TOXICOLOGICAL REVIEW

OF

ACRYLAMIDE

(CAS No. 79-06-1)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

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NOTICE

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ABBREVIATIONS AND ACRONYMS

AA	acrylamide
AAMA	N-acetyl-S-(2-carbamoylethyl)-L-cysteine
AAVal	acrylamide-hemoglobin-terminal-valine adduct, N-(2-carbamoylethyl)valine
ABT	1-aminobenzotriazole
ADAF	age-dependent adjustment factor
AIC	Akaike's Information Criterion
ALT	alanine aminotransferase
AUC	area under the curve
BB	Big Blue
BMD	benchmark dose
BMDL	95% lower bound on BMD
BMDS	benchmark dose software
BMR	benchmark response
bw, BW	body weight
C-C	control dams with control pups
CERHR	National Toxicology Program / Center for the Evaluation of Risks to Human Reproduction
CFR	Code of Federal Regulations
CI	confidence interval
CIR	Cosmetic Industry Review Expert Panel (4) (ref.)
CNS	central nervous system
C-T	control dams with treated pups
dAdo	2'-deoxyadenosine
dCyd	2'-deoxycytidine
dGua	2'-deoxyguanosine
dThd	2'-deoxythymidine
ED	effective dose
ENMG	electroneuromyographic
EPA	Environmental Protection Agency
FAO	Food and Agricultural Organization
FDA	U.S. Food and Drug Administration
FISH	fluorescence in situ hybridization
GA	glycidamide
GABA	gamma-aminobutyric acid
GAMA	N-(R,S)-acetyl-S-(carbamoyl-2-hydroxyethyl)-L-cysteine

GAVal	glycidamide-hemoglobin-terminal-valine adduct, N-(2-carbamoyl-2-hydroxyethyl)valine
GC-MS	gas chromatography-mass spectrometry
GD	gestational day
GSH	glutathione
Hb	hemoglobin
HBSS	Hanks' balanced salt solution
HEC	human equivalent concentration
HID	highest ineffective dose/concentration
HSDB	Hazardous Substances Data Bank
i.p.	intraperitoneal or intraperitoneally
i.v.	intravenous or intravenously
IARC	International Agency for Research on Cancer
IRB	Institute Review Board
IRIS	Integrated Risk Information System
IRMM	Institute for Reference Materials and Measurements
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JIFSAN	Joint Institute for Food Safety and Applied Nutrition
LD_{50}	median lethal dose
LED	95% lower bound on ED
LFB/PAS	luxol fast blue-periodic acid Schiff (59)
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSD	Fisher's Least Significant Difference Test
MF	mutant frequency
MLE	maximum likelihood estimate
MN	micronucleus or micronuclei
MN-RET	micronucleated reticulocytes
MOA	mode of action
MPDS	mortality and population data system; maintained at the University of Pittsburgh
N3-GA-Ade	N3-(2-carbamoyl-2-hydroxyethyl)adenine
NFCS	National Food Consumption Survey (Netherlands)
NIOSH	National Institute of Occupational Safety and Health
NMA	N-methylolacrylamide
NOAEL	no-observed-adverse-effect level
OR	odds ratio

OSHA	Occupational Safety and Health Administration
PBTK	physiologically based toxicokinetic (as in PBTK model)
PCNA	proliferating cell nuclear antigen
PEL	permissible exposure limit
PKA	protein kinase A
PND	postnatal day
POD	point of departure
R	risk
REL	recommended exposure limit
RfC	reference concentration
RfD	reference dose
SCF	Scientific Committee on Food of the European Commission
SEM	standard error of the mean
SHE	Syrian hamster embryo
SMR	standardized mortality ratio
SNFA	Swedish National Food Agency
SNT	Statens næringsmiddeltilsy; the Norwegian Food Control Authority
T3	triiodothyronine
T4	thyroxin
T-C	treated dams with control pups
TPA	12-O-tetradecanoyl-phorbol-13-acetate
TSH	thyroid stimulating hormone
T-T	treated dams with treated pups
TVM	tunica vaginalis mesothelioma
UCL	upper confidence limit
UCLE	upper confidence limit estimate
UDS	unscheduled DNA synthesis
UF	uncertainty factor

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to acrylamide. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of acrylamide.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of acrylamide. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (possibly threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the "oral slope factor" is an upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a "unit risk" is an upper bound on the estimate of risk per $\mu g/m^3$ air breathed .

Development of these hazard identification and dose-response assessments for acrylamide has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA Guidelines and Risk Assessment Forum Technical Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996b), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing*

Susceptibility from Early-Life Exposure to Carcinogens (U.S. EPA, 2005b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a, 2005c), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000d), and *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature through August 2007 is included in the assessment.

Estimates of risk for acrylamide derived by other organizations are compiled by the National Libraries of Medicine and can be found on the TOXNET webpage at http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?iter . Additionally, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) information on acrylamide risk and toxicity is available at: http://www.who.int/foodsafety/chem/chemicals/acrylamide/en/ .

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

2.1. CHEMICAL AND PHYSICAL INFORMATION

Acrylamide (AA) is an odorless, white, crystalline solid. Synonyms include acrylic amide, acrylic acid amide, ethylenecarboxamide, propenamide, and propenoic acid amide. The structure of AA is shown below in Figure 2-1 (carbons are numbered).

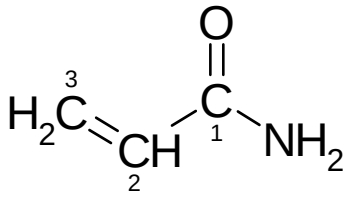

Figure 2-1. Chemical structure of acrylamide (AA) with carbon numbers indicated.

References for the selected chemical and physical properties of acrylamide listed below or in the subsequent text include HSDB, 2005; Budavari, 2001; Verschueren, 2001; Lide, 2000; Lewis, 1997; Hansch et al., 1995; IARC, 1994a; and Petersen et al., 1985.

CAS number:	79-06-1 (Verschueren, 2001)
Molecular weight:	71.08 (Verschueren, 2001)
Chemical Formula:	C_3H_5NO (Verschueren, 2001)
Boiling point:	192.6°C (Verschueren, 2001)
Melting point:	84.5°C (Verschueren, 2001)
Vapor pressure:	0.007 mm Hg at 25°C (HSDB, 2005)
Density:	1.12 g/mL at 30°C (Budavari, 2001)
Vapor density:	2.46 (air = 1) (Verschueren, 2001)
Water solubility:	2.155 g/mL at 30°C (Verschueren, 2001)
Other solubilities at 30°C:	Acetone (0.631 g/mL), chloroform (0.027 g/mL), diethyl ether (0.862 g/mL), ethanol (0.862 g/mL), ethyl acetate (0.126 g/mL), methanol (1.55 g/mL), heptane (0.068 g/mL) (Budavari, 2001; Lide, 2000)
Partition coefficient (K_{ow}):	log K_{ow} = –0.67 (octanol/water) (Hansch et al., 1995)
Partition coefficient (K_{oc}):	log K_{oc} = 1 (organic carbon/water) (HSDB, 2005)
pH:	5.0–6.5 (50% aqueous solution) (HSDB, 2005)
Henry's law constant:	1.7×10^{-9} atm-m^3/mol at 25°C (HSDB, 2005)
Bioconcentration factor:	1 for fingerling trout (Petersen et al., 1985)
Stability	Stable at room temperature but may polymerize violently on melting (HSDB, 2005)
Conversion factor:	1 mg/m^3 = 0.34 ppm, 1 ppm = 2.95 mg/m^3 (Verschueren, 2001)

Acrylamide is a highly water-soluble α,β-unsaturated amide that reacts with nucleophilic sites in macromolecules in Michael-type additions (Calleman, 1996; Segerbäck et al., 1995). Monomeric AA readily participates in radical-initiated polymerization reactions, whose products form the basis of most of its industrial applications (Calleman, 1996).

2.2. SOURCES OF EXPOSURE, FATE AND TRANSPORT

Acrylamide from industrial sources

Acrylamide was initially produced for commercial purposes by reaction of acrylonitrile with hydrated sulfuric acid and separation of the product from its sulfate salt. Relatively high levels of impurities resulted from this process, which was replaced in the 1970s by catalytic hydration with copper metal or a Raney copper catalyst and lower levels of impurities. With catalytic hydration, a solution of acrylonitrile in water is passed over a fixed bed of copper catalyst at 85°C to produce AA. A third production method, developed in 1985, uses microorganisms to convert acrylonitrile into acrylamide by enzymatic hydration (HSDB, 2005; IARC, 1994a). Direct uses of acrylamide include photopolymerization systems, adhesives and grouts, and polymer cross-linking. The primary use of AA is in the production of polyacrylamides, which are used for enhanced oil recovery in water flooding, in oil well drilling fluids, in fracturing aids, in sewage treatment flocculants, in soil conditioning and stabilization, in papermaking aids and thickeners, in adhesion-promoting polymers, in dye acceptors, in textile additives, and in paint softeners (HSDB, 2005; IARC, 1994a).

Release of AA to the environment may occur during its production and use or in the production of polyacrylamide. Products and compounds containing polyacrylamide may serve as sources of exposure to residues of acrylamide. Examples include polyacrylamide compounds used in oil well drilling operations (well drilling muds), as flocculents in water treatment, coagulants in food processing, sealing grouts and some coatings, and as foam builders, lubricants, and emollients in some personal care and grooming products (CFR, 2005; CIR, 1991). Localized contamination may arise from the use of acrylamide in grouting operations (HSDB, 2005). U.S. EPA (2003) requires drinking water authorities to certify that, for polyacrylamides used as coagulants or flocculents in drinking water treatment, the level of acrylamide monomer in the polymer does not exceed 0.05% and the application rate for the polymer does not exceed 1 mg/L. The National Sanitation Foundation /American National Standards Institute (NSF/ANSI) Standard 60 for Drinking Water Treatment Chemicals - Health Effects provides the restrictions for the use of polyacrylamides in well drilling muds and grouts for potable water wells based on acrylamide monomer levels.

If released to air, the vapor pressure of 0.007 mm Hg at 25°C indicates AA will exist solely as a vapor in the ambient atmosphere. Vapor-phase AA will be degraded in the

atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 1.4 days. The half-life for the reaction of vapor-phase AA with ozone is estimated to be 6.5 days. Acrylamide is not expected to be susceptible to direct photolysis in sunlight since it does not absorb light with wavelengths >290 nm (HSDB, 2005).

