ultrastructural evaluations included only 3 of 10 rats/group and were reported only as the total numbers of fields (per group) with ultrastructural changes as axolemma invaginations or Schwann cells without axons and/or with degenerating myelin. This reporting of the electron microscopy data does not support a statistical comparison of the incidence of changes between the exposed and control groups because the study report lacked information regarding the distribution of fields exhibiting alterations among the three rats within any particular dose group. Therefore, a BMD approach was not feasible.

PBPK modeling was used to estimate rat internal dose metrics for blood acrylamide and glycidamide at the acrylamide applied rat dose NOAEL of 0.2 mg/kg/day and for estimating HEDs. Code for acrylamide and glycidamide rat and human PBPK models was provided by L.M. Sweeney along with documentation of parameter values (Sweeney et al. 2010). The Sweeney et al. (2010) model is based on the acrylamide and glycidamide rat model reported by Kirman et al. (2003). Refer to Section 3.4.5 of the Toxicological Profile for Acrylamide for a detailed description of the PBPK model of Sweeney et al. (2010).

For the 90-day drinking water study of Burek et al. (1980), the model was used for interspecies extrapolation of rat internal dosimetry to humans using the following procedure:

1. The rat model was used to predict the internal dose metric, TWA concentration of acrylamide and glycidamide in mixed venous blood, corresponding to the administered acrylamide dose NOAEL of 0.2 mg/kg/day, where TWA is calculated as follows:

$$TWA = \left( \int_{t=0}^{t=i} C_{Blood} \right) \div t_i$$

where $C_{Blood}$ is the mixed venous blood concentration of acrylamide or glycidamide (mM) and $t_i$ is the exposure time (hours).

2. The oral dose (0.2 mg/kg/day) was assumed to be delivered during a daily 12-hour period (e.g., the daily food and water consumption period).

3. Rat internal doses (blood TWA) were estimated for the 90-day exposure duration (2,160 hours).

4. Rat body weight used in the simulation was the EPA (1988) subchronic reference body weight for the male F344 rat (0.180 kg) because quantitative body weight data were not included in the principal study report (Burek et al. 1980).

5. The human model was used to predict the daily human equivalent dose (HED in mg/kg/day) corresponding to the rat NOAEL of 0.2 mg/kg/day.

6. A body weight of 70 kg was assumed for humans.

7. The daily dose (mg/kg/day) in humans was assumed to be delivered in 12 consecutive hourly doses, separated by 12-hour intervals, 7 days/week for a duration of 365 days (upper end of the range for intermediate-duration MRL).

8. Human external doses corresponding to the PBPK model-predicted rat blood TWA acrylamide and TWA glycidamide doses at the rat NOAEL of 0.2 mg/kg/day were estimated for the 365-day exposure duration.

Based on PBPK model-predicted rat blood TWA acrylamide dose metric at the NOAEL of 0.2 mg acrylamide/kg/day, the HED is 0.038 mg acrylamide/kg/day. Based on the PBPK model-predicted rat blood TWA glycidamide dose metric at the NOAEL of 0.2 mg acrylamide/kg/day, the HED is 0.28 mg acrylamide/kg/day. Using a conservative approach, derivation of an intermediate-duration oral MRL for acrylamide was performed using the lowest HED of 0.038 mg acrylamide/kg/day. A total uncertainty factor of 30 (3 for interspecies extrapolation using a PBPK model and 10 for human variability) was applied to the HED of 0.038 mg/kg/day, resulting in an intermediate-duration oral MRL of 0.001 mg/kg/day.

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[x] 3 for extrapolation from animals to humans using dosimetric adjustment
[x] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information that lend support to this MRL: Results of available animal studies demonstrate that neurological effects in males and females and reproductive effects in males are the most sensitive noncancer effects associated with intermediate-duration oral exposure to acrylamide (Burek et al. 1980; Chapin et al. 1995; Johnson et al. 1984, 1985, 1986; NTP 2011b;

Sakamoto and Hashimoto 1986; Smith et al. 1986; Tyl et al. 2000a, 2000b; Zenick et al. 1986). The lowest dose level reported to induce male-mediated implantation losses was 2.8 mg/kg/day in male Long-Evans rats receiving acrylamide from the drinking water for 80 days; this study identified a NOAEL of 1 mg/kg/day (Smith et al. 1986). Ultrastructural degenerative peripheral nerve changes were observed at a dose level as low as 1 mg/kg/day in male F344 rats receiving acrylamide from the drinking water for up to 93 days; this study identified a NOAEL of 0.2 mg/kg/day (Burek et al. 1980). Available data suggest that neurological effects represent a more sensitive point of departure than reproductive effects for deriving intermediate- and chronic-duration oral MRLs for acrylamide. Therefore, degenerative nerve change was selected as the critical effect for deriving an intermediate-duration oral MRL for acrylamide. The study of Burek et al. (1980) was selected as the principal study because it identified the lowest LOAEL for the critical effect. A NOAEL/LOAEL approach was selected because results of the ultrastructural evaluations included only 3 of 10 rats/group and were reported only as the total numbers of fields (per group) with ultrastructural changes as axolemma invaginations or Schwann cells without axons and/or with degenerating myelin. This reporting of the electron microscopy data does not support a statistical comparison of the incidence of changes between the exposed and control groups because it is unknown within any exposure group how the numbers of changes were distributed among the three rats (i.e., whether the apparent increase in incidence of fields with changes was due to one, two, or all three rats in the 1, 5, and 20 mg/kg-day groups). Therefore, a BMD approach was not feasible.

Interim data from a chronic toxicity and carcinogenicity bioassay of male and female F344 rats receiving acrylamide from the drinking water for up to 2 years (Johnson et al. (1984, 1985, 1986) provide support to the findings of Burek et al. (1980). In the 2-year study, interim sacrifices were performed at 3, 6, 12, and 18 months on some rats from each exposure group. One aspect of interim sacrifices was to evaluate the condition of selected peripheral nerve sections using light and electron microscopy. The most significant noncancer effects were increased incidences of axolemma invaginations (observed by electron microscopy) in the tibial branch of the sciatic nerve of male rats following 3 and 6 months of treatment and increased prevalence of "moderate" to "severe" degeneration (observed by light microscopy) in both males and females following 2 years of treatment. This study identified a NOAEL of 0.5 mg/kg/day and a LOAEL of 2.0 mg/kg/day for light and electron microscope evidence of peripheral nerve fiber degeneration in the male rats. Electron microscope evaluations at interims ≥12 months were not considered meaningful due to age-related degenerative effects that could not be distinguished from acrylamide-induced effects.

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# MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Acrylamide
CAS Numbers: 79-06-1
Date: May 2012
Profile Status: Post-Public Comment Draft 3
Route: [ ] Inhalation [x] Oral
Duration: [ ] Acute [ ] Intermediate [x] Chronic
Graph Key: 154
Species: Rat

Minimal Risk Level: 0.001 [x] mg/kg/day [ ] ppm

Reference: Friedman MA, Dulak LH, Stedham MA. 1995. A lifetime oncogenicity study in rats with acrylamide. Fundam Appl Toxicol 27(1):95-105.

Experimental design:

Groups of male and female F344 rats (≥50/sex/group) were exposed to acrylamide in the drinking water for 2 years at concentrations resulting in calculated doses of 0, 0.1, 0.5, or 2.0 mg/kg/day for the males and 0, 0.5, or 3.0 mg/kg/day for the females. The study included two control groups for each sex to assess variability in background tumor responses and 204 male rats in the 0.1 mg/kg/day group to increase the statistical power sufficient to detect a 5% increase in incidence of scrotal sac mesotheliomas over an expected background incidence of this tumor for F344 rats of about 1%. The study also had different dose group spacing for female rats to improve the characterization of the dose-response relationships. An additional group of 25 rats/sex was observed during the course of this study for signs of viral infections. Water and food intakes and body weights were monitored throughout the study. All animals were observed twice daily for mortality, morbidity, and obvious clinical signs of toxicity; periodic physical examinations were performed. Complete postmortem gross pathologic examinations were performed on all rats in the study. Brain, liver, kidneys, and testes were excised and weighed. Group mean organ weights and organ-to-body weight ratios were calculated. Representative sections from all major organs and tissues (including the sciatic nerve) were processed for histopathologic examination. Initially, light microscopic examination was completed only on high-dose and control rats. Based on histopathologic results in these groups, examinations were performed on specific tissues harvested from rats of lower dose groups. Histopathologic examination was performed on thyroid, brain (three levels, females only), mammary glands (females), and testes (males) in all rats. In addition, spinal cord (three levels), uterus, and gross lesions were evaluated in all control and high-dose females, and in low-dose female rats found dead or sacrificed moribund. Brain (three levels), spinal cord (three levels), and gross lesions were examined in all control and high-dose males and in low- and mid-dose male rats found dead or sacrificed moribund. No special staining methods were used to enhance light microscopic detection of degenerative changes in nervous tissues.

Effect noted in study and corresponding doses: The most sensitive noncancer effect was degenerative changes in the sciatic nerve as detected by light microscopy. The study identified a NOAEL of 0.5 mg/kg/day and a LOAEL of 2.0 mg/kg/day for the males and a NOAEL of 1.0 mg/kg/day and a LOAEL of 3.0 mg/kg/day for the female rats. Incidence data for degenerative changes in the sciatic nerve of the male and female rats are presented in Table A-5.

## Table A-5. Incidence Data for Degenerative Changes in Sciatic Nerve Preparations from F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years

| Sciatic nerve degenerative changes | Dose (mg/kg/day) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 0 | 0.1 | 0.5 | 1.0 | 2.0 | 3.0 |
| Males | 30/83 | 29/88 | 21/65 | 13/38 | | 26/49[a] | |
| Females | 7/37 | 12/43 | | | 2/20 | | 38/86[b] |

[a]Significantly ($p<0.05$) different from control incidence by Fisher's exact test performed by SRC, Inc.; incidences for the two control groups were pooled.
[b]Significantly ($p<0.01$) different from control incidence by Fisher's exact test performed by SRC, Inc.; incidences for the two control groups were pooled.

Source: Friedman et al. 1995

Dose and end point used for MRL derivation: The MRL is based on a $BMDL_{05}$ of 0.000240096 mM (PBPK model-predicted rat blood TWA acrylamide dose) for degenerative sciatic nerve changes in male F344 rats administered acrylamide in the drinking water for up to 2 years.

[ ] NOAEL [ ] LOAEL [x] $BMDL_{05}$

PBPK modeling was used to estimate rat internal dose metrics for blood acrylamide and glycidamide at the each of the administered acrylamide doses and for estimating HEDs. Code for acrylamide and glycidamide rat and human PBPK models was provided by L.M. Sweeney along with documentation of parameter values (Sweeney et al. 2010). Refer to Section 3.4.5 for a detailed description of the PBPK model of Sweeney et al. (2010).

For the 2-year drinking water study of Friedman et al. (1995), the model was used for interspecies extrapolation of rat internal dosimetry to humans using the following procedure:

1. The rat model was used to simulate external rat dosages and to predict the corresponding internal dose metric, TWA concentrations of acrylamide and glycidamide in mixed venous blood, where TWA is calculated as follows:

$$TWA = \left( \int_{t=0}^{t=i} C_{Blood} \right) \div t_i$$

where $C_{Blood}$ is the mixed venous blood concentration of acrylamide or glycidamide (mM) and $t_i$ is the exposure time (hours).