With a K_{oc} of 10, AA is expected to be highly mobile in soils. Volatilization of AA from dry or moist soil surfaces is not expected to be an important fate process, based on its vapor pressure and estimated Henry's law constant of 1.7×10^{-9} atm-m^3/mol (HSDB, 2005). Acrylamide is expected to degrade in soil. Degradation in the range of 74–94% within 14 days and 79–80% in 6 days was reported for AA in several soils that had been moistened to field capacity (Abdelmagid and Tabatabai, 1982). Half-lives of 18–45 hours were observed for four central New York soils that had been moistened to 70% field capacity (Lande et al., 1979).

If released to water, AA is not expected to adsorb to suspended solids or sediment, based on the K_{oc} (HSDB, 2005). In a river die-away test, 90% of AA disappeared in approximately 150 hours (Croll et al., 1974). The hydrolysis half-life of acrylamide has been reported as >38 years (HSDB, 2005). Volatilization of acrylamide from water surfaces is not expected, based on the compound's Henry's law constant. An estimated bioconcentration factor of 1 for fingerling trout (Petersen et al., 1985) suggests that bioconcentration in aquatic organisms is low (HSDB, 2005). Microbial degradation of acrylamide can occur under light or dark, aerobic or anaerobic conditions (Brown et al., 1980; Lande et al., 1979; Croll et al., 1974).

Acrylamide was formerly thought to only be present as an industrially manufactured chemical and not a naturally occurring contaminant (IARC, 1994a). It is now known that acrylamide is present in cigarette smoke, and can form in certain foods during cooking or processing.

Acrylamide in cigarette smoke

Acrylamide is a component of cigarette smoke, and AA content in mainstream cigarette smoke has been estimated at 1.1–2.34 μg per cigarette (Smith et al., 2000). Smoking is a source of human inhalation exposure, and secondhand smoke could contribute to AA in indoor air, although no data were found on indoor air levels of acrylamide from environmental tobacco smoke. Boettcher et al. (2005) measured the AA and AA metabolites in human urine, and reported median levels in smokers (n=13) about four times higher than in non-smokers (n=16) indicating that cigarette smoke is clearly an important source of acrylamide exposure.

Acrylamide formation in foods during processing

In early 2002, high concentrations of AA were reported in certain fried, baked, and deep-fried foods (Swedish National Food Agency, 2002). This discovery dramatically increased the interest in nonindustrial sources of acrylamide exposure to the general public. Subsequent

research in many European countries and the United States determined that AA is formed primarily in carbohydrate-rich foods prepared or cooked at high temperatures (i.e., >120º C) (Tareke et al., 2002, 2000). The predominant chemistry involves a Maillard reaction, a nonenzymatic browning reaction that occurs by a condensation of the amino group of the amino acid, asparagine, and the carbonyl group of reducing sugars (fructose and glucose) during high-temperature heating (Mottram et al., 2002; Stadler et al., 2002). Thus, browned crispy crusts in foods like French fries, potato chips, crackers, pretzel-like snacks, cereals, and browned breads tend to have the highest levels of AA. Acrylamide has been detected in some food products that are processed at temperatures in the 98º - 116º C range and in high moisture conditions (e.g., canned black olives [not oil cured] and prune juice) [Roach et al., 2003]), so there are other pathways of formation that do not involve temperatures over 120º C and crispiness, and these are being further evaluated (JIFSAN, 2004). It is worth noting that, since AA appears to form from standard cooking methods like baking, frying, and roasting, it has been in the human diet for many thousands of years.

Dybing et al. (2005) list AA concentrations in various foods in the United States as determined by the U.S. Food and Drug Administration (U.S. FDA, 2006a) in Table 2-1 and, in Table 2-2, in foods in Europe from data compiled by the Institute for Reference Materials and Measurements (IRMM, 2004).

Estimates of acrylamide exposure based on diet and acrylamide content in foods

The FDA has estimated overall daily intake levels of acrylamide from exposures in the U.S. diet to be around 0.4 µg/kg-day with a 90th percentile of 0.95 µg/kg-day (U.S. FDA, 2006a). Table 2-3 is a compilation by Dybing et al. (2005) of exposure estimates from many different national organizations. Estimated daily intake in populations around the world are reasonably similar to FDA's estimate, with the variability assumed to result from cultural differences in food preferences (i.e., different composition of diet among populations), processing methods (i.e., that result in different AA levels among local foods), and consumption levels.

A 2004 expert panel review of risk for human reproductive toxicity from exposure to AA compiled a table of estimates for total exposures, presented here as Table 2-4 (NTP/CERHR, 2004).

Table 2-1. Summary of acrylamide levels in food (ppb) derived from the FDA data collected from 2002 through October 1, 2003)

Food commodity	n	Minimum	25%	Median	75%	Maximum	St. Dev.
Baby food and infant formula	36	0.0	0.0	10.0	31.8	130.0	36.6
French fries and chips	97	20.0	220.0	318.0	462.0	2762.0	427.9
Protein foods	21	0.0	0.0	10.0	25.0	116.0	27.7
Breads and bakery products[a]	49	0.0	15.0	34.0	96.0	432.0	107.9
Cereals and muesli	23	11.0	49.0	77.0	166.0	1057.0	249.1
Crackers and snack foods	32	12.0	92.5	169.0	302.3	1243.0	331.1
Gravies and seasonings	13	0.0	0.0	0.0	0.0	151.0	43.4
Nuts and butters	13	0.0	28.0	89.0	236.0	457.0	143.0
Chocolate products	14	0.0	2.5	20.5	84.3	909.0	243.6
Canned fruits and vegetables	33	0.0	0.0	10.0	70.0	1925.0	411.7
Coffee, ground	59	37.0	158.0	205.0	299.0	539.0	106.3
Coffee, brewed	20	3.0	6.0	6.5	8.0	13.0	2.4
Miscellaneous[b]	41	0.0	0.0	10.0	43.0	5399.0	1018.8

[a]Includes cookies, pies and pastry, bagels.
[b]Hot beverages other than coffee (Postum, caffeine-free coffee substitute), frozen vegetables, dried foods, dairy, juice and other miscellaneous.

Data were calculated from the data published by the FDA on the Internet ("Exploratory Data on Acrylamide in Food," March 2004 [http://www.cfsan.fda.gov/~dms/acrydata.html]). The database contains data collected from 2002 through October 1, 2003. The categories were used as given by the FDA. For coffee, only data for roasted coffee were used (total sample number [n] = 439).

Source: Dybing et al. (2005).

Table 2-2. Acrylamide levels in food (ppb) as collected by the European Union Joint Research Center (updated June 2004)

Food commodity	n	Min	25%	Median	75%	Max
French fries	741	5.0	90.0	178.0	326.0	2228.0
Chips	569	5.0	378.0	600.0	980.0	3770.0
Potato fritter[a]	75	15.0	215.0	492.0	797.6	2779.0
Fine bakery ware	485	5.0	67.0	160.0	366.0	3324.0
Gingerbread	414	5.0	152.0	298.5	650.7	7834.0
Crispbread	261	5.0	81.0	251.0	602.0	2838.0
Infant biscuits	63	5.0	64.3	90.0	275.1	910.0
Diabetics' cakes and biscuits	212	5.0	92.5	291.5	772.3	3044.0
Breakfast cereals	162	5.0	30.0	60.0	152.5	846.0
Coffee, roasted	102	79.0	192.0	264.0	337.0	975.0
Coffee, substitutes	50	115.6	439.4	739.0	1321.8	2955.0

[a]Grated potatoes fried into a pancake.

Note: Data were calculated from the monitoring database on acrylamide levels in food (http://www.irmm.jrc.be/) maintained by the IRMM, together with the Directorate General for Health and Consumer Affairs. This database comprises 3442 samples of acrylamide levels in food products throughout the EU, including the data collection from the Confédération des Industries Agro-Alimentaires de l'Union Européenne. The categories were used as given in the data collection.

Source: Dybing et al. (2005).

Table 2-3. Exposure estimates from 2002–2006

Exposure assessment	Daily intake µg/kg-day		Source
	Mean (age group)	Percentile[a,b]	
FAO/WHO (2000)	0.3–0.8		http://www.who.int/foodsafety/publications/chem/en/acrylamide_full. pdf
SCF, European Union (2002)	0.2–0.4		http://europa.eu.int/comm/food/fs/sc/scf/out131_en.pdf
BfR, Germany (2002)	1.1 (15–18)	3.4[a]	http://www.bfr.bund.de/cm/208/Abschaetzung_der_Acrylamid_Aufnahme_durch_hochbelastete_Nahrungsmittel_in_Deutschland_Studie.pdf
BAG, Switzerland (2002)	0.28 (16–57)		http://www.bag.admin.ch/verbrau/aktuell/d/DDS%20acrylamide%20preliminary%20communication.pdf
AFSSA, France (2002)	0.5 (>15) 1.4 (2–14)	1.1[a] 2.9[a]	http://www.afssa.fr/ftp/afssa/basedoc/acrylpoint2sansannex.pdf
FDA, United States (2002)	0.7		http://www.cfsan.fda.gov/~dms/acryexpo.html
FDA, United States (2004)	0.43 (>2) 1.06 (2–5)	0.92[b] 2.31[b]	http://www.cfsan.fda.gov/~dms/acryexpo.html
FDA, United States (2006)	0.40 (>2) 1.07 (2–5)	0.95[a] 2.33[b]	http://www.cfsan.fda.gov/~dms/acryexpo.html
NFCS, Netherlands	0.48 (1–97) 1.04 (1–6) 0.71 (7–18)	0.6[a] 1.1[a] 0.9[a]	Konigs et al. (2003)
SNFA, Sweden (2002)	0.45 (18–74)	1.03	Svensson et al. (2003)
SNT, Norway (2003)	0.49 (males) 0.46 (females) 0.36 (9, boys) 0.32 (9, girls) 0.52 (13, boys) 0.49 (13, girls) 0.53 (16–30, males) 0.50 (16–30, females)	1.01[b] 0.86[b] 0.72[b] 0.61[b] 1.35[b] 1.2[b]	Dybing and Sanner (2003)

[a] = 95th percentile.
[b] = 90th percentile.