2. The rat oral doses were assumed to be delivered during a daily 12-hour period.

3. Rat internal doses (blood TWA) were estimated for the 2-year exposure duration (17,250 hours).

4. Rat body weights used in the simulation were the time-weighted average body weights for each dose group calculated from quantitative body weight data provided in Dulak et al. (1989).

5. The human model was used to predict the daily dosage (mg/kg/day) corresponding to the 95% lower confidence limit (BMDL) on the BMD for TWA acrylamide or glycidamide blood concentration in the rat.

6. A body weight of 70 kg was assumed for humans.

7. The daily dose (mg/kg/day) in humans was assumed to be delivered in 12 consecutive hourly doses, separated by 12-hour intervals, 7 days/week for a duration of 366 days (lower end of the chronic MRL duration for humans and sufficient duration to achieve unchanging values for the human dose with increasing exposure duration).

8. Human external doses corresponding to the BMDLs were estimated for the 366-day exposure duration.

Administered dose, predicted corresponding rat blood TWA acrylamide and glycidamide doses, and degenerative sciatic nerve incidence data from the study of Friedman et al. (1995) are shown in Table A-6.

**Table A-6. PBPK Model-Predicted Rat TWA Blood Acrylamide and Glycidamide Doses and Incidences of Degenerative Sciatic Nerve Changes in Male and Female F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years**

| Ingested acrylamide dose (mg/kg/day) | Rat blood TWA acrylamide dose (mM) | Rat blood TWA glycidamide dose (mM) | Number of rats | Number of rats affected |
|---|---|---|---|---|
| Males | | | | |
| 0 | 0 | 0 | 171 | 59 |
| 0.1 | 0.000135 | 0.0000732 | 65 | 21 |
| 0.5 | 0.000675 | 0.000365 | 38 | 13 |
| 2.0 | 0.00268 | 0.00142 | 49 | 26 |
| Females | | | | |
| 0 | 0 | 0 | 80 | 19 |
| 1.0 | 0.00119 | 0.000652 | 20 | 2 |
| 3.0 | 0.00358 | 0.00191 | 86 | 38 |

PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

Source: Friedman et al. 1995

The chronic-duration oral MRL for acrylamide was derived using a BMD modeling approach. All dichotomous models in the EPA Benchmark Dose Software (Version 2.1.2) were fit to the incidence data for degenerative sciatic nerve changes in the male and female F344 rats of the principal study (Friedman et al. 1995) using predicted rat blood TWA acrylamide as the dose metric and also using rat blood TWA glycidamide as the dose metric. A BMR of 10% extra risk was selected for initial BMD modeling. Adequate model fit was judged by three criteria: $X^2$ goodness-of-fit p-value ($p>0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Comparing across models within a particular dataset, the best fit to the data is generally indicated by the model with the lowest AIC when BMDLs for all models providing adequate fit to the data differ

from one another by <2–3-fold; otherwise, the model with the lowest BMDL is selected as the best-fitting model if it is not considered to be an outlier. The best-fitting model for each dataset was then fit to the incidence data for degenerative sciatic nerve changes using a BMR of 5% extra risk because sufficient numbers of rats were used (≥38/dose group with the exception of 20 for the 1.0 mg/kg/day group of female rats). The BMD modeling results for the male and female rats using blood TWA acrylamide as the dose metric are presented in Tables A-7 and A-8, respectively. The BMD modeling results for the male and female rats using blood TWA glycidamide as the dose metric are presented in Tables A-9 and A-10, respectively.

**Table A-7. Benchmark Results for PBPK Model-Predicted Rat Blood TWA Acrylamide and Incidence of Degenerative Changes in Sciatic Nerve Preparations from Male F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years in the Study of Friedman et al. (1995)**

| | $\chi^2$ | Scaled residuals[b] | | | | Rat blood TWA acrylamide dose (mM)[c] | |
|---|---|---|---|---|---|---|---|
| Model | Goodness of fit p-value[a] | Dose below BMD | Dose above BMD | Overall largest | AIC | $BMD_{10}$ | $BMDL_{10}$ |
| Gamma[d] | 0.75 | 0.002 | 0.00 | -0.271 | 424.82 | 0.00174413 | 0.000493189 |
| Logistic | 0.80 | -0.45 | 0.12 | -0.45 | 423.16 | 0.000982251 | 0.000613461 |
| LogLogistic[e] | 0.75 | 0.004 | 0.00 | -0.272 | 424.82 | 0.00180899 | 0.000411211 |
| LogProbit[e] | 0.75 | 0.00 | 0.00 | -0.271 | 424.82 | 0.00163947 | 0.000816335 |
| Multistage (1-degree)[f] | 0.75 | -0.54 | 0.18 | -0.54 | 423.29 | 0.000868289 | 0.000471031 |
| Multistage (2-degree)[f] | 0.94 | -0.12 | 0.01 | -0.26 | 422.84 | 0.00148538 | 0.000492016 |
| ***Multistage (3-degree)[f,g]*** | ***0.95*** | ***-0.005*** | ***0.00*** | ***-0.27*** | ***422.82*** | ***0.00180927*** | ***0.000493176*** |
| Probit | 0.80 | -0.46 | 0.12 | -0.46 | 423.16 | 0.000974583 | 0.000607024 |
| Weibull[d] | 0.75 | 0.004 | 0.00 | -0.27 | 424.82 | 0.00185484 | 0.000493183 |
| | | | | | | $BMD_{05}$ | $BMDL_{05}$ |
| ***Multistage (3-degree)[f,g]*** | ***0.95*** | ***-0.005*** | ***0.00*** | ***-0.27*** | ***422.82*** | ***0.0014233*** | ***0.000240096*** |

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Rat blood TWA acrylamide doses predicted by the PBPK model of Sweeney et al. (2010) based on oral doses estimated by Friedman et al. (1995).
[d]Power restricted to ≥1.
[e]Slope restricted to ≥1.
[f]Betas restricted to ≥0.
[g]Selected model. All models provided adequate fit to the data as judged by $X^2$ goodness-of-fit p-value (p>0.1), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. BMDLs for models providing adequate fit differed by <2–3-fold; therefore, the model with the lowest AIC was selected as the best-fitting model (Multistage 3-degree).

AIC = Akaike's Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: e.g., $_{10}$ = exposure concentration associated with 10% extra risk); PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

## Table A-8. Benchmark Results for PBPK Model-Predicted Rat Blood TWA Glycidamide and Incidence of Degenerative Changes in Sciatic Nerve Preparations from Male F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years in the Study of Friedman et al. (1995)

| | $\chi^2$ | Scaled residuals[b] | | | | Rat blood TWA glycidamide dose (mM)[c] | |
|---|---|---|---|---|---|---|---|
| Model | Goodness of fit p-value[a] | Dose below BMD | Dose above BMD | Overall largest | AIC | $BMD_{10}$ | $BMDL_{10}$ |
| Gamma[d] | 0.75 | 0.002 | 0.00 | -0.27 | 424.82 | 0.000929055 | 0.000262417 |
| Logistic | 0.80 | -0.46 | 0.12 | -0.46 | 423.17 | 0.000521154 | 0.000325441 |
| LogLogistic[e] | 0.75 | 0.003 | 0.00 | -0.27 | 424.82 | 0.000963337 | 0.000219382 |
| LogProbit[e] | 0.75 | 0.00 | 0.00 | -0.27 | 424.82 | 0.000874854 | 0.000433150 |
| Multistage (1-degree)[f] | 0.75 | -0.55 | 0.19 | 0.55 | 423.31 | 0.000461251 | 0.000250180 |
| Multistage (2-degree)[f] | 0.94 | -0.13 | 0.01 | -0.25 | 422.84 | 0.000787078 | 0.000261731 |
| ***Multistage (3-degree)[f,g]*** | ***0.95*** | ***-0.008*** | ***0.00*** | ***-0.27*** | ***422.82*** | ***0.000958606*** | ***0.000262409*** |
| Probit | 0.80 | -0.47 | 0.12 | -0.47 | 423.18 | 0.000517105 | 0.000322016 |
| Weibull[d] | 0.75 | 0.004 | 0.00 | -0.27 | 424.82 | 0.000987529 | 0.000262414 |
| | | | | | | $BMD_{05}$ | $BMDL_{05}$ |
| ***Multistage (3-degree)[f,g]*** | ***0.95*** | ***-0.008*** | ***0.00*** | ***-0.27*** | ***422.82*** | ***0.00075411*** | ***0.00012775*** |

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Rat blood TWA glycidamide doses predicted by the PBPK model of Sweeney et al. (2010) based on oral doses estimated by Friedman et al. (1995).
[d]Power restricted to ≥1.
[e]Slope restricted to ≥1.
[f]Betas restricted to ≥0.
[g]Selected model. All models provided adequate fit to the data as judged by $X^2$ goodness-of-fit p-value (p>0.1), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. BMDLs for models providing adequate fit differed by <2–3-fold; therefore, the model with the lowest AIC was selected as the best-fitting model (Multistage 3-degree).

AIC = Akaike's Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: e.g., $_{10}$ = exposure concentration associated with 10% extra risk); PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

## Table A-9. Benchmark Results for PBPK Model-Predicted Rat Blood TWA Acrylamide and Incidence of Degenerative Changes in Sciatic Nerve Preparations from Female F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years in the Study of Friedman et al. (1995)

| | $\chi^2$ | Scaled residuals[b] | | | | Rat blood TWA acrylamide dose (mM)[c] | |
|---|---|---|---|---|---|---|---|
| Model | Goodness of fit p-value[a] | Dose below BMD | Dose above BMD | Overall largest | AIC | $BMD_{10}$ | $BMDL_{10}$ |
| ***Gamma[d,e]*** | ***0.18*** | ***-1. 21*** | ***0.00*** | ***-1.21*** | ***224.85*** | ***0.00296422*** | ***0.00110863*** |
| Logistic | 0.06 | -1.73 | 0.25 | -0.73 | 226.85 | 0.00146242 | 0.00109037 |
| LogLogistic[f] | NA | -1.21 | 0.00 | -1.21 | 226.85 | 0.00324757 | 0.00111234 |
| LogProbit[f] | NA | -1.21 | 0.00 | -1.21 | 226.85 | 0.00301355 | 0.00139081 |
| Multistage (1-degree)[g] | 0.05 | 0.51 | 0.40 | -1.87 | 227.45 | 0.00123757 | 0.00077607 |
| Multistage (2-degree)[g] | 0.11 | -1.46 | 0.12 | -1.46 | 225.68 | 0.00200634 | 0.00092149 |
| Probit | 0.06 | -1.75 | 0.26 | -1.75 | 226.92 | 0.00143256 | 0.00105280 |
| Weibull[d] | NA | -1.21 | 0.00 | -1.21 | 226.85 | 0.00327830 | 0.00110853 |
| | | | | | | $BMD_{05}$ | $BMDL_{05}$ |
| ***Gamma[d,e]*** | ***0.18*** | ***-1.21*** | ***0.00*** | ***-1.21*** | ***224.85*** | ***0.00268972*** | ***0.000542603*** |

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Rat blood TWA acrylamide doses predicted by the PBPK model of Sweeney et al. (2010) based on oral doses estimated by Friedman et al. (1995).
[d]Power restricted to ≥1.
[e]Selected model. The only models that provided adequate fit to the data were the Gamma and Multistage 2-degree ($\chi^2$ goodness-of-fit p-value >0.1). BMDLs for models providing adequate fit differed by <2–3-fold; therefore, the model with the lowest AIC was selected as the best-fitting model (Gamma).
[f]Slope restricted to ≥1.
[g]Betas restricted to ≥0.