Source: For all exposures estimates from 2002-2004 Dybing et al. (2005) except the FDA estimates; FDA exposure estimates 2002- 2006 (directly from the FDA website: http://www.cfsan.fda.gov/~dms/acryexpo.html

Table 2-4. Summary of exposure estimates (µg/kg-day) by sources and population groups

Source of exposure	Mean or median[a]	90th percentile or upper boundary[a]
Diet: general population	0.43	0.92
2- to 5-year-olds	1.06	2.31
Drinking water	No data	<0.01
Personal care products	~0.5	1.1 (female)
Cigarette smoking	0.67 (from cigarette data) 2.6 (from adduct data)[b]	1.3 ~6
Occupational exposures	1.4–18	43 (based on PEL[e])
Totals (adults)		
General population		
Nonsmokers	0.98[c] 0.85 (from adduct data)	2.0
Smokers	1.7 (from cigarette data) 3.6 (from adduct data)	3.2
Occupational exposure[d]		45–52
Nonsmokers	2.4–19	45
Smokers	3.1–20 (cigarette data) 5–22 (adduct data)	46 51

[a]Dose levels in experimental animal studies are expressed as mg/kg-day, human exposures are expressed as µg/kg-day. To convert figures in table to mg/kg-day, divide by 1000.
[b]Acrylamide exposure in smokers based on adduct formation was estimated by taking the value for total exposure in smokers (3.4 µg/kg-day) and subtracting the value for total exposure in nonsmokers (0.85 µg/kg-day).
[c]Estimated from diet, water, and personal care products. The adduct-derived estimates are considered more comprehensive.
[d]Occupational exposures include monomer and polymer production and grouting applications.
[e]PEL = permissible exposure limit. The Occupational Safety and Health Administration (OSHA) permissible exposure level (PEL) for acrylamide is 0.3 mg/m^3. Based on a geometric means of 0.01–0.13 mg/m^3 and an upper bound exposure of 0.3 mg/m^3 (PEL), the NTP/CERHR Expert Panel estimated mean and upper bound workplace acrylamide inhalation exposures at 1.4–18.6 µg/kg bw/day and 43 µg/kg bw/day, respectively.

Source: NTP/CERHR (2004).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

Much of the information in this section describes interactions of acrylamide (AA) and its principal and toxicologically significant (epoxide) metabolite, glycidamide (GA) with various biologically significant targets such as cellular thiols (e.g., glutathione), various proteins and bases in DNA. The chemical basis for these interactions is strongly associated with the degree of electrophilicity (electron deficiency) of such agents as AA and GA with nucleophilic centers (i.e., unshared electrons) that may be present in biological targets. 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 AA 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 (Lopachin and DeCaprio, 2005; Dearfield et al, 1995). A recent evaluation of soft-soft interactions based on frontier molecular orbital characteristics (as defined by the quantum mechanical parameters for softness [sigma] and chemical potential [mu]) suggest that the thiolate state of cysteine residues is the corresponding adduct target for AA (Lopachin et al., 2007). This information is useful in understanding the differences discussed in this section between the types of adducts formed by AA and GA (e.g., hemoglobin and/or DNA) and the binding rates.

3.1. ABSORPTION

Hemoglobin adducts as a biomarker of exposure/absorption

Numerous studies, including a recent study by Fennell et al. (2005), support the use of acrylamide hemoglobin adducts as a biomarker of exposure. (See the Metabolism Section 3.3 for a detailed discussion of the chemistry of acrylamide [AA] and glycidamide [GA] hemoglobin adducts, and glycidamide DNA adducts). Estimates of exposure using hemoglobin adduct levels 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. Fennell et al. (2005) calculated acrylamide exposure by using the results of the toxicokinetic study described above in 24 volunteer adult males. The estimated average daily background exposure to acrylamide was 1.26 μg/kg-day based on the subject's preexposure background acrylamide-hemoglobin-terminal-valine adduct levels (AAVal) (averaging about 80 fmol/mg globin). In an occupational exposure study, Hagmar et al. (2001) reported a background range of 20–70 fmol AAVal/mg globin in the unexposed reference group. Using the Hagmar et al. (2001) lower range and their observed average as an upper value (i.e., a range of

20–80 fmol AAVal/mg globin), Fennell et al. (2005) estimated a daily acrylamide intake of 0.31–1.26 μg/kg-day. For a 70 kg adult this translates into a total daily intake of 22–88 μg of acrylamide. As can be seen in Table 2-3, many of the estimates of daily intakes in adults based on exposure estimates in foods are in the 0.4–0.8 μg/kg-day range, suggesting that adults with higher adduct levels may be exposed to acrylamide from sources other than food (e.g., smoking, occupational, or from an as yet unknown source).

Detection of hemoglobin adducts of AA in workers exposed via inhalation and dermal exposure provides qualitative evidence of absorption by these routes and suggests that dermal exposure was the predominant route of absorption in these workers (Hagmar et al., 2001; Bergmark et al., 1993). Hemoglobin adduct levels were measured in 41 Chinese workers who were exposed to acrylamide for 0.1–8 years (Bergmark et al., 1993). Adducts measured in this study were those at N-terminal valine residues in hemoglobin. 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. Hemoglobin adducts of AA were not detected in blood samples from 10 control workers from the same city who had not been exposed to acrylamide (or acrylonitrile). Blood samples from 5 of the 41 exposed workers were also analyzed for hemoglobin adducts of glycidamide (a principal metabolite of acrylamide in both humans and animals) (see Section 3.3). There was a statistically significant linear relationship between levels of hemoglobin adducts of AA and GA in these 5 workers; the ratio between GA and AA adducts was approximately 3:10. Average levels of AA 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, however, showed average hemoglobin adduct levels of acrylamide of 7.3 ± 3.4 nmol/g hemoglobin (n = 12, polymerization) and 14.7 ± 10.6 nmol/g hemoglobin (n = 14, synthesis). The study authors calculated the levels of hemoglobin adducts of AA in these workers that would have resulted from the observed exposure concentrations, based on an assumption that exposure was only via inhalation (as well as additional assumptions)[1], and derived levels of 0.93 (instead of 7.3) nmol/g hemoglobin for the polymerization workers and 0.44 (instead of 14.7) nmol/g hemoglobin for synthesis workers. Thus, Bergmark et al. (1993) state that the observed and predicted adduct levels were inconsistent with exposure only via inhalation and hypothesize that dermal exposure was the predominant route of absorption in these workers.

Hagmar et al. (2001) measured hemoglobin adducts in a group of 210 tunnel construction workers who were occupationally exposed for 2 months without personal protection devices to a chemical grouting agent containing AA and N-methylolacrylamide. An important caveat in

[1] The calculation assumed that (1) adducts are stable during the life of erythrocytes; (2) the life span of human erythrocytes is about 120 days (17 weeks); (3) the second-order reaction rate constant for the reaction of

interpreting the hemoglobin adduct data relative to AA absorption is that both AA and N-methylolacrylamide form the same N-(2-carbamoylethyl)valine adduct in hemoglobin and subsequent chemical measures of adduct levels cannot distinguish which parent compound formed the adduct (Fennell et al., 2003) (see additional discussion in the next section). Blood samples were drawn within a month after construction work was completed and analyzed for levels of N-terminal valine adducts. Workers were expected to have experienced dermal exposure to varying extents, as well as inhalation 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 AA 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 globin; 36 with 0.3–1.0 nmol/g globin; and 38 with 1.0–17.7 nmol/g globin. 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.

Human oral/dermal exposure

Fennell et al. (2005) evaluated metabolism and hemoglobin adduct formation following oral and dermal administration of AA to 24 adult male volunteers. The 24 volunteers were all male Caucasians (with the exception of one Native American), weighing between 71 and 101 kg, and between 26 and 68 years of age. All volunteers were aspermic (i.e., clinically sterile because of the potential for adverse effects of AA on sperm), and all volunteers had not used tobacco products for the past 6 months. The study was conducted in accordance with the Code of Federal Regulations (CFRs) governing protection of human subjects (21 CFR 50), Institute Review Board (IRB) (21 CFR 56), and retention of data (21 CFR 312) as applicable and consistent with the Declaration of Helsinki. The study used radiolabeled [1,2,3-^{13}C]-acrylamide, and, prior to the conduct of exposures in humans, a low-dose study protocol was evaluated in rats administered 3 mg/kg [1,2,3-^{13}C]-acrylamide by gavage. The [1,2,3-^{13}C]-acrylamide human study protocol was reviewed and approved by IRBs both at the researchers' facility (Research Triangle Institute International), where the sample analysis occurred, and by the clinical research center conducting the study (Covance Clinical Research Unit [CRU]). The health of the volunteers, exposed under controlled conditions, was continually monitored.

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acrylamide with N-terminal valine residues in human hemoglobin is 4.4×10^{-6} L/g Hb/hour (based on in vitro experiments); (4) the human ventilation rate is 0.2 L/min-kg; and 5) inhaled acrylamide is 100% absorbed.

Acrylamide was administered orally in an aqueous solution (single dose of 0.5, 1.0, or 3.0 mg/kg) or dermally (three daily doses of 3.0 mg/kg) to the male volunteers. Approximately 34% of the administered dose of AA was recovered in the total urinary metabolites within 24 hours of administration, representing a lower bound on total absorption from the oral route. No other estimate of total absorption from an oral exposure was reported.

The results of the dermal exposure in Fennell et al. (2005) indicate much lower levels of AAVal and glycidamide-hemoglobin-terminal-valine adduct (GAVal) formed than with an equivalent dose via the oral route. Based on total amount administered, formation of AAVal after dermal exposure was much lower than after oral administration (4.9 nmol/g globin/mmol AA/kg vs. 74.7 nmol/g globin/mmol AA/kg). These numbers can be used to estimate that approximately 6.6% of the dermally administered dose was absorbed compared to a comparable orally administered dose, assuming that there was 100% oral absorption. Similarly, dermal exposure also resulted in much lower formation of GAVal, 9.7% of that formed following oral exposure. However, approximately 66% of the dermally administered dose of AA was recovered in the occluding solutions (data not included in the report) and thus was not systemically absorbed on dermal administration. This suggests that a maximum of 3% of the dermally applied dose could have been absorbed. An estimate of dermal absorption based on the formation of AAVal adducts normalized to the absorbed dose yields a value of 17.0% of the amount formed following oral exposure (12.7 nmol/g globin/mmol AA/kg for dermal vs. 74.7 nmol/g globin/mmol AA/kg for oral). Similarly, GAVal formation following dermal exposure was 25.3% of that formed on oral administration (7.3 pmol/g globin/mmol AA/kg for dermal vs. 28.9 pmol/g globin/mmol AA/kg for oral). This suggests that as much as 83% of the AA penetrating the skin was not available systemically. An alternative hypothesis is that AA and GA clearance is different following dermal exposure, resulting in a lower area under the curve (AUC) and lower adduct formation on a mg/kg basis. Ongoing study of urinary metabolites in dermally exposed individuals may help resolve the reason(s) for these differences.

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, 60.3 ± 11.2% of the dose was recovered in the urine. Although no glycidamide was found, unchanged acrylamide, AAMA, and GAMA accounted for urinary excretion of (mean ± SD) 4.4 ± 1.5%, 50.0 ± 9.4%, and 5.9 ± 1.2% of the dose, respectively. These results indicate that most of the acrylamide ingested with food is absorbed in humans.

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

Bjellaas et al. (2007) reported urinary mercapturic acid derivatives of AA and in a clinical study comprising of 53 subjects. Median intakes (range) of AA were estimated based on 24 h dietary recall as 21 (13-178) μg for non-smokers 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 h was 16 (7-47) μg acrylamide for non-smokers and 74 (38-106) μg acrylamide for smokers. In a multiple linear regression analysis, the urinary excretion of acrylamide metabolites correlated statistically significant with intake of aspartic acid, protein, starch and coffee. Consumption of citrus fruits correlated negatively with excretion of acrylamide metabolites.

Animal oral exposure

Studies in rats indicate that orally administered AA is rapidly and extensively absorbed by the gastrointestinal tract (Doerge et al., 2005b; Fennell et al., 2005; Kadry et al., 1999; Dow Chemical Co., 1984; Dixit et al., 1982; Miller et al., 1982).

Doerge et al. (2005b) compared the toxicokinetics of AA and GA in serum and tissues of male and female B6C3F1 mice following a single dose by intravenous (i.v.) injection or gavage of 0.1 mg/kg AA or a comparable dose of 0.1 mg/kg AA from a feeding exposure for 30 minutes. Study groups also received an equimolar amount of GA from either an i.v. injection or gavage dose. AA was rapidly absorbed following oral dosing, widely distributed to tissues, and efficiently converted to GA. Liver levels of GA-DNA adducts were increased at 8 hours post dosing, which is a time point where AA has been eliminated from the serum. Oral GA dosing also resulted in rapid absorption, wide distribution to tissues, and liver DNA adduct levels that were approximately 40% higher than those from an equimolar dose of orally administered AA. Based on the kinetics of AA following i.v. injection, oral administration from the diet attenuated AA bioavailability to 23% of the i.v. dose, and aqueous gavage attenuated AA bioavailability to 32–52%. In contrast, oral exposure resulted in higher relative internal levels of GA compared with levels following an i.v. exposure, likely due to a first-pass effect but possibly the result of some other kinetic change.

Fennell et al. (2005) administered 3 mg/kg [1,2,3-^{13}C]-AA by gavage to male F344 rats (n= 4). The total amount of AA metabolites recovered in urine by 24 hours after dosing was 50%, which is similar to that reported by Miller et al. (1982) and by Kadry et al. (1999).

The time course and extent of urinary elimination of radioactivity from male F344 rats (n= 3) during a 7-day period following administration of either a single oral gavage or an i.v. dose of 10 mg/kg [2,3-^{14}C]-acrylamide (in water vehicle) was essentially the same, indicating that 100% of the oral dose was absorbed (Miller et al., 1982). The time courses of urinary elimination of radioactivity for groups of rats (n = 3) given single oral doses of 1, 10, or 100 mg/kg [2,3-^{14}C]-acrylamide were also similar, indicating that the extent of absorption was not affected by dose level in this experimental 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 with male Sprague-Dawley rats (n = 5–7) given single oral doses of 50 mg/kg [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).

Animal inhalation exposure

Animal studies indicate that inhaled AA 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 AA. The remaining rats and mice were monitored for 24-hour elimination of radiolabeled AA 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; less than 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; less than 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.[2]

[2] If reference minute ventilation rates for rats (0.7 cm^3/min-gram) or mice (1.5 cm^3/min-gram) and midpoints of the reported ranges of the experimental animal body weights (211 grams, rats, and 30 grams, mice) are used, the amounts of acrylamide inhaled in the 6-hour exposure period are calculated to be 6.5 and 2 µmol acrylamide/exposure period for rats and mice, respectively. Given that the measured amounts of recovered acrylamide equivalents were about three- to fourfold higher than these calculated values, it is expected that the animals had much higher minute ventilation rates during exposure than reference values. Sample calculations:

Animal dermal exposure

Studies on dermal absorption in animals indicate that considerable amounts of AA can be absorbed by the skin within short time frames (Sumner et al., 2003; Frantz et al., 1995; Dow Chemical Co., 1984).

In male F344 rats, 14–30% (mean 22%) of an occluded dermal dose of [2,3-^{14}C]-labeled acrylamide (162 mg/kg in distilled water) was absorbed during a 24-hour exposure period (Sumner et al., 2003). By 24 hours post application, approximately 44% of recovered radioactivity (excluding material from dermal patch and wash of application site at termination of exposure) was in the urine, feces, and cage washes; 3% was in exhaled air; and 53% remained in tissues.

Frantz et al. (1995) applied a 0.5% aqueous solution of [^{14}C]-labeled acrylamide to the skin of male F344 rats at a single dose level of 2 mg/kg. The test material penetrated the skin and was systemically distributed in male F344 rats within 24 hours; about 31% of the applied dose penetrated the skin at the dosing site (was not removed by washing) and was considered available for further absorption.

Peak plasma concentrations of radioactivity occurred at about 2 and 5 hours after dermal administration of 2 and 50 mg/kg to F344 rats, respectively, indicating rapid absorption by the skin (Dow Chemical Co., 1984). Aqueous solutions (1%) of [1,3-^{14}C]-labeled acrylamide in a nonionic detergent were applied at 2 or 50 mg/kg to areas of clipped skin on the backs of groups of three male F344 rats. Radioactivity was measured in plasma and urine samples collected for 48 hours following administration. The peak concentration following administration of 50 mg/kg was about 20-fold higher than the peak concentration following administration of 2 mg/kg. Following attainment of peak concentrations, plasma concentrations declined with time, showing slopes that were similar to slopes of curves following i.v. administration of 2 or 50 mg/kg doses of [1,3-^{14}C]-labeled acrylamide. The fraction of dermally applied compound that was absorbed was not reported.

Results of several in vitro studies describe dermal absorption of acrylamide. Frantz et al. (1995) applied a 0.5% [^{14}C]-labeled acrylamide in aqueous solution to excised skin discs from male F344 rats and noted considerable dermal penetration after 24 hours. Approximately 54% of the radioactivity was recovered in effluents and 13% was retained in washed skin. Diembeck et al. (1998) applied a 0.5% [^{14}C]-labeled acrylamide in aqueous solution to excised sections of female pig skin for 24 hours. Approximately 6% of the applied dose was found on the skin surface; 17.5% in the horny layer, 2% in the epidermis, 52.5% in the dermis, and 22% in the receptor fluid. Marty and Vincent (1998) applied [^{14}C]-labeled acrylamide (in an aqueous gel of

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3 ppm × 71.08/24.45 = 8.7 mg/m^3; (8.7mg/m^3) × (0.7cm^3/min-gram) × (60 min/hour) × (6 hours/exposure) × (211 grams/rat) × (m^3/10^6 cm^3) × (mmol/71.08 mg) × (10^{3}µmol/mmol) = 6.5 µmol/rat-exposure period.

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, were 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 collectively from the receptor phase, epidermis, and dermis.

3.2. DISTRIBUTION

No human data on distribution of acrylamide were identified. Results from several animal studies indicate that, following absorption, radioactivity from radiolabeled AA is distributed among tissues with no specific accumulation in any tissues other than red blood cells (Barber et al., 2001; Kadry et al., 1999; Crofton et al., 1996; Marlowe et al., 1986; Ikeda et al., 1985; Dow Chemical Co., 1984; Miller et al., 1982; Edwards, 1975; Hashimoto and Aldridge, 1970) and late-staged spermatids (Sega et al., 1989).

Animal oral exposure

Following 13 daily oral doses of [1,3-^{14}C]-labeled acrylamide (at levels of 0.05 or 30 mg/kg), tissue concentrations of acrylamide in male F344 rats were similar among tissues with the exception of red blood cells, which showed higher concentrations, presumably due to the formation of hemoglobin adducts of AA or GA (Dow Chemical Co., 1984). In rats exposed to 30 mg/kg, mean concentrations (µg equivalents [^{14}C]-acrylamide per gram of tissue) were as follows: red blood cells, 383.70; liver, 87.74; kidneys, 70.43; epididymides, 70.60; testes, 67.14; sciatic nerve, 54.00; brain, 53.52; carcass, 47.56; skin, 39.11; and plasma, 16.45. In rats exposed to 0.05 mg/kg, the mean concentration in red blood cells was 1.26 µg/g [^{14}C]-acrylamide equivalents (approximately 61% of the dose that was recovered from all tissues) compared with a range of 0.07–0.13 µg/g [^{14}C]-acrylamide equivalents in the other tissues (Dow Chemical Co., 1984).

In Sprague-Dawley rats given single oral doses of 50 mg/kg [1-^{14}C]-labeled acrylamide, tissue concentrations of radioactivity, 28 and 144 hours after administration, were indicative of wide distribution of AA metabolites among tissues with no evidence for accumulation in toxicity targets, i.e., AA bound, but did not accumulate in erythrocytes or neural tissue (Kadry et al., 1999). At 28 hours, brain, thyroid, testes, adrenal, pancreas, thymus, liver, kidney, heart, and spleen showed a narrow range of mean concentrations (based on values for 5 rats), 0.05–0.10% of initial dose/g. Higher concentrations were noted in the skin, bone marrow, stomach, and lung, ranging from 0.15 to 0.18% of initial dose/g, and only the gastric contents showed a markedly higher concentration, 1.37% of 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 initial dose/g, with the exception of skin, bone marrow, and lung, which had mean concentrations of 0.06, 0.08, and 0.19% of initial dose/g, respectively.

Animal dermal exposure

Following 24-hour dermal exposure of male F344 rats to [^{14}C]-labeled acrylamide (150 mg/kg), blood cells had the highest concentration of AA equivalents (excluding skin at the site of exposure), about 1 μmol/g (71 μg equivalents/g), followed by skin at the nondosing site (~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).

Animal inhalation exposure

Immediately following a 6-hour inhalation exposure of male F344 rats to 3 ppm [^{14}C]/[^{13}C]-labeled acrylamide vapor, blood cells had the highest concentration (~7 μg/g), followed by concentrations in testes, skin, liver, and kidneys (~6 μg/g) and brain, spleen, lung, and epididymis (~4 μg/g) (Sumner et al., 2003). Immediately following a 6-hour inhalation exposure to the same concentration, male B6C3F1 mice showed the following order of decreasing AA equivalent concentrations: testes (~14 μg/g), skin and liver (~11 μg/g), kidney (~10 μg/g), epididymis (~8 μg/g), brain (~7 μg/g), lung and blood (~6 μg/g), and fat (~5 μg/g). These differences in distribution pattern between rats and mice following inhalation exposure are unexplained, but more data are needed to support a consistent difference and to determine the kinetic determinants.

Animal intravenous or intraperitoneal administration

Similar results were reported in male albino Porton rats injected with single i.v. doses of 100 mg/kg [1-^{14}C]-labeled AA (Hashimoto and Aldridge, 1970). Twenty-four hours and 14 days after dosing, tissue concentrations of radioactivity (μg equivalents/g) were as follows: whole blood, 90.9 and 54.7; kidney, 36.1 and 6.5; liver, 26.1 and 4.0; brain, 18.6 and 5.1; spinal cord, 12.4 and 5.0; sciatic nerve, 10.6 and 4.0; and plasma, 4.5 and 0.4 (Hashimoto and Aldridge, 1970).