AIC = Akaike's Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: e.g., $_{10}$ = exposure concentration associated with 10% extra risk); PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

## Table A-10. Benchmark Results for PBPK Model-Predicted Rat Blood TWA Glycidamide and Incidence of Degenerative Changes in Sciatic Nerve Preparations from Female F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years in the Study of Friedman et al. (1995)

| Model | $\chi^2$ Goodness of fit p-value[a] | Scaled residuals[b] Dose below BMD | Dose above BMD | Overall largest | AIC | Rat blood TWA glycidamide dose (mM)[c] $BMD_{10}$ | $BMDL_{10}$ |
|---|---|---|---|---|---|---|---|
| ***Gamma[d,e]*** | ***0.18*** | ***-1.21*** | ***0.00*** | ***-1.21*** | ***224.85*** | ***0.001581470*** | ***0.000608521*** |
| Logistic | 0.06 | -1.74 | 0.26 | -0.74 | 226.91 | 0.000781896 | 0.000582625 |
| LogLogistic[f] | NA | -1.21 | 0.00 | -1.21 | 226.85 | 0.001736800 | 0.000610443 |
| LogProbit[f] | NA | -1.21 | 0.00 | -1.21 | 226.85 | 0.001612570 | 0.000747637 |
| Multistage (1-degree)[g] | 0.05 | 0.51 | 0.41 | -1.89 | 227.52 | 0.000663165 | 0.000415425 |
| Multistage (2-degree)[g] | 0.11 | -1.47 | 0.13 | -1.47 | 225.73 | 0.001071610 | 0.000495119 |
| Probit | 0.06 | -1.76 | 0.27 | -1.76 | 226.98 | 0.000765892 | 0.000562600 |
| Weibull[d] | NA | -1.21 | 0.00 | -1.21 | 226.85 | 0.001752310 | 0.000608403 |
| | | | | | | $BMD_{05}$ | $BMDL_{05}$ |
| ***Gamma[d,e]*** | ***0.18*** | ***-1.21*** | ***0.00*** | ***-1.21*** | ***224.85*** | ***0.00143502*** | ***0.000302968*** |

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Rat blood glycidamide doses predicted by the PBPK model of Sweeney et al. (2010) based on oral doses estimated by Friedman et al. (1995).
[d]Power restricted to ≥1.
[e]Selected model. The only models that provided adequate fit to the data were the Gamma and Multistage 2-degree ($\chi^2$ goodness-of-fit p-value >0.1). BMDLs for models providing adequate fit differed by <2–3-fold; therefore, the model with the lowest AIC was selected as the best-fitting model (Gamma).
[f]Slope restricted to ≥1.
[g]Betas restricted to ≥0.

AIC = Akaike's Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: e.g., $_{10}$ = exposure concentration associated with 10% extra risk); PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

The best-fitting model results for incidence of light microscope-detected degenerative sciatic nerve changes in the male and female F344 rats from the principal study (Friedman et al. 1995) using rat blood TWA acrylamide as the dose metric and rat blood TWA glycidamide as the dose metric are summarized in Table A-11. The human PBPK model (Sweeney et al. 2010) model was then used to predict the daily HED (in mg/kg/day) corresponding to the 95% lower confidence limit ($BMDL_{10}$ and $BMDL_{05}$) on the $BMD_{10}$ and $BMD_{05}$ for PBPK model-predicted rat blood TWA acrylamide dose and for PBPK model-predicted rat blood TWA glycidamide dose. The corresponding PBPK model-predicted rat external doses and HEDs are also presented in Table A-11.

## Table A-11. Summary of BMDL Values and Corresponding Rat External Doses and HEDs from the Best-Fitting Models for Incidences of Degenerative Changes in Sciatic Nerves Using Rat PBPK Model-Predicted Blood TWA Acrylamide as the Dose Metric and Glycidamide as the Dose Metric for the Male and Female F344 Rats from the Principal Study (Friedman et al. (1995)

| Parameter | Rat blood TWA acrylamide-based BMDL (mM) | Rat dose[a] (mg/kg/day) | HED (mg/kg/day) | Rat blood TWA glycidamide-based BMDL (mM) | Rat dose[a] (mg/kg/day) | HED (mg/kg/day) |
|---|---|---|---|---|---|---|
| Male rat $BMDL_{10}$ | 0.000493176 | 0.37 | 0.085 | 0.000262417 | 0.36 | 0.49 |
| Female rat $BMDL_{10}$ | 0.00110863 | 0.93 | 0.19 | 0.000608521 | 0.93 | 1.17 |
| Male rat $BMDL_{05}$ | 0.000240096 | 0.18 | 0.042 | 0.00012775 | 0.18 | 0.24 |
| Female rat $BMDL_{05}$ | 0.000542603 | 0.46 | 0.094 | 0.000302968 | 0.46 | 0.57 |

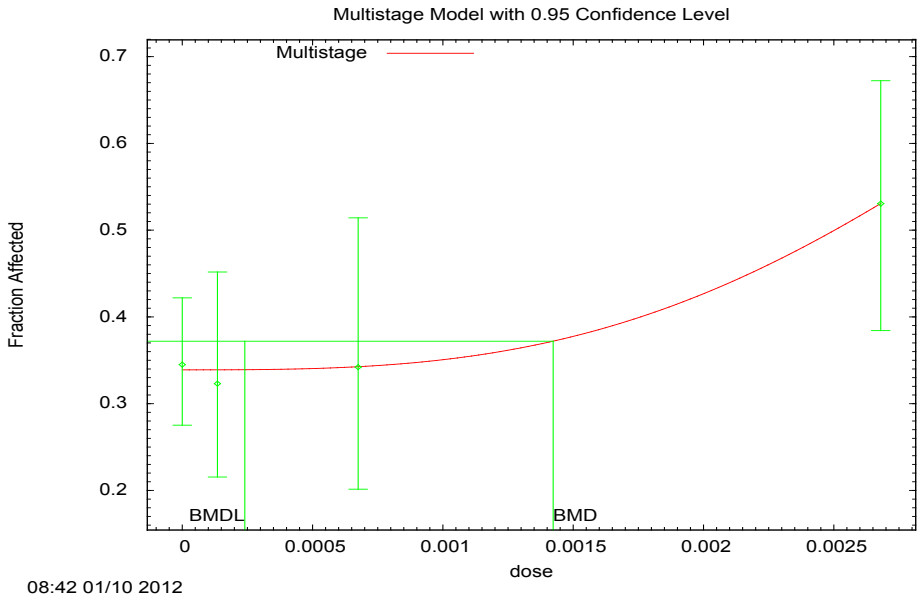
[a]Rat dose is the PBPK model-predicted dose of acrylamide corresponding to the PBPK model-predicted HED at the BMD-predicted rat blood TWA acrylamide- or glycidamide-based BMDL.

BMDL = 95% lower confidence limit on the benchmark dose (BMD), which is the maximum likelihood estimate of the exposure concentration associated with the selected benchmark response (subscripts denote benchmark response: e.g., $_{05}$ = exposure concentration associated with 5% extra risk); HED = human equivalent dose; TWA = time-weighted average

As stated previously, a BMR of 5% extra risk is justified because the principal study (Friedman et al. 1995) used sufficient numbers of animals. Comparing the PBPK model-predicted HEDs for the male and female rats of the principal study using blood TWA acrylamide as the dose metric and blood TWA glycidamide as the dose metric, the lowest HED is 0.042 mg/kg/day based on PBPK model-predicted blood TWA acrylamide ($BMDL_{05}$ of 0.000240096 mM) for the male rats. The HED of 0.042 mg/kg/day was selected as the POD for deriving a chronic-duration oral MRL for acrylamide because it represents the most public health protective POD. The HED of 0.042 mg/kg/day was divided by a total uncertainty factor of 30 (3 for interspecies extrapolation using a PBPK model and 10 for human variability), resulting in a chronic-duration oral MRL of 0.001 mg/kg/day for acrylamide. An uncertainty factor of 10 for human variability is justified based on findings that key metabolic enzymes for acrylamide are CYP2E1, GST, and microsomal EH for which human polymorphisms are known (Huang et al. 2011a) and wide variation in human CYP2E1 expression as reviewed by Bolt et al. (2003).

Figure A-7 shows the plotted results from the best-fitting model (Multistage 3-degree; Table A-7) for PBPK model-predicted blood TWA acrylamide and degenerative sciatic nerve changes in the male rats from the study of Friedman et al. (1995).

**Figure A-7. Fit of Multistage (3-degree) Benchmark Dose Model to Data on the Incidence of Degenerative Peripheral Nerve Changes in Male F344 Rats Exposed to Acrylamide in the Drinking Water for up to 2 Years Using Time-Weighted Average Acrylamide Blood Concentration (mM) as the Dose Metric and a BMR of 5% Extra Risk**




Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[x] 3 for extrapolation from animals to humans using dosimetric adjustment
[x] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information that lend support to this MRL: Two other chronic toxicity and carcinogenicity drinking water studies that employed male and female F344 rats (Johnson et al. 1986; NTP 2011b) reported acrylamide-induced degenerative peripheral nerve changes at doses in the same range as those eliciting degenerative sciatic nerve changes in the rats of the principal study (Friedman et al. 1995). The incidence data for degenerative peripheral nerve changes in the rats from these additional studies were subjected to the same PBPK modeling and BMD analysis as those employed using results from the principal study (Friedman et al. 1995). Relevant study details and results of PBPK modeling and BMD modeling of the data from the studies of Johnson et al. (1986) and NTP (2011b) follow.

In the chronic toxicity and carcinogenicity study of Johnson et al. (1986), groups of F344 rats (90/sex/treatment group) were exposed acrylamide in the drinking water at concentrations calculated to

provide doses of 0, 0.01, 0.1, 0.5, or 2.0 mg/kg-day for up to 2 years. Ten rats/sex/treatment group were randomly selected for interim sacrifices after 6, 12, or 18 months of treatment. The remaining rats (60/sex/treatment group) were subjected to comprehensive histopathological evaluations at death or terminal sacrifice. The evaluations included light and electron microscope examinations of three separate peripheral nerves (tibial nerve and two unspecified nerves), three locations of the spinal cord, and six sections through the brain and olfactory bulbs.

Light microscopic examination of peripheral nerve section revealed degenerative changes that consisted of focal swelling of individual nerve fibers with fragmentation of the myelin and axon and formation of vacuoles containing small round eosinophilic globules and macrophages. The study authors graded nerve degeneration as very slight, slight, moderate, or severe but did not further characterize the grading scheme. "Minimal" tibial nerve degeneration was observed in control and all treated groups beginning at the 12-month necropsy. Incidences of nerve degeneration increased in controls and treated groups alike throughout the remainder of the treatment period. Table A-12 summarizes the light microscopic findings in tibial nerve sections of the groups of rats from the main study that were treated for up to 2 years. Pairwise comparisons between acrylamide-treated groups and controls revealed no significant differences regarding incidences of histopathologic nerve lesions in any group of treated males or females at treatment levels <2.0 mg/kg/day. A statistically significant increase in pooled incidence of slight-to-moderate degeneration was noted in tibial nerves for 2.0 mg/kg/day females.