Doerge et al. (2005a) measured DNA adducts following a single intraperitoneal (i.p.) administration of AA and GA to adult B6C3F1 mice and F344 rats at 50 mg AA/kg or an equimolar dose of GA (61 mg/kg). GA-derived DNA adducts of adenine and guanine were formed in all tissues examined for both AA and GA dosing, including both target tissues identified in rodent carcinogenicity bioassays and nontarget tissues (including liver, brain, thyroid, leukocytes, mammary gland, and testis in rats), and in liver, lung, kidney, leukocytes, and testis in mice,; indicating widespread distribution.

Concentrations of radiolabel did not differ in neural tissues (brain, sciatic nerve, spinal cord) and nonneural tissues (fat, liver, kidney, testes, lung, small intestine, skin, muscle), following single i.v. injections of 10 mg/kg [2,3-^{14}C]-labeled AA into groups of three male F344 rats sacrificed at time intervals ranging from 15 minutes to 7 days after dosing (Miller et al., 1982). Radioactivity was rapidly distributed to all tissues and eliminated from most tissues (and plasma) with biphasic kinetics showing half-lives of elimination of about 5 hours or less for the first phase and about 8 days or less for the second phase. Peak concentrations of radiolabel were observed by 1 hour after dose administration in liver, fat, kidney, nervous tissues, and testes. Red blood cells did not show an elimination of the radioactivity with time up to 70 hours after dose administration, consistent with the formation of AA and GA adducts with hemoglobin. Less than 1% of the dose was contained in the brain, spinal cord, or sciatic nerve at any time point, indicating no special accumulation of AA or metabolites in these targets of AA toxicity (Miller et al., 1982).

Following i.p. injection of [^{14}C]-labeled acrylamide (125 mg/kg) into male (C3H × 101)F1 mice, peak levels of radioactivity appeared 8–12 days postdosing in sperm heads recovered from the vasa deferentia and caudal epididymides from a 3-week period of monitoring (Sega et al., 1989). Essentially all of the covalently bound radioactivity in spermheads was shown to be alkylated protamine; alkylation of DNA represented generally <0.5% of the sperm-head alkylation radioactivity. The time course of alkylation of sperm-head protamine paralleled the time course of AA-induced dominant lethality in mice injected with the same dose (125 mg/kg) of AA (Sega et al., 1989). In another study using whole-body autoradiography of Swiss-Webster mice orally exposed to [^{14}C]-labeled acrylamide, (120 mg/kg), radioactivity moved through the testis and the reproductive tract in a sequence that paralleled the movement of spermatids (Marlowe et al., 1986).

Further evidence that AA does not accumulate in most tissues is provided by observations that, 30 minutes after the final i.p. dose in a daily repeated exposure of from 10–90 days, at dose levels between 3.3 and 30 mg/kg-day, AA concentrations in rat sciatic nerves or in serum were similar to concentrations in rats exposed to that dose for the first time (Crofton et al., 1996). The ranges and durations of exposure to groups of three male Long-Evans hooded rats in this study were 0, 7.5, 15, or 30 mg/kg-day for 10 days of exposure; 0, 5, 10, 15, or 20 mg/kg-day for 30 days; and 0, 3.3, 6.7, or 10 mg/kg-day for 90 days.

Results from studies with pregnant animals indicate that absorbed AA is distributed across the placenta (Marlowe et al., 1986; Ikeda et al., 1985, 1983). Two hours following i.v. administration of 5 mg/kg [1-^{14}C]-labeled AA to pregnant beagle dogs (n = 6), concentrations of radioactivity in blood, brain, heart, and lung were similar in both maternal and fetal tissues (Ikeda et al., 1985). Average concentrations of radioactivity in maternal tissues were only about 1.1- to 1.2-fold higher than those in fetal tissues. Comparable results were found with pregnant

miniature pigs treated similarly (Ikeda et al., 1985). Whole-body radiographs of pregnant Swiss-Webster mice, 3 or 24 hours following gavage administration of 120 mg/kg [2,3-^{14}C]-labeled AA on gestation day (GD) 13 or 17, showed uniform distribution of radioactivity among fetal tissues that was similar to that seen in maternal tissues, with the exception of increased label in fetal brain regions at 13 days and in fetal skin regions at 17 days (Marlowe et al., 1986). The autoradiographic technique used, however, provided only qualitative information.

3.3. METABOLISM

Human metabolism

In the Fennell et al. (2005) study on 24 adult male volunteers previously discussed in the absorption section, approximately 86% of the urinary metabolites were derived from glutathione (GSH) conjugation and excreted as N-acetyl-S-(3-amino-3-oxopropyl)cysteine and its S-oxide. Glycidamide (GA), glyceramide (2,3-dihydroxypropionamide), and low levels of N-acetyl-S-(3-amino-2-hydroxy-3-oxopropyl)cysteine were detected in urine. On oral administration, a linear dose response was observed for AAVal and GAVal in hemoglobin. The authors reported that the urinary metabolites of AA in humans showed similarities and differences with data obtained previously in the rat and mouse. The main pathway of metabolism in humans was via direct glutathione conjugation, forming N-acetyl-S-(3-amino-3-oxopropyl)cysteine, as observed in the rat and mouse, and its S-oxide, which has not been reported previously. Epoxidation to GA was the other important pathway, with glyceramide formed as a major metabolite in humans. GA was detected in low amounts. The glutathione conjugation of GA, which is a major pathway in rodents, appeared to occur at very low levels in humans. Metabolism via GA (i.e., derived from GA and glyceramide) in humans was approximately 12% of the total urinary metabolites. This is considerably lower than the amount of GA derived metabolites reported for oral administration of AA in rats (28% at 50 mg/kg, [Sumner et al., 2003]) and in mice (59% at 50 mg/kg [Sumner et al., 1992]).

The Fennell et al. (2005) study also provided data on the amount of hemoglobin adducts derived from AA and GA following administration of a defined dose of AA to adult male volunteers. Both AAVal and GAVal increased linearly with increasing dose of AA administered orally, suggesting that, over the range of 0.5–3.0 mg/kg, there is no saturation of metabolism of AA to GA. The ratio of GAVal:AAVal produced by administration of AA 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 [^{13}C]-GAVal: [^{13}C]-AAVal in humans was lower (0.44 ± 0.06) than in rats (0.84 ± 0.07), and the absolute amount (i.e., not scaled to body weight) of [^{13}C]-AAVal formed in humans was approximately 2.7-fold higher than in the rat. The absolute amount of [^{13}C]-GAVal was approximately 1.4-fold higher than that formed in the rat.

Fennell et al. (2005) calculated the expected amount of adduct that would accumulate in adult male humans from continuous exposure based on the amount of adduct formed/day of exposure, and from the life span of the erythrocyte. Exposure via oral intake to 1 µg/kg AA (1.05 fmol AAVal/mg globin/day) for the life span 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 AA (0.18 fmol AAVal/mg globin/day) for the life span of the erythrocyte (120 days) would result in the accumulation of adducts to 10.8 fmol AAVal/mg globin. With workplace exposure of 5 days/week, this would decrease to approximately 7.8 fmol AAVal/mg globin.

Fennell et al. (2005) also derived second order rate constants (see Table 3-1) for the reaction of acrylamide or glycidamide with the N-terminal valine residue of hemoglobin in rats and humans. These rate constants are of particular use for physiologically based toxicokinetic (PBTK) models of acrylamide and glycidamide (see Section 3.5).

Table 3-1. Second order rate constants for reaction of acrylamide or glycidamide with the N-terminal valine residue of hemoglobin

	AAVal L/g Hb[a]/hour	GAVal L/g Hb[a]/hour	AAVal/GAVal
Rat	3.82×10^{-6}	4.96×10^{-6}	0.77
Human	4.27×10^{-6}	6.72×10^{-6}	0.64
Rat/human	0.89	0.73	

[a]Hb = hemoglobin.

Source: Fennell et al. (2005).

Boettcher et al. (2005) measured the mercapturic acid of AA and its epoxide GA, i.e., N-acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA) and N-(R,S)-acetyl-S-(carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA) in human urine as biomarkers of the internal exposure to acrylamide in the general population. The median levels in smokers (n = 13) were found to be about four times higher than in nonsmokers (n = 16) with median levels of 127 µg/L vs. 29 µg/L for AAMA and 19 µg/L vs. 5 µg/L for GAMA. The level of AAMA in the occupationally nonexposed collective (n = 29) ranged from 3 to 338 µg/L, the level of GAMA from below level of detection to 45 µg/L. The authors noted that the ratio of GAMA:AAMA varied from 0.03 to 0.53; the median was 0.16, which is in reasonable agreement with results of different studies on rats. They concluded that the metabolic conversion of AA to its genotoxic epoxide GA seems to occur to a comparable extent in rats and humans. They also measured the hemoglobin adducts of AA and GA in the blood of 26 participants. These results were compared with those of the mercapturic acids to deduce a steady state for AA uptake and demonstrate a higher reactivity of GA in comparison to AA towards hemoglobin compared to GSH in humans.

Boettcher et al. (2006a) investigated the human metabolism of AA to AAMA and GAMA in a healthy male volunteer who received a single dose of about 1 mg deuterium-labelled

acrylamide (d(3)-AA), representing 13 µg/kg body weight, in drinking water. Urine samples before dosing and within 46 h after the dose were analysed for d(3)-AAMA and d(3)-GAMA by LC-ESI-MS/MS. Total recovery in urine after 24 h 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 bw (Doerge et al., 2007). After 2 days AAMA accounted for 52% of the total AA dose, and was the major metabolite of AA in humans. GAMA accounted for 5%, and appeared as a minor metabolite of AA. 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., 2005a). The authors conclude that the metabolic fate of AA in humans was more similar to that in rats than in mice as previously demonstrated in terms of haemoglobin adducts.

Fuhr et al. (2006) evaluated the urinary levels of AA, AAMA, GA, and GAMA 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 (mean ± SD) 4.4 ± 1.5%, 50.0 ± 9.4%, and 5.9 ± 1.2% of the dose, respectively. Conjugation with glutathione exceeded the formation of the reactive metabolite glycidamide. The data suggests an at least 2-fold and 4-fold lower relative internal exposure for glycidamide from dietary acrylamide in humans compared with rats or mice, respectively.

Previous studies in people who are occupationally exposed or who smoke established that AA and GA form hemoglobin adducts (Bergmark, 1997; Calleman et al., 1994; Bergmark et al., 1993). AA was reported to form the N-(2-carbamoylethyl) valine and GA the N-(2-carbamoyl-2-hydroxyethyl)valine and the N-(1-carbamoyl-2- hydroxyethyl)-valine (Bergmark, 1997; Bergmark et al., 1993; Calleman et al., 1994). The detection of GA adducts of hemoglobin in AA-exposed workers demonstrated the transformation of acrylamide to glycidamide in humans (Bergmark et al., 1993). Hemoglobin adducts were first proposed as biomarkers of exposure to acrylamide by WHO (1985), and the initial analytical techniques were developed by Bailey et al. (1986). Other related compounds like acrylonitrile and N-methylolacrylamide (NMA) also form hemoglobin adducts. These confounders should be discussed if they are potentially present in studies that use AA hemoglobin adducts as the basis for estimating exposure to acrylamide. This is further discussed at the end of this section under the heading "Potential confounders for the hemoglobin adduct biomarker of acrylamide exposure."

Animal studies

Results from rat and mouse studies also indicate that acrylamide is rapidly metabolized and excreted predominantly in the urine as metabolites (Twaddle et al., 2004; Sumner et al., 2003, 1999, 1992; Dow Chemical Co., 1984; Dixit et al., 1982; Miller et al., 1982; Edwards, 1975). Formation of AA and GA hemoglobin adducts in rats has also been reported (Bergmark

et al., 1991). The hemoglobin binding index of AA to cysteine was found to be 6400 pmol/g Hb/μmol AA/kg, higher than for any other substance studied so far in the rat, and the hemoglobin binding index of GA to cysteine was 1820 pmol/g Hb/μmol GA/kg (Bergmark et al., 1991). The difference between AA and GA rates was proposed as being due primarily to a lower reactivity of GA than AA toward Hb-cysteine and a shorter half-life for GA in blood (based on determinations of these values in this study).

A metabolic scheme for acrylamide, based on results from these and other studies, is illustrated in Figure 3-1. AA 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. 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]-labeled acrylamide (Sumner et al., 1992).

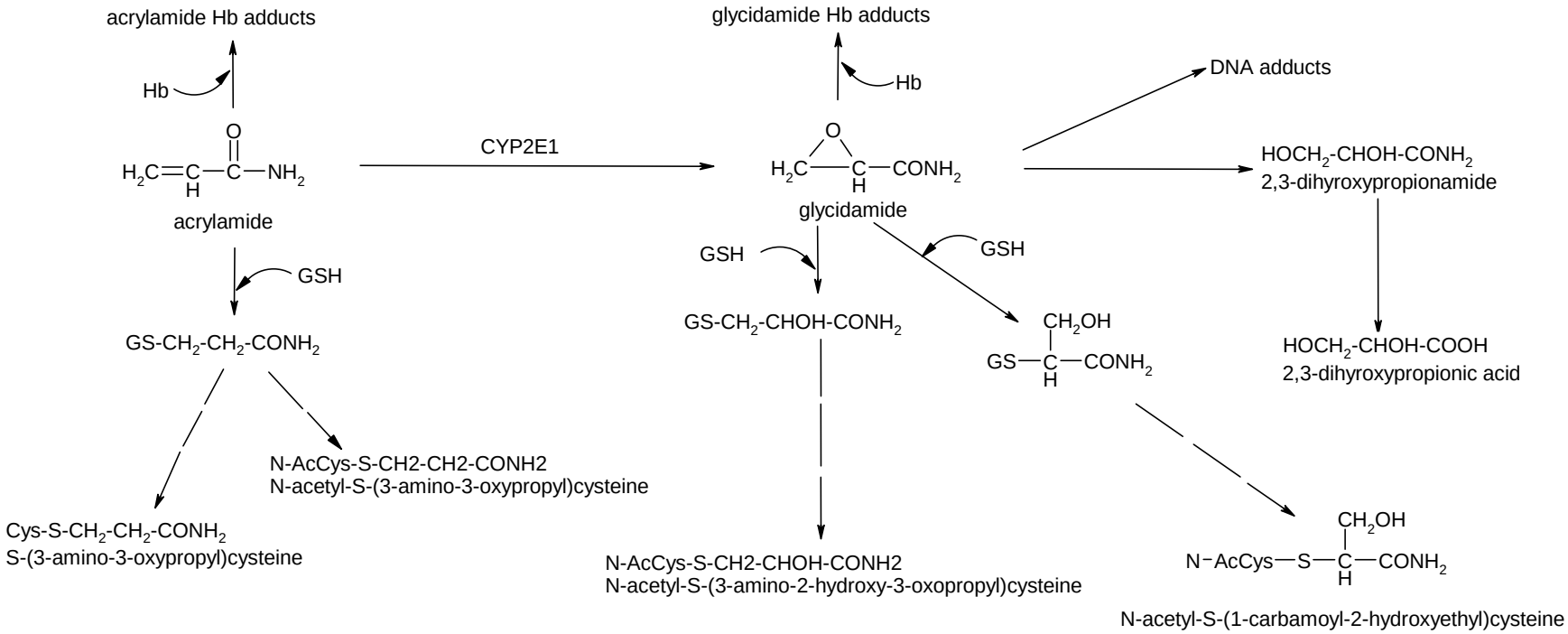

Figure 3-1. Metabolic scheme for acrylamide (AA) and its metabolite glycidamide (GA).

Note: Processes involving several steps are represented with broken arrows. Abbreviations: Hb, hemoglobin; GSH, reduced glutathione; N-AcCys, N-acetylcysteine.

Sources: Adapted from Sumner et al. (1999); Calleman (1996); IARC (1994a).

Table 3-2 lists the relative amounts of AA metabolites determined by ^{13}C-NMR analysis of urine collected for 24 hours in the latter of these studies. In another study with wild-type C57BL/6N × Sv129 mice exposed to 50 mg/kg [1,2,3-^{13}C]-labeled acrylamide, N-acetyl-S-(3-amino-3-oxopropyl)cysteine and S-(3-amino-3-oxopropyl)cysteine accounted for 29% and 20% of total metabolites excreted within 24 hours in the urine (Sumner et al., 1999).

Table 3-2. Metabolites detected in urine collected for 24 hours following oral administration of [1,2,3-^{13}C]-labeled acrylamide (50 mg/kg) to male F344 rats or male B6C3F1 mice

	% of total metabolites excreted in urine in 24 hours (mean ± SD, n = 3)	
Metabolite[a]	**Rat**	**Mouse**[b]
From AA precursor		
N-acetyl-S-(3-amino-3-oxopropyl)cysteine	67.4 ± 3.6	41.2 ± 2.2
Glycidamide	5.5 ± 1.0	16.8 ± 2.1
From GA precursor		
N-acetyl-S-(3-amino-2-hydroxy-3-oxopropyl)cysteine	15.7 ± 1.3	21.3 ± 0.6
N-acetyl-S-(1-carbamoyl-2-hydroxyethyl)cysteine	9.0 ± 1.1	11.7 ± 0.6
2,3-Dihydroxypropionamide	2.4 ± 0.7	5.3 ± 1.2

[a]^{13}C-NMR analysis was used to detect, identify, and quantify metabolites in urine. Urinary metabolites accounted for about 50% of the administered dose in both species. Unchanged acrylamide was detected in urine but was not quantified. In other studies with F344 rats exposed to [2,3-^{14}C]-labeled acrylamide, less than 2% of administered radiolabel was excreted in urine and bile as unchanged acrylamide (Miller et al., 1982).
[b]In mice, an epoxide degradation product accounted for 4% of the total metabolites excreted.

Source: Sumner et al. (1992).

Another initial step, catalyzed by CYP2E1, involves oxidation of acrylamide to the epoxide derivative, glycidamide. Gylcidamide (either at the number 2 or 3 carbon) can react with GSH 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 (Sumner et al., 1992, 1999), leading to the formation of 2,3-dihydroxypropionamide and 2,3-dihydroxypropionic acid. Glycidamide and metabolites (or degradation products) derived from it accounted for about 33 and 59% of the total metabolites excreted in rat and mouse urine within 24 hours, respectively (Table 3-2), indicating that, under these test conditions, the rate of transformation from acrylamide to glycidamide is about twofold greater in mice than in rats. Similar results were reported in a study of metabolites in urine collected for 24 hours after 6-hour inhalation exposure (nose only) to 3 ppm acrylamide (Sumner et al., 2003). Glycidamide and metabolites derived from it accounted for 36 ± 2.4% and 73 ± 3.7% of total metabolites excreted in rat and mouse urine within 24 hours, respectively (Sumner et al., 2003).

Results from mouse studies indicate that mouse CYP2E1 is the only CYP isozyme that catalyzes the oxidative formation of GA from AA. Following oral administration of single 50 mg/kg doses of [1,2,3-^{13}C]-labeled acrylamide, no evidence of metabolites formed through GA was found by ^{13}C-NMR analysis of urine collected for 24 hours from C57BL/6N × Sv129 mice devoid of CYP2E1 (CYP2E1 null) or wild-type mice of the same strain treated with the CYP2E1 inhibitor, aminobenzotriazole (ABT) (50 mg/kg i.p. injection 2 hours preexposure) (Sumner et al., 1999). In contrast, urine collected from wild-type mice contained considerable amounts of metabolites derived from GA (Sumner et al., 1999). With wild-type mice in this study, 22% of excreted metabolites were accounted for by metabolites 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% of excreted metabolites were accounted for by glycidamide and its hydrolysis products (2,3-dihydroxypropionamide and 2,3-dihydroxypropionic acid). 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 AA via direct conjugation with GSH.

Information on human specificity of CYP2E1 to AA oxidation is lacking. It is noted, however, that age related increases in human CYP2E1 expression have been reported. Johnsrud et al. (2003) evaluated the content of CYP2E1 in human hepatic microsomes from samples spanning fetal (n = 73, 8–37 weeks) and postnatal (n = 165, 1 day–18 years) ages. Measurable immunodetectable CYP2E1 was seen in 18 of 49 second-trimester fetal samples (93–186 gestational days; median level = 0.35 pmol/mg microsomal protein) and 12 of 15 third-trimester samples (>186 days, median level = 6.7 pmol/mg microsomal protein). CYP2E1 in neonatal samples was low and less than that of infants 31 to 90 days of age, which was less than that of older infants, children, and young adults (median [range] = 8.8 [0–70]; 23.8 [10–43]; 41.4 [18–95] pmol/mg microsomal protein, respectively; each $p < 0.001$, analysis of variance, post hoc). Among those older than 90 days of age, CYP2E1 content was similar. A fourfold or greater intersubject variation was observed among samples from each age group, with the greatest variation, 80-fold, seen among neonatal samples. These results suggest that infants less than 90 days old may have decreased clearance of CYP2E1 substrates compared with older infants, children, and adults. However, actual differences will depend upon the delivery rate and substrate concentration relative to the value of the Michaelis-Menten constant (Km) for CYP2E1, which will determine the total amount metabolized (or parent compound cleared) (Lipscomb, 2004; Lipscomb et al., 2003). The higher the substrate concentration relative to Km, the more marked will be the influence of enzyme level (i.e., maximum activity level) on total clearance for a saturable enzyme like CYP2E1.