**Table A-12. Incidence Data for Degenerative Changes in Tibial Nerve Preparations from F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years**

| Severity of degenerative change | Dose (mg/kg/day) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.01 | 0.1 | 0.5 | 2.0 |
| Males | | | | | |
| Very slight | 30/60 | 29/60 | 23/60 | 25/60 | 19/60 |
| Slight | 19/60 | 22/60 | 21/60 | 19/60 | 21/60 |
| Moderate | 8/60 | 5/60 | 12/60 | 13/60 | 12/60 |
| Severe | 1/60 | 1/60 | 0/60 | 0/60 | 4/60 |
| Moderate + severe[a] | 9/60 | 6/60 | 12/60 | 13/60 | 16/60 |
| Females | | | | | |
| Very slight | 45/60 | 43/60 | 45/60 | 42/60 | 37/61 |
| Slight | 3/60 | 7/60 | 5/60 | 7/60 | 13/61 |
| Moderate | 0/60 | 0/60 | 0/60 | 0/60 | 3/61 |
| Slight + moderate | 3/60 | 7/60 | 5/60 | 7/60 | 16/61[b] |

[a]Statistically significant trend for increased incidence with increasing dose by Mantel-Haenszel extension of the Cochran-Armitage test; p<0.01.
[b]Significantly different from control incidence by Fisher's exact test performed by SRC, Inc.; p<0.01.

Source: Johnson et al. 1986

Electron microscopic examinations of peripheral nerve sections from rats in the groups destined for independent neuropathologic assessment revealed slightly increased incidences of axolemma invaginations in 2.0 mg/kg-day male (but not female) rats, relative to controls, at 3- and 6-month interim sacrifices. There were no indications of treatment-related degenerative effects at lower treatment levels. At 12-month interim examination, degenerative myelin and axonal changes were observed in controls as well as all treatment groups and were considered to be the result of aging. High background incidences of

degenerative changes at 18 and 24 months precluded the usefulness of electron microscopic analysis to detect differences between control and exposed groups.

The chronic study of Johnson et al. (1986) identified a NOAEL of 0.5 mg/kg/day and a LOAEL of 2.0 mg/kg/day for significantly increased incidences of degenerative tibial nerve changes in the female rats. Although the male rats exhibited a significant trend for increasing incidences of degenerative tibial nerve changes with increasing dose, incidences in the acrylamide-treated males were not significantly different from the incidence in the control males.

The PBPK model-predicted rat blood TWA acrylamide and glycidamide doses and corresponding incidence data for degenerative tibial nerve changes in male and female F344 rats are shown in Table A-13. BMD modeling results from the study of Johnson et al. (1986) using blood TWA acrylamide as the dose metric and using blood TWA glycidamide as the dose metric are presented in Tables A-14 and A-15, respectively for the male rats, and Tables A-16 and A-17, respectively, for the female rats.

**Table A-13. PBPK Model-Predicted Rat TWA Blood Acrylamide and Glycidamide Doses and Incidences of Degenerative Tibial Nerve Changes in Male and Female F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years**

| Ingested acrylamide dose (mg/kg/day) | Rat blood TWA acrylamide dose (mM) | Rat blood TWA glycidamide dose (mM) | Number of rats | Number of rats affected[a] |
|---|---|---|---|---|
| Males | | | | |
| 0 | 0 | 0 | 60 | 9 |
| 0.01 | 0.0000135 | 0.00000737 | 60 | 6 |
| 0.1 | 0.000135 | 0.0000735 | 60 | 12 |
| 0.5 | 0.000678 | 0.000366 | 60 | 13 |
| 2.0 | 0.00273 | 0.00144 | 60 | 16 |
| Females | | | | |
| 0 | 0 | 0 | 60 | 3 |
| 0.01 | 0.0000135 | 0.00000652 | 60 | 7 |
| 0.1 | 0.000135 | 0.0000656 | 60 | 5 |
| 0.5 | 0.000678 | 0.000325 | 60 | 7 |
| 2.0 | 0.00273 | 0.00128 | 61 | 16 |

[a]Incidences in males are for the combined severity categories of moderate+severe; incidences in females are for the combined severity categories of slight+moderate.

PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

Source: Johnson et al. 1986

## Table A-14. Benchmark Results for PBPK Model-Predicted Rat Blood TWA Acrylamide and Incidence of Degenerative Changes in Tibial Nerve Preparations from Male F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years in the Study of Johnson et al. (1986)

| | $X^2$ | Scaled residuals[b] | | | | Rat blood TWA acrylamide dose (mM)[c] | |
|---|---|---|---|---|---|---|---|
| Model | Goodness of fit p-value[a] | Dose below BMD | Dose above BMD | Overall largest | AIC | $BMD_{10}$ | $BMDL_{10}$ |
| Gamma[d] | 0.48 | 0.60 | -0.25 | -1.15 | 288.66 | 0.00175067 | 0.000880355 |
| Logistic | 0.44 | 0.70 | -0.18 | -1.21 | 288.89 | 0.00201959 | 0.00123132 |
| ***LogLogistic[e,f]*** | ***0.49*** | ***0.56*** | ***-0.27*** | ***-1.13*** | ***288.59*** | ***0.00166439*** | ***0.000774339*** |
| LogProbit[e] | 0.33 | 0.98 | -0.09 | -1.34 | 289.67 | 0.00235132 | 0.00144244 |
| Multistage (1-degree)[g] | 0.48 | 0.60 | -0.25 | -1.15 | 288.66 | 0.00175071 | 0.000880355 |
| Multistage (2-degree)[g] | 0.48 | 0.60 | -0.25 | -1.15 | 288.66 | 0.00175071 | 0.000880355 |
| Multistage (3-degree)[g] | 0.48 | 0.60 | -0.25 | -1.15 | 288.66 | 0.00175071 | 0.000880355 |
| Multistage (4-degree)[g] | 0.48 | 0.60 | -0.25 | -1.15 | 288.66 | 0.00175071 | 0.000880355 |
| Probit | 0.45 | 0.69 | -0.19 | -1.20 | 288.86 | 0.00198498 | 0.00118383 |
| Weibull[d] | 0.48 | 0.60 | -0.25 | -1.15 | 288.66 | 0.00175071 | 0.000880355 |
| | | | | | | $BMD_{05}$ | $BMDL_{05}$ |
| ***LogLogistic[e,f]*** | ***0.49*** | ***0.56*** | ***-0.27*** | ***-1.13*** | ***288.59*** | ***0.000788397*** | ***0.000366792*** |

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Rat blood TWA acrylamide doses predicted by the PBPK model of Sweeney et al. (2010) based on oral doses estimated by Johnson et al. (1986).
[d]Power restricted to ≥1.
[e]Slope restricted to ≥1.
[f]Selected model. All models provided adequate fit to the data as judged by $X^2$ goodness-of-fit p-value (p>0.1), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. BMDLs for models providing adequate fit differed by <2–3-fold; therefore, the model with the lowest AIC was selected as the best-fitting model (LogLogistic).
[g]Betas restricted to ≥0.

AIC = Akaike's Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: e.g., $_{10}$ = exposure concentration associated with 10% extra risk); PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

## Table A-15. Benchmark Results for PBPK Model-Predicted Rat Blood TWA Glycidamide and Incidence of Degenerative Changes in Tibial Nerve Preparations from Male F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years in the Study of Johnson et al. (1986)

| | $\chi^2$ | Scaled residuals[b] | | | | Rat blood TWA glycidamide dose (mM)[c] | |
|---|---|---|---|---|---|---|---|
| Model | Goodness of fit p-value[a] | Dose below BMD | Dose above BMD | Overall largest | AIC | $BMD_{10}$ | $BMDL_{10}$ |
| Gamma[d] | 0.48 | 0.59 | -0.25 | -1.14 | 288.64 | 0.00092035 | 0.000463817 |
| Logistic | 0.45 | 0.69 | -0.18 | -1.21 | 288.87 | 0.00106268 | 0.000649044 |
| ***LogLogistic[e,f]*** | ***0.50*** | ***0.55*** | ***-0.27*** | ***-1.12*** | ***288.57*** | ***0.000874893*** | ***0.000408089*** |
| LogProbit[e] | 0.33 | 0.98 | -0.10 | -1.34 | 289.66 | 0.00123711 | 0.000759144 |
| Multistage (1-degree)[g] | 0.48 | 0.59 | -0.25 | -1.14 | 288.64 | 0.000920351 | 0.000463817 |
| Multistage (2-degree)[g] | 0.48 | 0.59 | -0.25 | -1.14 | 288.64 | 0.000920351 | 0.000463817 |
| Multistage (3-degree)[g] | 0.48 | 0.59 | -0.25 | -1.14 | 288.64 | 0.000920351 | 0.000463817 |
| Multistage (4-degree)[g] | 0.48 | 0.59 | -0.25 | -1.14 | 288.64 | 0.000920351 | 0.000463817 |
| Probit | 0.45 | 0.68 | -0.19 | 0.68 | 288.84 | 0.00104432 | 0.000623954 |
| Weibull[d] | 0.48 | 0.59 | -0.25 | -1.14 | 288.64 | 0.000920351 | 0.000463817 |
| | | | | | | $BMD_{05}$ | $BMDL_{05}$ |
| ***LogLogistic[e,f]*** | ***0.50*** | ***0.55*** | ***-0.27*** | ***-1.12*** | ***288.57*** | ***0.000414423*** | ***0.000193305*** |

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Rat blood TWA glycidamide doses predicted by the PBPK model of Sweeney et al. (2010) based on oral doses estimated by Johnson et al. (1986).
[d]Power restricted to ≥1.
[e]Slope restricted to ≥1.
[f]Selected model. All models provided adequate fit to the data as judged by $X^2$ goodness-of-fit p-value (p>0.1), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. BMDLs for models providing adequate fit differed by <2–3-fold; therefore, the model with the lowest AIC was selected (LogLogistic).
[g]Betas restricted to ≥0.