Figure 3-1 does not include a possible minor pathway hypothesized to result in the release of CO_2 from hydrolysis products of GA. This pathway is not included because of conflicting results from several studies. Following i.v. 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 F344 rats, no radioactivity was detected in exhaled breath (Miller et al., 1982). Sumner et al. (1992) noted that these results may be consistent with the existence of a minor pathway involving metabolism of 2,3-dihydroxypropionamide (glyceramide) to glycerate and hydroxypyruvate with the subsequent release of CO_2 and production of glycolaldehyde, but they did not detect labeled two-carbon metabolites in urine of mice exposed to [1,2,3-^{13}C]-labeled acrylamide. In other experiments, no exhaled $^{14}CO_2$ was detected following oral administration of 50 mg/kg [1-^{14}C]-labeled acrylamide to male Sprague-Dawley rats (Kadry et al., 1999), whereas 3–4% of i.v. injected [1,3-^{14}C]-acrylamide (2 or 100 mg/kg) was detected as $^{14}CO_2$ in exhaled breath in male F344 rats (Dow Chemical Co., 1984). During a 24-hour period following a 24-hour dermal exposure of male F344 rats to 162 mg/kg [2,3-^{14}C]-labeled acrylamide, $^{14}CO_2$ in exhaled breath accounted for 1.8 ± 0.2% of radioactivity recovered in exhaled air, urine, feces, and tissues (Sumner et al., 2003). Similarly, $^{14}CO_2$ in exhaled breath accounted for 1.7 ± 0.1% and 0.9 ± 0.2% of radioactivity recovered in exhaled air, urine, feces, and tissues in male B6C3F1 mice and F344 rats, respectively, following nose-only inhalation exposure to 3 ppm of a mixture of [1,2,3-^{13}C]-acrylamide and [2,3-^{14}C]-acrylamide (Sumner et al., 2003).

Results from a rat kinetic study by Sumner et al. (2003) indicate an intraparenteral (i.p.). or gavage route of exposure had a small effect on the percentage of acrylamide conjugated to GSH vs. the percentage of AA converted to GA. Following i.p. or gavage administration of 50 mg/kg [1,2,3-^{13}C]-acrylamide to male F344 rats, 69 ± 0.9% or 71 ± 3.8% of total urinary metabolites, respectively, were metabolites associated with direct conjugation of AA with glutathione. In contrast, metabolites associated with direct conjugation of AA with glutathione accounted for 64 ± 2.4% of metabolites in urine collected for 24 hours following 6-hour inhalation (nose only) exposure of male F344 rats to 3 ppm of a mixture of radiolabeled [1,2,3-^{13}C]- and [2,3-^{14}C]-acrylamide. The percentages of total urinary metabolites associated with GA formation were 31 ± 0.9%, 28 ± 3.8%, and 36 ± 2.4% following i.p., gavage, and inhalation exposure, respectively. The percentages of urinary metabolites associated with GA formation following i.p. and gavage exposure were reported to be statistically significantly smaller than the value for inhalation exposure. These results indicate that a smaller percentage of AA is converted to GA following oral or intraparenteral exposure compared with inhalation exposure. The biological significance of this small difference is uncertain. These results in

F344 rats are in contrast to the increased percentage of GA formation observed in mice from an oral gavage or dietary exposure vs. an i.v. exposure (Doerge et al., 2005b).

Lehning et al. (1998) report that repeated oral exposures of 26–45 days to AA at relatively low doses (e.g., 20 mg/kg-day from drinking water concentrations of 20 mM) induces axonal degeneration, but shorter-term (11 days) exposure to higher i.p. doses (50 mg/kg-day) does not. Barber et al. (2001) compared AA metabolism and toxicokinetics for these dosing regimens, but did not find differences that provided a clear explanation for the occurrence of degeneration with the longer oral dosing regimen. In this study, plasma concentrations of radioactivity in AA and GA were determined from tail-vein blood samples that were collected from groups of five to seven Sprague-Dawley rats at nine intervals from 0 to 580 minutes following a single administration of [2,3-^{14}C]-labeled acrylamide by gavage (24 hours after the last dose of a drinking water solution of 20 mg/kg-day nonlabeled acrylamide for 34 days) or by a single i.p. injection (on day 11 of the i.p. administration of 50 mg/kg-day for 11 days). The authors noted that the toxicokinetics from a single gavage dose had been evaluated in separate experiments, and in the opinion of the authors, gave a reasonable estimate of the AUCs and half-life of a drinking water exposure that was simulated with multiple smaller doses (i.e., data not shown).

Barber et al. (2001) also measured the activities of CYP2E1 and epoxide hydrolase in liver microsomes, as well as concentrations of AA-hemoglobin and GA-hemoglobin adducts before treatment, after i.p. exposure for 5 or 11 days and after 15, 34, and 47 days of oral exposure. With both dosing regimens, AA appeared rapidly in plasma and rose to peak concentrations within 60–90 minutes, followed by peak levels of GA. Respective plasma half-lives ($t_{½}$) were approximately 2 hours and peak plasma levels for each route were directly related to the magnitude of the respective daily dose (i.e., the i.p. dose and resulting C_{max} were both 2.5 times larger than comparable oral parameters). The only differences found in metabolic or toxicokinetic parameters for the two dosing regimens involved some, but not all, parameters that determined GA formation and metabolism. Derived areas under the plasma concentration vs. time curves (AUCs) indicated that a larger proportion of plasma AA was converted to GA following the longer oral regimen (30%) compared with the 11-day i.p. regimen (8%), although the GA AUCs were similar (822 units for 20 mg/kg-day subchronic oral vs. 730 units for 50 mg/kg-day 11-day i.p.) and no correlation was found to the different enzyme activities involved in GA formation (CYP2E1) or metabolism (epoxide hydrolase). Concentrations of AA-hemoglobin adducts with the longer oral regimen were about 30% less than concentrations from the i.p. regimen, and concentrations of GA-hemoglobin adducts were about twofold higher than those from the i.p. regimen. Barber et al. (2001) noted that, although it has been proposed that GA might mediate axonal degeneration, peak concentrations of free GA with the subchronic oral regime were relatively low and other studies showed that GA is only a weak neurotoxicant. It

was concluded that the mechanism of axonal degeneration did not appear to involve route- or dose-rate differences in metabolism or disposition of acrylamide.

Doerge et al. (2005b) compared the toxicokinetics of AA and GA in serum and tissues of male and female B6C3F1 mice following a single dose by i.v. injection or gavage of 0.1 mg/kg AA, or a comparable dose of 0.1 mg/kg AA from a feeding exposure for 30 minutes. Study groups also received an equimolar amount of GA from either an i.v. injection or gavage dose. Oral exposure to AA resulted in higher relative internal levels of GA compared with levels following an i.v. exposure, likely due to a first-pass effect, but possibly the result of some other kinetic change. In comparing the results of this study with previous studies by this laboratory at a 500-fold higher concentration (Twaddle et al., 2004), an increase in relative internal GA levels was observed, suggesting that as dose rate decreases, the conversion of AA to GA in mice is more efficient.

Differences in mouse and rat metabolism

Twaddle et al. (2004) administered AA at approximately 50 mg/kg via gavage to adult male and female B6C3F1 mice. Serum concentrations of AA and GA were taken at 0.5, 1, 2, 4, and 8 hours postdosing. Livers were removed from control and AA-treated mice at all exposure times, and analyzed for GA derived DNA adducts. The results indicated no systematic sex differences in AA and GA serum levels at each time point for the species and doses in this study. Twaddle et al. (2004) estimated an AA half-life of elimination from plasma at 0.73 hours in these B6C3F1 mice. 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 AA in drinking water for 34 days or subacute i.p. 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 AA in rat blood following a 10 mg/kg i.v. dose. For GA, Twaddle et al. (2004) report that the mice had an elimination half-life for GA of 1.9 hours, which is identical to that measured by Barber et al. (2001) in rats. Barber et al. (2001) also reported a GA/AA-AUC ratio of 0.18 for Sprague-Dawley rats treated with 20 mg/kg AA by gavage. This contrasts to Twaddle et al.'s (2004) observation of equal AUCs for AA and GA in B6C3F1 mice. Since rats and mice had a comparable GA elimination half-life, this approximately five-fold difference in internal exposure to GA for mice compared with rats (i.e., a GA/AA-AUC ratio of 1 in mice vs. a GA/AA-AUC ratio of 0.18 in rats) is considered to be the result of an increased rate of GA formation in the mouse.

Formation of DNA adducts

Doerge et al. (2005a) measured DNA adducts following a single i.p. administration of AA to adult B6C3F1 mice and F344 rats at 50 mg acrylamide/kg, or an equimolar dose of GA (61 mg/kg). They report GA-derived DNA adducts of adenine and guanine formed in all tissues

examined, including both target tissues identified in rodent carcinogenicity bioassays and nontarget tissues, including liver, brain, thyroid, leukocytes, mammary gland, and testis in rats, and in liver, lung, kidney, leukocytes, and testis in mice. Dosing rats and mice with an equimolar amount of GA typically produced higher levels of DNA adducts than those observed from the AA dose.

Doerge et al. (2005a) also measured DNA adduct formation following oral administration of a single dose of AA (50 mg/kg), and accumulation from repeat dosing at 1 mg/kg-day. The formation of DNA adducts was consistent with the previously reported mutagenicity of AA and GA in vitro, which involved reaction of GA with adenine and guanine bases. These results provide support for a mutagenic mechanism of AA carcinogenicity in rodents.