AIC = Akaike's Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: e.g., $_{10}$ = exposure concentration associated with 10% extra risk); PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

## Table A-16. Benchmark Results for PBPK Model-Predicted Rat Blood TWA Acrylamide and Incidence of Degenerative Changes in Tibial Nerve Preparations from Female F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years in the Study of Johnson et al. (1986)

| | $X^2$ | Scaled residuals[b] | | | | Rat blood TWA acrylamide dose (mM)[c] | |
|---|---|---|---|---|---|---|---|
| Model | Goodness of fit p-value[a] | Dose below BMD | Dose above BMD | Overall largest | AIC | $BMD_{10}$ | $BMDL_{10}$ |
| Gamma[d] | 0.41 | -0.01 | 0.00 | 0.98 | 222.69 | 0.00130613 | 0.000704889 |
| Logistic | 0.62 | 0.10 | -0.02 | 0.95 | 220.69 | 0.00146511 | 0.00110148 |
| LogLogistic[e] | 0.41 | 0.00 | 0.00 | 0.98 | 222.69 | 0.00129772 | 0.000636942 |
| LogProbit[e] | 0.59 | 0.45 | -0.08 | -1.04 | 220.94 | 0.00154931 | 0.00108329 |
| Multistage (1-degree)[f] | 0.59 | -0.23 | 0.09 | 1.08 | 220.75 | 0.00115579 | 0.000701405 |
| Multistage (2-degree)[f] | 0.41 | 0.00 | 0.00 | 0.99 | 222.68 | 0.00135129 | 0.000705558 |
| Multistage (3-degree)[f] | 0.41 | 0.01 | 0.00 | 1.00 | 222.68 | 0.00140517 | 0.000705578 |
| Multistage (4-degree)[f] | 0.41 | 0.01 | 0.00 | 1.00 | 222.68 | 0.00140517 | 0.000705578 |
| ***Probit[g]*** | ***0.62*** | ***0.06*** | ***-0.01*** | ***0.97*** | ***220.68*** | ***0.00141533*** | ***0.0010388*** |
| Weibull[d] | 0.41 | -0.01 | 0.00 | 0.98 | 222.69 | 0.00131215 | 0.000704948 |
| | | | | | | $BMD_{05}$ | $BMDL_{05}$ |
| ***Probit[g]*** | ***0.62*** | ***0.06*** | ***-0.01*** | ***0.97*** | ***220.68*** | ***0.000798991*** | ***0.000586137*** |

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Rat blood TWA acrylamide doses predicted by the PBPK model of Sweeney et al. (2010) based on oral doses estimated by Johnson et al. (1986).
[d]Power restricted to ≥1.
[e]Slope restricted to ≥1.
[f]Betas restricted to ≥0.
[g]Selected model. All models provided adequate fit to the data as judged by $X^2$ goodness-of-fit p-value (p>0.1), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. BMDLs for models providing adequate fit differed by <2–3-fold); therefore, the model with the lowest AIC was selected (Probit).

AIC = Akaike's Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: e.g., $_{10}$ = exposure concentration associated with 10% extra risk); PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

## Table A-17. Benchmark Results for PBPK Model-Predicted Rat Blood TWA Glycidamide and Incidence of Degenerative Changes in Tibial Nerve Preparations from Female F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years in the Study of Johnson et al. (1986)

| | $X^2$ | Scaled residuals[b] | | | | Rat blood TWA glycidamide dose (mM)[c] | |
|---|---|---|---|---|---|---|---|
| Model | Goodness of fit p-value[a] | Dose below BMD | Dose above BMD | Overall largest | AIC | $BMD_{10}$ | $BMDL_{10}$ |
| Gamma[d] | 0.41 | 0.00 | 0.00 | 0.98 | 222.69 | 0.000711912 | 0.000381903 |
| Logistic | 0.62 | 0.09 | -0.02 | 0.95 | 220.69 | 0.00079139 | 0.000595247 |
| LogLogistic[e] | 0.41 | 0.00 | 0.00 | 0.98 | 222.69 | 0.000707432 | 0.000345627 |
| LogProbit[e] | 0.59 | 0.44 | -0.08 | -1.04 | 220.93 | 0.000836071 | 0.000584963 |
| Multistage (1-degree)[f] | 0.59 | -0.24 | 0.10 | 1.08 | 220.76 | 0.000625365 | 0.00037971 |
| Multistage (2-degree)[f] | 0.41 | 0.00 | 0.00 | 0.99 | 222.68 | 0.000736191 | 0.000382278 |
| Multistage (3-degree)[f] | 0.41 | 0.01 | 0.00 | 1.00 | 222.68 | 0.000766549 | 0.000382291 |
| Multistage (4-degree)[f] | 0.41 | 0.01 | 0.00 | 1.00 | 222.68 | 0.000766549 | 0.000382291 |
| ***Probit[g]*** | ***0.62*** | ***0.05*** | ***-0.01*** | ***0.97*** | ***220.68*** | ***0.000764564*** | ***0.000561417*** |
| Weibull[d] | 0.41 | -0.01 | 0.00 | 0.98 | 222.69 | 0.000715356 | 0.000381935 |
| | | | | | | $BMD_{05}$ | $BMDL_{05}$ |
| ***Probit[g]*** | ***0.62*** | ***0.05*** | ***-0.01*** | ***0.97*** | ***220.68*** | ***0.000431712*** | ***0.000316857*** |

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Rat blood TWA glycidamide doses predicted by the PBPK model of Sweeney et al. (2010) based on oral doses estimated by Johnson et al. (1986).
[d]Power restricted to ≥1.
[e]Slope restricted to ≥1.
[f]Betas restricted to ≥0.
[g]Selected model. All models provided adequate fit to the data as judged by $X^2$ goodness-of-fit p-value (p>0.1), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. BMDLs for models providing adequate fit differed by <2–3-fold; therefore, the model with the lowest AIC was selected (Probit).

AIC = Akaike's Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: e.g., $_{10}$ = exposure concentration associated with 10% extra risk); PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

In the chronic toxicity and carcinogenicity study of NTP (2011b), groups of male and female F344 rats 48/sex/group) were exposed to acrylamide in the drinking water for up to 2 years at concentrations resulting in calculated doses of 0, 0.33, 0.66, 1.32, or 2.71 mg/kg/day for the males and 0, 0.44, 0.88, 1.84, or 4.02 mg/kg/day for the females. Complete necropsies were performed on all animals that died, those that were sacrificed moribund, and those that survived to terminal sacrifice. A comprehensive set of tissues was prepared for microscopic evaluation. Special care was taken to assess neurological tissues that included brain (cerebrum, cerebellum, brain stem), sciatic nerve, and spinal cord (cervical, thoracic, lumbar).

Significantly increased incidences of axonal degeneration in the sciatic nerve of the male and female rats were observed. Table A-18 presents the incidence data for axonal degeneration in the sciatic nerves from the rats of the NTP (2011b) study.

**Table A-18. Incidence Data for Axonal Degeneration in Sciatic Nerve Preparations from F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years**

| | Dose (mg/kg/day), males | | | | |
|---|---|---|---|---|---|
| | 0 | 0.33 | 0.66 | 1.32 | 2.71 |
| Lesion incidence, males | 5/48 | 7/48 | 7/48 | 11/48 | 23/48[a] |
| | Dose (mg/kg/day), females | | | | |
| | 0 | 0.44 | 0.88 | 1.84 | 4.02 |
| Lesion incidence, females | 4/48 | 348 | 1/48 | 4/48 | 19/48[a] |

[a]Significantly ($p<0.001$) different from control incidence by Fisher's exact test performed by SRC, Inc.

Source: NTP 2011b

The chronic study of NTP (2011b) identified a NOAEL of 1.32 mg/kg/day and a LOAEL of 2.71 mg/kg/day for significantly increased incidences of axonal degeneration in the sciatic nerve of the male rats; the female rats exhibited a NOAEL of 1.84 mg/kg/day and a LOAEL of 4.02 mg/kg/day.

The PBPK model-predicted rat blood TWA acrylamide and glycidamide doses and corresponding incidence data for degenerative sciatic nerve changes in male and female F344 rats are shown in Table A-19. Benchmark modeling results from the study of NTP (2011b) using blood TWA acrylamide as the dose metric and using blood TWA glycidamide as the dose metric are presented in Tables A-20 and A-21, respectively, for the male rats, and Tables A-22 and A-23, respectively, for the female rats.

## Table A-19. PBPK Model-Predicted Rat TWA Blood Acrylamide and Glycidamide Doses and Incidences of Degenerative Sciatic Nerve Changes in Male and Female F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years

| Ingested acrylamide dose (mg/kg/day) | Rat blood TWA acrylamide dose (mM) | Rat blood TWA glycidamide dose (mM) | Number of rats | Number of rats affected |
|---|---|---|---|---|
| Males | | | | |
| 0 | 0 | 0 | 48 | 5 |
| 0.33 | 0.000454 | 0.000245 | 48 | 7 |
| 0.66 | 0.000912 | 0.000491 | 48 | 7 |
| 1.32 | 0.00182 | 0.000971 | 48 | 11 |
| 2.71 | 0.00377 | 0.00197 | 48 | 23 |
| Females | | | | |
| 0 | 0 | 0 | 48 | 4 |
| 0.44 | 0.000540 | 0.000296 | 48 | 3 |
| 0.88 | 0.00108 | 0.000590 | 48 | 1 |
| 1.84 | 0.00226 | 0.00122 | 48 | 4 |
| 4.02 | 0.00494 | 0.00260 | 48 | 19 |

PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

Source: NTP 2011b

## Table A-20. Benchmark Results for PBPK Model-Predicted Rat Blood TWA Acrylamide and Incidence of Degenerative Changes in Sciatic Nerve Preparations from Male F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years in the Study of NTP (2011b)

| | $X^2$ | Scaled residuals[b] | | | | Rat blood TWA acrylamide dose (mM)[c] | |
|---|---|---|---|---|---|---|---|
| Model | Goodness of fit p-value[a] | Dose below BMD | Dose above BMD | Overall largest | AIC | $BMD_{10}$ | $BMDL_{10}$ |
| Gamma[d] | 0.87 | -0.07 | -0.08 | 0.42 | 236.25 | 0.00156524 | 0.000661132 |
| ***Logistic[e]*** | ***0.98*** | ***-0.27*** | ***-0.14*** | ***0.35*** | ***234.18*** | ***0.00133804*** | ***0.00109874*** |
| LogLogistic[f] | 0.87 | -0.06 | -0.07 | 0.41 | 236.25 | 0.00157891 | 0.000634105 |
| LogProbit[f] | 0.84 | 0.04 | -0.08 | 0.42 | 236.33 | 0.00163506 | 0.00104555 |
| Multistage (1-degree)[g] | 0.65 | 0.17 | -0.66 | 0.72 | 235.64 | 0.00085085 | 0.000600222 |
| Multistage (2-degree)[g] | 0.90 | -0.16 | -0.07 | 0.37 | 236.17 | 0.00148901 | 0.000666046 |
| Multistage (3-degree)[g] | 0.93 | -0.23 | 0.05 | 0.28 | 236.11 | 0.00148479 | 0.000669336 |
| Probit | 0.96 | -0.31 | -0.22 | 0.35 | 234.26 | 0.00126026 | 0.00102925 |
| Weibull[d] | 0.88 | -0.11 | -0.06 | 0.40 | 236.21 | 0.00154616 | 0.000663332 |
| | | | | | | $BMD_{05}$ | $BMDL_{05}$ |
| ***Logistic[e]*** | ***0.98*** | ***-0.27*** | ***-0.14*** | ***0.35*** | ***234.18*** | ***0.000754069*** | ***0.000611743*** |

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Rat blood TWA acrylamide doses predicted by the PBPK model of Sweeney et al. (2010) based on oral doses estimated by NTP (2011b).
[d]Power restricted to ≥1.
[e]Selected model. All models provided adequate fit to the data as judged by $X^2$ goodness-of-fit p-value (p>0.1), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. BMDLs for models providing adequate fit differed by <2–3-fold; therefore, the model with the lowest AIC was selected as the best-fitting model (Logistic).
[f]Slope restricted to ≥1.
[g]Betas restricted to ≥0.

AIC = Akaike's Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: e.g., $_{10}$ = exposure concentration associated with 10% extra risk); NTP = National Toxicology Program; PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

## Table A-21. Benchmark Results for PBPK Model-Predicted Rat Blood TWA Glycidamide and Incidence of Degenerative Changes in Sciatic Nerve Preparations from Male F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years in the Study of NTP (2011b)

| Model | $\chi^2$ Goodness of fit p-value[a] | Scaled residuals[b] Dose below BMD | Dose above BMD | Overall largest | AIC | Rat blood TWA glycidamide dose (mM)[c] $BMD_{10}$ | $BMDL_{10}$ |
|---|---|---|---|---|---|---|---|
| Gamma[d] | 0.86 | -0.06 | -0.08 | 0.42 | 236.26 | 0.000841019 | 0.000352709 |
| ***Logistic[e]*** | ***0.97*** | ***-0.28*** | ***-0.17*** | ***0.35*** | ***234.21*** | ***0.000702251*** | ***0.00057741*** |
| LogLogistic[f] | 0.87 | -0.05 | -0.07 | 0.42 | 236.25 | 0.000847186 | 0.000344671 |
| LogProbit[f] | 0.83 | 0.05 | -0.08 | 0.42 | 236.33 | 0.000876216 | 0.000552102 |
| Multistage (1-degree)[g] | 0.62 | 0.16 | -0.72 | 0.76 | 235.77 | 0.000448601 | 0.000316751 |
| Multistage (2-degree)[g] | 0.90 | -0.16 | -0.09 | 0.34 | 236.18 | 0.000796366 | 0.000355351 |
| Multistage (≥3-degree)[g] | 0.93 | -0.23 | 0.05 | 0.29 | 236.11 | 0.000796521 | 0.000357633 |
| Probit | 0.95 | -0.32 | -0.26 | 0.36 | 234.30 | 0.000661509 | 0.000540898 |
| Weibull[d] | 0.88 | -0.10 | -0.06 | 0.41 | 236.22 | 0.000829981 | 0.000354012 |
| | | | | | | $BMD_{05}$ | $BMDL_{05}$ |
| ***Logistic[e]*** | ***0.97*** | ***-0.28*** | ***-0.17*** | ***0.35*** | ***234.21*** | ***0.000396239*** | ***0.000321782*** |

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Rat blood TWA glycidamide doses predicted by the PBPK model of Sweeney et al. (2010) based on oral doses estimated by NTP (2011b).
[d]Power restricted to ≥1.
[e]Selected model. All models provided adequate fit to the data as judged by $X^2$ goodness-of-fit p-value (p>0.1), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. BMDLs for models providing adequate fit differed by <2–3-fold; therefore, the model with the lowest AIC was selected as the best-fitting model (Logistic).
[f]Slope restricted to ≥1.
[g]Betas restricted to ≥0.

AIC = Akaike's Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: e.g., $_{10}$ = exposure concentration associated with 10% extra risk); NTP = National Toxicology Program; PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

## Table A-22. Benchmark Results for PBPK Model-Predicted Rat Blood TWA Acrylamide and Incidence of Degenerative Changes in Sciatic Nerve Preparations from Female F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years in the Study of NTP (2011b)

| | $\chi^2$ | Scaled residuals[b] | | | | Rat blood TWA acrylamide dose (mM)[c] | |
|---|---|---|---|---|---|---|---|
| Model | Goodness of fit p-value[a] | Dose below BMD | Dose above BMD | Overall largest | AIC | $BMD_{10}$ | $BMDL_{10}$ |
| Gamma[d] | 0.39 | 0.09 | -0.01 | 0.81 | 159.83 | 0.00322124 | 0.00229724 |
| Logistic | 0.16 | -0.78 | 0.37 | 1.63 | 160.82 | 0.00249874 | 0.00208354 |
| LogLogistic[e] | 0.39 | 0.13 | -0.01 | 0.80 | 159.86 | 0.00330861 | 0.00229704 |
| LogProbit[e] | 0.39 | 0.05 | -0.01 | 0.83 | 159.80 | 0.00311592 | 0.0022417 |
| Multistage (1-degree)[f] | 0.01 | -1.83 | -1.45 | -1.83 | 167.54 | 0.00180843 | 0.00123452 |
| Multistage (2-degree)[f] | 0.26 | -0.86 | 0.52 | -1.26 | 160.06 | 0.00254487 | 0.00201577 |
| ***Multistage (3-degree)[f,g]*** | **0.54** | **-0.22** | **0.09** | **0.92** | **158.11** | **0.00307014** | **0.00228873** |
| Multistage (4-degree)[f] | 0.39 | 0.20 | -0.02 | 0.78 | 159.88 | 0.00343689 | 0.00232663 |
| Probit | 0.11 | -0.94 | 0.52 | 1.69 | 161.63 | 0.00233731 | 0.00192611 |
| Weibull[d] | 0.39 | 0.15 | -0.01 | 0.80 | 159.88 | 0.00338563 | 0.00232739 |
| | | | | | | $BMD_{05}$ | $BMDL_{05}$ |
| ***Multistage (3-degree)[f,g]*** | **0.54** | ***-0.22*** | ***0.09*** | ***0.92*** | ***158.11*** | ***0.0024152*** | ***0.00154042*** |

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Rat blood TWA acrylamide doses predicted by the PBPK model of Sweeney et al. (2010) based on oral doses estimated by NTP (2011b).
[d]Power restricted to ≥1.
[e]Slope restricted to ≥1.
[f]Betas restricted to ≥0.
[g]Selected model. All models except the Multistage 1-degree provided adequate fit to the data ($X^2$ goodness-of-fit p-value >0.1). BMDLs for models providing adequate fit differed by <2–3-fold; therefore, the model with the lowest AIC was selected as the best-fitting model (Multistage 3-degree).

AIC = Akaike's Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: e.g., $_{10}$ = exposure concentration associated with 10% extra risk); NTP = National Toxicology Program; PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

## Table A-23. Benchmark Results for PBPK Model-Predicted Rat Blood TWA Glycidamide and Incidence of Degenerative Changes in Sciatic Nerve Preparations from Female F344 Rats Administered Acrylamide in the Drinking Water for up to 2 Years in the Study of NTP (2011b)

| | $\chi^2$ | Scaled residuals[b] | | | | Rat blood TWA glycidamide dose (mM)[c] | |
|---|---|---|---|---|---|---|---|
| Model | Goodness of fit p-value[a] | Dose below BMD | Dose above BMD | Overall largest | AIC | $BMD_{10}$ | $BMDL_{10}$ |
| Gamma[d] | 0.39 | 0.09 | -0.01 | -1.08 | 159.82 | 0.00171592 | 0.00123645 |
| Logistic | 0.15 | -0.82 | 0.39 | 1.67 | 161.02 | 0.00132224 | 0.00110363 |
| LogLogistic[e] | 0.39 | 0.12 | -0.01 | -1.09 | 159.86 | 0.00176227 | 0.00123714 |
| LogProbit[e] | 0.40 | 0.04 | -0.01 | -1.06 | 159.79 | 0.00166352 | 0.00120814 |
| Multistage (1-degree)[f] | 0.01 | -1.85 | -1.47 | -1.85 | 167.89 | 0.000966702 | 0.000659626 |
| Multistage (2-degree)[f] | 0.24 | -0.91 | 0.56 | -1.28 | 160.29 | 0.00134688 | 0.00107179 |
| Multistage (3-degree)[f] | 0.52 | -0.27 | 0.11 | -1.10 | 158.17 | 0.00161831 | 0.00122539 |
| ***Multistage (4-degree)[f,g]*** | ***0.59*** | ***0.16*** | ***-0.01*** | ***-1.10*** | ***157.88*** | ***0.00181181*** | ***0.00125584*** |
| Probit | 0.10 | -0.98 | 0.56 | 1.72 | 161.87 | 0.00123781 | 0.00102087 |
| Weibull[d] | 0.39 | 0.14 | -0.01 | -1.10 | 159.87 | 0.00180181 | 0.00125287 |
| | | | | | | $BMD_{05}$ | $BMDL_{05}$ |
| ***Multistage (4-degree)[f,g]*** | ***0.59*** | ***0.16*** | ***-0.01*** | ***-1.10*** | ***157.88*** | ***0.00151342*** | ***0.000860953*** |

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Rat blood TWA glycidamide doses predicted by the PBPK model of Sweeney et al. (2010) based on oral doses estimated by NTP (2011b).
[d]Power restricted to ≥1.
[e]Slope restricted to ≥1.
[f]Betas restricted to ≥0.
[g]Selected model. All models except the Multistage 1-degree provided adequate fit to the data ($X^2$ goodness-of-fit p-value >0.1). BMDLs for models providing adequate fit differed by <2–3-fold; therefore, the model with the lowest AIC was selected as the best-fitting model (Multistage 4-degree).

AIC = Akaike's Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: e.g., $_{10}$ = exposure concentration associated with 10% extra risk); NTP = National Toxicology Program; PBPK = physiologically based pharmacokinetic; TWA = time-weighted average

The best-fitting model results for incidence of light microscope-detected degenerative peripheral nerve changes in the male and female F344 rats from the studies of Johnson et al. (1986) and NTP (2011b) using rat blood TWA acrylamide as the dose metric and rat blood TWA glycidamide as the dose metric are presented in Table A-24 for comparison. The human PBPK model (Sweeney et al. 2010) model was used to predict the daily human equivalent dose (HED in mg/kg/day) corresponding to the 95% lower confidence limit ($BMDL_{10}$ and $BMDL_{05}$) on the $BMD_{10}$ and $BMD_{05}$ associated with the rat PBPK model-predicted blood TWA acrylamide dose and rat PBPK model-predicted blood TWA glycidamide dose. The corresponding rat external doses and HEDs are also shown in Table A-24.

**Table A-24. Summary of BMDL Values and Corresponding Rat External Doses and HEDs from the Best-Fitting Models for Incidences of Degenerative Changes in Peripheral Nerves Using Rat PBPK Model-Predicted Blood TWA Acrylamide as the Dose Metric and Glycidamide as the Dose Metric for the Male and Female F344 Rats from the Chronic Studies of Johnson et al. (1986) and NTP (2011b)**

| Parameter | Rat blood TWA acrylamide-based BMDL (mM) | Rat dose[a] (mg/kg/day) | HED (mg/kg/day) | Rat blood TWA glycidamide-based BMDL (mM) | Rat dose[a] (mg/kg/day) | HED (mg/kg/day) |
|---|---|---|---|---|---|---|
| Johnson et al. (1986) study | | | | | | |
| Male rat $BMDL_{10}$ | 0.000774339 | 0.57 | 0.13 | 0.000408089 | 0.56 | 0.77 |
| Female rat $BMDL_{10}$ | 0.0010388 | 0.88 | 0.18 | 0.000561417 | 0.86 | 1.07 |
| Male rat $BMDL_{05}$ | 0.000366792 | 0.23 | 0.063 | 0.000193305 | 0.26 | 0.36 |
| Female rat $BMDL_{05}$ | 0.000586137 | 0.50 | 0.10 | 0.000316857 | 0.49 | 0.59 |
| NTP (2011b) study | | | | | | |
| Male rat $BMDL_{10}$ | 0.00109874 | 0.80 | 0.19 | 0.00057741 | 0.78 | 1.10 |
| Female rat $BMDL_{10}$ | 0.00228873 | 1.86 | 0.39 | 0.00125584 | 1.89 | 2.53 |
| Male rat $BMDL_{05}$ | 0.000611743 | 0.44 | 0.11 | 0.000302968 | 0.41 | 0.57 |
| Female rat $BMDL_{05}$ | 0.00154042 | 1.26 | 0.27 | 0.000860953 | 1.29 | 1.68 |

[a]Rat dose is the PBPK model-predicted dose of acrylamide corresponding to the PBPK model-predicted HED at the BMD-predicted rat blood TWA acrylamide- or glycidamide-based BMDL.

BMDL = 95% lower confidence limit on the benchmark dose (BMD) which is the maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; (subscripts denote benchmark response: e.g., $_{05}$ = exposure concentration associated with 5% extra risk); HED = human equivalent dose; NTP = National Toxicology Program; TWA = time-weighted average

Figure A-8 shows the plotted results from the best-fitting model (Log-logistic; Table A-14) for PBPK model-predicted blood TWA acrylamide and degenerative tibial nerve changes in the male rats from the study of Johnson et al. (1986) using a BMR of 5% extra risk.

**Figure A-8. Fit of Logistic Model to Data on the Incidence of Degenerative Tibial Nerve Changes in Male F344 Rats Exposed to Acrylamide in the Drinking Water for up to 2 Years Using Time-Weighted Average Acrylamide Blood Dose (mM) as the Dose Metric and a Benchmark Response of 5% Extra Risk in the Study of Johnson et al. (1986)**

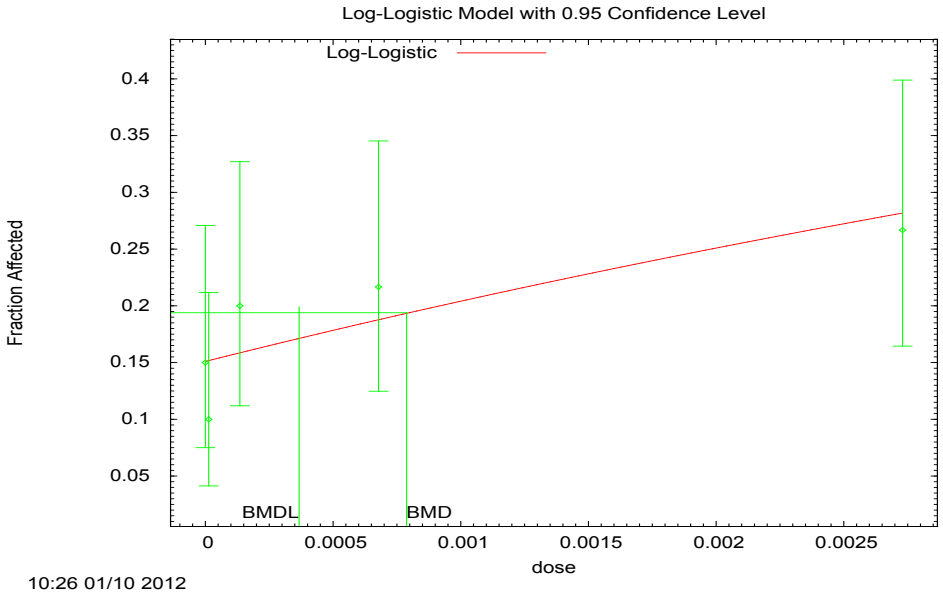
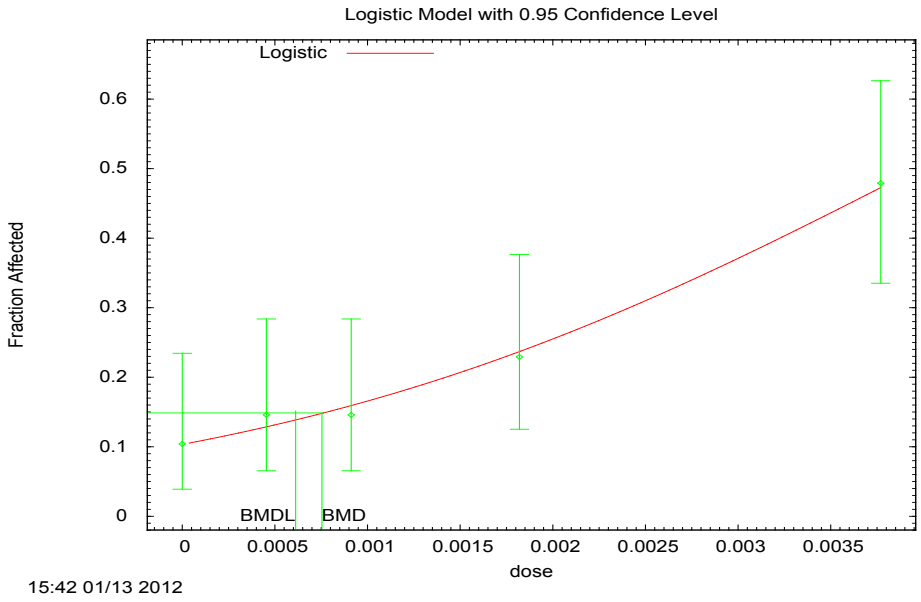



Figure A-9 shows the plotted results from the best-fitting model (Logistic; Table A-20) for PBPK model-predicted blood TWA acrylamide and degenerative sciatic nerve changes in the male rats from the study of NTP (2011b) using a BMR of 5% extra risk.

**Figure A-9. Fit of Log-logistic Model to Data on the Incidence of Degenerative Sciatic Nerve Changes in Male F344 Rats Exposed to Acrylamide in the Drinking Water for up to 2 Years Using Time-Weighted Average Acrylamide Blood Dose (mM) as the Dose Metric and a Benchmark Response of 5% Extra Risk in NTP (2011b)**

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# APPENDIX B. USER'S GUIDE

## Chapter 1

### Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public, especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

## Chapter 2

### Relevance to Public Health

This chapter provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The chapter covers end points in the same order that they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, and dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal Risk Levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

## Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, ATSDR has derived MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables.

## Chapter 3

## Health Effects

## Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, MRLs to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

**LEGEND**

**See Sample LSE Table 3-1 (page B-6)**

(1) Route of Exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Tables 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures.

(2) Exposure Period. Three exposure periods—acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more)—are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect. The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in sample Figure 3-1).

(5) Species. The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration. The duration of the study and the weekly and daily exposure regimens are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to “Chemical x” via inhalation for 6 hours/day, 5 days/week, for 13 weeks. For a more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Nitschke et al. 1981).

(7) System. This column further defines the systemic effects. These systems include respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered

in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system, which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less Serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference. The complete reference citation is given in Chapter 9 of the profile.

(11) CEL. A CEL is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates that the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

**LEGEND**

**See Sample Figure 3-1 (page B-7)**

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the acute and intermediate exposure periods are illustrated.

(14) Health Effect. These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in $mg/m^3$ or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL. In this example, the open circle designated 18r identifies a NOAEL critical end point in the rat upon which an intermediate inhalation exposure MRL is based. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL. Key number 38m is one of three studies for which CELs were derived. The diamond symbol refers to a CEL for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels. This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels ($q_1$*).

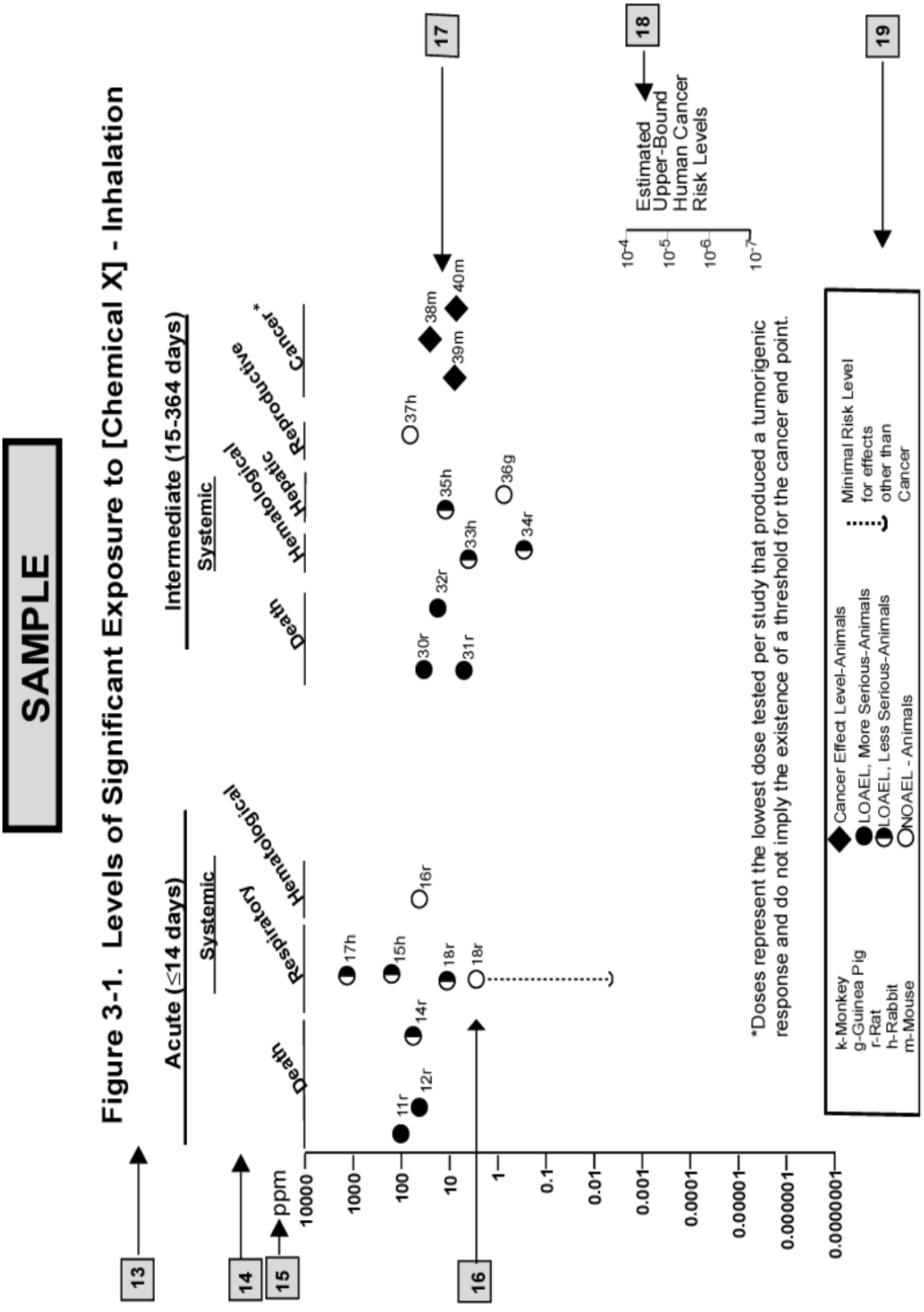
(19) Key to LSE Figure. The Key explains the abbreviations and symbols used in the figure.

**SAMPLE**

1 → **Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

| | Key to figure[a] | Species | Exposure frequency/ duration | System | NOAEL (ppm) | LOAEL (effect) Less serious (ppm) | LOAEL (effect) Serious (ppm) | Reference |
|---|---|---|---|---|---|---|---|---|
| 2 → | INTERMEDIATE EXPOSURE | | | | | | | |
| | | 5 | 6 | 7 | 8 | 9 | | 10 |
| 3 → | Systemic | ↓ | ↓ | ↓ | ↓ | ↓ | | ↓ |
| 4 → | 18 | Rat | 13 wk<br>5 d/wk<br>6 hr/d | Resp | 3[b] | 10 (hyperplasia) | | Nitschke et al. 1981 |
| | CHRONIC EXPOSURE | | | | | | | |
| | Cancer | | | | | 11 | | |
| | | | | | | ↓ | | |
| | 38 | Rat | 18 mo<br>5 d/wk<br>7 hr/d | | | 20 | (CEL, multiple organs) | Wong et al. 1982 |
| | 39 | Rat | 89–104 wk<br>5 d/wk<br>6 hr/d | | | 10 | (CEL, lung tumors, nasal tumors) | NTP 1982 |
| | 40 | Mouse | 79–103 wk<br>5 d/wk<br>6 hr/d | | | 10 | (CEL, lung tumors, hemangiosarcomas) | NTP 1982 |

12 → [a] The number corresponds to entries in Figure 3-1.
[b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of $5x10^{-3}$ ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).


SAMPLE
Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation
Acute (≤14 days)
Intermediate (15-364 days)
Systemic
Death
Respiratory
Hematological
Hepatic
Reproductive
Cancer *
ppm
10000
1000
100
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
0.0000001
11r
12r
14r
15h
16r
17h
18r
18r
30r
31r
32r
33h
34r
35h
36g
37h
38m
39m
40m
Estimated Upper-Bound Human Cancer Risk Levels
$10^{-4}$
$10^{-5}$
$10^{-6}$
$10^{-7}$
*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer end point.
k-Monkey
g-Guinea Pig
r-Rat
h-Rabbit
m-Mouse
Cancer Effect Level-Animals
LOAEL, More Serious-Animals
LOAEL, Less Serious-Animals
NOAEL - Animals
Minimal Risk Level for effects other than Cancer
13
14
15
16
17
18
19

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# APPENDIX C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

| | |
|---|---|
| ACGIH | American Conference of Governmental Industrial Hygienists |
| ACOEM | American College of Occupational and Environmental Medicine |
| ADI | acceptable daily intake |
| ADME | absorption, distribution, metabolism, and excretion |
| AED | atomic emission detection |
| AFID | alkali flame ionization detector |
| AFOSH | Air Force Office of Safety and Health |
| ALT | alanine aminotransferase |
| AML | acute myeloid leukemia |
| AOAC | Association of Official Analytical Chemists |
| AOEC | Association of Occupational and Environmental Clinics |
| AP | alkaline phosphatase |
| APHA | American Public Health Association |
| AST | aspartate aminotransferase |
| atm | atmosphere |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| AWQC | Ambient Water Quality Criteria |
| BAT | best available technology |
| BCF | bioconcentration factor |
| BEI | Biological Exposure Index |
| BMD/C | benchmark dose or benchmark concentration |
| $BMD_X$ | dose that produces a X% change in response rate of an adverse effect |
| $BMDL_X$ | 95% lower confidence limit on the $BMD_X$ |
| BMDS | Benchmark Dose Software |
| BMR | benchmark response |
| BSC | Board of Scientific Counselors |
| C | centigrade |
| CAA | Clean Air Act |
| CAG | Cancer Assessment Group of the U.S. Environmental Protection Agency |
| CAS | Chemical Abstract Services |
| CDC | Centers for Disease Control and Prevention |
| CEL | cancer effect level |
| CELDS | Computer-Environmental Legislative Data System |
| CERCLA | Comprehensive Environmental Response, Compensation, and Liability Act |
| CFR | Code of Federal Regulations |
| Ci | curie |
| CI | confidence interval |
| CL | ceiling limit value |
| CLP | Contract Laboratory Program |
| cm | centimeter |
| CML | chronic myeloid leukemia |
| CPSC | Consumer Products Safety Commission |
| CWA | Clean Water Act |
| DHEW | Department of Health, Education, and Welfare |
| DHHS | Department of Health and Human Services |
| DNA | deoxyribonucleic acid |
| DOD | Department of Defense |
| DOE | Department of Energy |
| DOL | Department of Labor |

| | |
|---|---|
| DOT | Department of Transportation |
| DOT/UN/ NA/IMDG | Department of Transportation/United Nations/ North America/Intergovernmental Maritime Dangerous Goods Code |
| DWEL | drinking water exposure level |
| ECD | electron capture detection |
| ECG/EKG | electrocardiogram |
| EEG | electroencephalogram |
| EEGL | Emergency Exposure Guidance Level |
| EH | epoxide hydrolase |
| EPA | Environmental Protection Agency |
| F | Fahrenheit |
| $F_1$ | first-filial generation |
| FAO | Food and Agricultural Organization of the United Nations |
| FDA | Food and Drug Administration |
| FEMA | Federal Emergency Management Agency |
| FIFRA | Federal Insecticide, Fungicide, and Rodenticide Act |
| FPD | flame photometric detection |
| fpm | feet per minute |
| FR | Federal Register |
| FSH | follicle stimulating hormone |
| g | gram |
| GC | gas chromatography |
| gd | gestational day |
| GLC | gas liquid chromatography |
| GPC | gel permeation chromatography |
| GST | glutathione S-transferase |
| HPLC | high-performance liquid chromatography |
| HRGC | high resolution gas chromatography |
| HSDB | Hazardous Substance Data Bank |
| IARC | International Agency for Research on Cancer |
| IDLH | immediately dangerous to life and health |
| ILO | International Labor Organization |
| IRIS | Integrated Risk Information System |
| Kd | adsorption ratio |
| kg | kilogram |
| kkg | metric ton |
| $K_{oc}$ | organic carbon partition coefficient |
| $K_{ow}$ | octanol-water partition coefficient |
| L | liter |
| LC | liquid chromatography |
| $LC_{50}$ | lethal concentration, 50% kill |
| $LC_{Lo}$ | lethal concentration, low |
| $LD_{50}$ | lethal dose, 50% kill |
| $LD_{Lo}$ | lethal dose, low |
| LDH | lactic dehydrogenase |
| LH | luteinizing hormone |
| LOAEL | lowest-observed-adverse-effect level |
| LSE | Levels of Significant Exposure |
| $LT_{50}$ | lethal time, 50% kill |
| m | meter |
| MA | *trans,trans*-muconic acid |

| | |
|---|---|
| MAL | maximum allowable level |
| mCi | millicurie |
| MCL | maximum contaminant level |
| MCLG | maximum contaminant level goal |
| MF | modifying factor |
| MFO | mixed function oxidase |
| mg | milligram |
| mL | milliliter |
| mm | millimeter |
| mmHg | millimeters of mercury |
| mmol | millimole |
| mppcf | millions of particles per cubic foot |
| MRL | Minimal Risk Level |
| MS | mass spectrometry |
| NAAQS | National Ambient Air Quality Standard |
| NAS | National Academy of Science |
| NATICH | National Air Toxics Information Clearinghouse |
| NATO | North Atlantic Treaty Organization |
| NCE | normochromatic erythrocytes |
| NCEH | National Center for Environmental Health |
| NCI | National Cancer Institute |
| ND | not detected |
| NFPA | National Fire Protection Association |
| ng | nanogram |
| NHANES | National Health and Nutrition Examination Survey |
| NIEHS | National Institute of Environmental Health Sciences |
| NIOSH | National Institute for Occupational Safety and Health |
| NIOSHTIC | NIOSH's Computerized Information Retrieval System |
| NLM | National Library of Medicine |
| nm | nanometer |
| nmol | nanomole |
| NOAEL | no-observed-adverse-effect level |
| NOES | National Occupational Exposure Survey |
| NOHS | National Occupational Hazard Survey |
| NPD | nitrogen phosphorus detection |
| NPDES | National Pollutant Discharge Elimination System |
| NPL | National Priorities List |
| NR | not reported |
| NRC | National Research Council |
| NS | not specified |
| NSPS | New Source Performance Standards |
| NTIS | National Technical Information Service |
| NTP | National Toxicology Program |
| ODW | Office of Drinking Water, EPA |
| OERR | Office of Emergency and Remedial Response, EPA |
| OHM/TADS | Oil and Hazardous Materials/Technical Assistance Data System |
| OPP | Office of Pesticide Programs, EPA |
| OPPT | Office of Pollution Prevention and Toxics, EPA |
| OPPTS | Office of Prevention, Pesticides and Toxic Substances, EPA |
| OR | odds ratio |
| OSHA | Occupational Safety and Health Administration |

| | |
|---|---|
| OSW | Office of Solid Waste, EPA |
| OTS | Office of Toxic Substances |
| OW | Office of Water |
| OWRS | Office of Water Regulations and Standards, EPA |
| PAH | polycyclic aromatic hydrocarbon |
| PBPD | physiologically based pharmacodynamic |
| PBPK | physiologically based pharmacokinetic |
| PCE | polychromatic erythrocytes |
| PEL | permissible exposure limit |
| pg | picogram |
| PHS | Public Health Service |
| PID | photo ionization detector |
| pmol | picomole |
| PMR | proportionate mortality ratio |
| ppb | parts per billion |
| ppm | parts per million |
| ppt | parts per trillion |
| PSNS | pretreatment standards for new sources |
| RBC | red blood cell |
| REL | recommended exposure level/limit |
| RfC | reference concentration |
| RfD | reference dose |
| RNA | ribonucleic acid |
| RQ | reportable quantity |
| RTECS | Registry of Toxic Effects of Chemical Substances |
| SARA | Superfund Amendments and Reauthorization Act |
| SCE | sister chromatid exchange |
| SGOT | serum glutamic oxaloacetic transaminase |
| SGPT | serum glutamic pyruvic transaminase |
| SIC | standard industrial classification |
| SIM | selected ion monitoring |
| SMCL | secondary maximum contaminant level |
| SMR | standardized mortality ratio |
| SNARL | suggested no adverse response level |
| SPEGL | Short-Term Public Emergency Guidance Level |
| STEL | short term exposure limit |
| STORET | Storage and Retrieval |
| $TD_{50}$ | toxic dose, 50% specific toxic effect |
| TLV | threshold limit value |
| TOC | total organic carbon |
| TPQ | threshold planning quantity |
| TRI | Toxics Release Inventory |
| TSCA | Toxic Substances Control Act |
| TWA | time-weighted average |
| UF | uncertainty factor |
| U.S. | United States |
| USDA | United States Department of Agriculture |
| USGS | United States Geological Survey |
| VOC | volatile organic compound |
| WBC | white blood cell |
| WHO | World Health Organization |

| | |
|---|---|
| > | greater than |
| ≥ | greater than or equal to |
| = | equal to |
| < | less than |
| ≤ | less than or equal to |
| % | percent |
| α | alpha |
| β | beta |
| γ | gamma |
| δ | delta |
| μm | micrometer |
| μg | microgram |
| $q_1^*$ | cancer slope factor |
| – | negative |
| + | positive |
| (+) | weakly positive result |
| (–) | weakly negative result |

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# APPENDIX D. INDEX