Acrylamide and glycidamide react with nucleophilic sites in macromolecules (including hemoglobin and DNA [Figure 3-2]) in Michael-type additions (Segerbäck et al., 1995; Bergmark et al., 1993, 1991; Solomon et al., 1985). Solomon et al. (1985) conducted in vitro studies for the reaction of acrylamide at pH 7.0 and 37°C for 10 and 40 days with 2'-deoxyadenosine (dAdo), 2'-deoxycytidine (dCyd), 2'-deoxyguanosine (dGua), and 2'-deoxythymidine (dThd), which resulted in the formation of 2-formamidoethyl and 2-carboxyethyl adducts via Michael addition. However, AA reacted extremely weakly with DNA (second order rate constant of 9×10^{-12} L/mg DNA-hour at pH 7 and 37°C for all adducts), even under in vitro conditions, producing significant levels of adducts only after incubations of several weeks with high acrylamide concentrations (Solomon et al., 1985). Based on the second order rate constant derived by Solomon et al. (1985), Segerbäck et al. (1995) estimated formation of 25 fmol/mg DNA for all adducts from an in vivo i.p. AA dose of 50 mg/kg. Only about 14% of these would be adducts to the N-7 atom of guanine. This amount was considered to be negligible compared with observed levels of N-7-(2-carbamoyl-2-hydroxyethyl)guanine adducts with glycidamide, which were in the 20–30 pmol/mg DNA range for the in liver of both mice and rats from a comparable (46–53 mg/kg) i.p. dose (Segerbäck et al., 1995). Two additional glycidamide-DNA adducts have been identified in vitro, N3-(2-carbamoyl-2-hydroxyethyl)adenine (N3-GA-Ade) and N1-(2-carboxy-2-hydroxyethyl)-2'-deoxyadenosine (Gamboa et al., 2003). Using liquid chromatography with tandem mass spectrometry and isotope dilution, Gamboa et al. (2003) measured DNA adduct formation in selected tissues of adult and whole body DNA of 3-day-old neonatal mice treated with AA and GA. In adult mice, DNA adduct formation was observed in liver, lung, and kidney with levels of N7-GA-Gua around 2000 adducts/10^8 nucleotides and N3-GA-Ade around 20 adducts/10^8 nucleotides. Adduct levels were modestly higher in adult mice dosed with GA as opposed to AA; however, treatment of neonatal mice with GA produced five- to sevenfold higher whole body DNA adduct levels than with AA. The authors suggest that this is due to lower oxidative enzyme activity in newborn mice. DNA adduct formation from

AA treatment in adult mice showed a supralinear dose-response relationship, consistent with saturation of oxidative metabolism at higher doses.

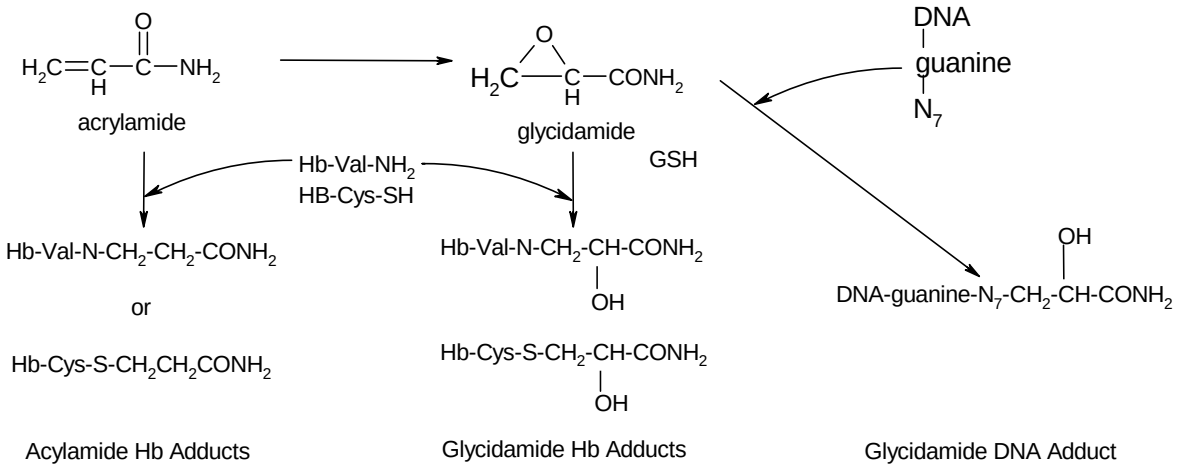

Figure 3-2. Hemoglobin and DNA adducts of acrylamide and glycidamide.

Sources: Dearfield et al. (1995); Bergmark et al. (1993, 1991).

Potential confounders for the hemoglobin adduct biomarker of acrylamide exposure

Other related compounds like acrylonitrile and N-methylolacrylamide (NMA) also form hemoglobin adducts. NMA is produced by the reaction of formaldehyde with acrylamide and, like acrylamide, is used in the production of grouting agents. Acrylonitrile can be used as a precursor in one method to manufacture acrylamide, and is also formed when acrylamide is dehydrogenated.

Studies that use AA hemoglobin adducts as a biomarker for exposure should address the potential presence of NMA. Acrylonitrile forms an N-(2-cyanoethyl)valine adduct that is distinguishable from the acrylamide N-(2-carbamoylethyl)valine adduct with gas chromatography/mass spectrometry (GC-MS) analysis after derivatization with pentafluorophenyl isothiocyanate (Bergmark et al., 1993). N-methylolacrylamide, however, forms the same adduct as acrylamide, the N-(2-carbamoylethyl)valine adduct. It is not known whether NMA undergoes loss of the hydroxymethyl group to form AA, which can then react with globin to form AAVal, or if NMA reacts directly with globin and then loses the hydroxymethyl group to form AAVal. Both reactions, involving loss of formaldehyde, could occur on a chemical basis without the involvement of metabolism (Fennell et al., 2003). There are also differing results on the relative rate of formation of the N-(2-carbamoylethyl) valine adduct from AA or NMA (Paulsson et al., 2002; Fennell et al., 2003).

Paulsson et al. (2002) measured hemoglobin adducts (and micronucleus [MN] frequencies) in mice and rats after acrylamide or NMA treatment. Male CBA mice were treated by i.p. injection of 0.35, 0.7, and 1.4 mmol/kg for both compounds (i.e., 25, 50, and 100 mg AA/kg, or 35, 71, and 142 mg NMA/kg). The rats were only treated with the highest dose of AA or NMA, 100 mg/kg or 142 mg/kg, respectively. Mice were sacrificed after 48 hours and blood was collected for hemoglobin adduct measurement. One group of rats was sacrificed after 24 hours and one group after 48 hours for the hemoglobin adduct analysis. The identical (N-[2-carbamoylethyl]valine) adduct and the respective epoxide metabolite (N-[2-carbamoyl-2-hydroxyethyl]valine) adduct were monitored for either the AA or NMA exposure. Per unit of administered amount, AA gave rise to three to six times higher hemoglobin adduct levels than NMA in mice and rats. Mice exhibited higher in vivo doses of the epoxy metabolites, compared with rats, indicating that AA and NMA were more efficiently metabolized in the mice. In mice the AA and NMA induced dose-dependent increases in both hemoglobin adduct level and MN frequency in peripheral erythrocytes. Per unit of administered dose, NMA showed only half the potency for inducing micronuclei compared with AA, although the MN frequency per unit of in vivo dose of measured epoxy metabolite was three times higher for NMA than for AA. No increase in MN frequency was observed in rat bone marrow erythrocytes after treatment with either compound. This is compatible with a lower sensitivity of the rat than of the mouse to the carcinogenic action of these compounds.

Fennell et al. (2003) also measured levels of N-(2-carbamoylethyl)valine adducts following gavage exposure of male F344 rats (4/group) to equimolar levels of acrylamide or NMA. The nominal dose of [1,2,3-^{13}C]-AA was 50 mg/kg, and NMA was administered at a nominal dose of 71 mg/kg. The AA and NMA dose solutions were prepared in distilled water and delivered at 1 mL/kg. In contrast to Paulsson et al. (2002), Fennell et al. (2003) reported that acrylamide exposure resulted in the formation of 21 ± 1.7 pmol/mg globin (mean ± SD), less than the equimolar dose of NMA that resulted in 41 ± 4.9 pmol/mg. Since rates of formation of the N-terminal valine adduct are not comparable (regardless of whether more or less) and both compounds form the same adduct, caution should be exercised when drawing conclusions about acrylamide exposure based on N-terminal valine levels if there is also a potential for concurrent exposure to NMA.

3.4. ELIMINATION

Human data

Boettcher et al. (2005) measured the mercapturic acid of AA and its epoxide glycidamide (GA) i.e. N-acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA) and N-(R,S)-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA) in human urine. Median levels in smokers (n=13) were found to be about four times higher than in non-smokers (n=16) with median levels

of 127 µg/L versus 29 µg/L for AAMA and µg/L versus µg/L for GAMA indicating that cigarette smoke is clearly an important source of acrylamide exposure. The level of AAMA in the occupationally non-exposed collective (n=29) ranged from 3 to 338 µg/L, the level of GAMA from <LOD to 45 µg/L.

Fennell et al. (2005) report that approximately 34% of the orally administered dose of 3 mg AA/kg to adult male volunteers was recovered in the total urinary metabolites within 24 hours of administration. A dermal exposure to humans was part of this study but no elimination data from the dermal exposure were reported.

Boettcher et al. (2006a) investigated the human metabolism of AA to AAMA and GAMA in a healthy male volunteer who received a single dose of about 1 mg deuterium-labelled acrylamide (d(3)-AA), representing 13 µg/kg body weight, in drinking water. Urine samples before dosing and within 46 h after the dose were analysed for d(3)-AAMA and d(3)-GAMA by LC-ESI-MS/MS. A first phase of increase in urinary concentration was found to last 18 h with a broad plateau between 8 and 18 h for AAMA, and 22 h for GAMA. Elimination half-lives of both AAMA and GAMA were estimated to be approximately 3.5 h for the first phase and more than 10 h up to few days for the second phase. Total recovery in urine after 24 h 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 bw (Doerge et al., 2007). After 2 days AAMA accounted for 52% of the total AA dose, and was the major metabolite of AA in humans. GAMA accounted for 5%, and appeared as a minor metabolite of AA.

Fuhr et al. (2006) measured AA 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. Overall, 60.3 ± 11.2% of the dose was recovered in the urine. Although no glycidamide was found, unchanged acrylamide, AAMA, and GAMA accounted for urinary excretion of (mean ± SD) 4.4 ± 1.5%, 50.0 ± 9.4%, and 5.9 ± 1.2% of the dose, respectively. Toxicokinetic variables were obtained by noncompartmental methods, with apparent terminal elimination half-lives for the unchanged acrylamide, AAMA, and GAMA of 2.4 ± 0.4, 17.4 ± 3.9, and 25.1 ± 6.4 hours, respectively.

Boettcher et al. (2006b) evaluated urinary mercapturic acid metabolites derived from AA in three healthy volunteers who fasted for 48 h. Urinary AA mercapturic acid metabolites were considerably reduced after 48 h of fasting, with levels well below the median level in non-smokers. These results indicate that, for nonsmokers, acrylamide in the diet is the main source of environmental AA exposure in humans.

Bjellaas et al. (2007) reported urinary mercapturic acid derivatives of AA and in a clinical study comprising of 53 subjects. Urinary metabolite levels were determined using solid-phase extraction and liquid chromatography with positive electrospray MS/MS detection. The median (range) total excretion of acrylamide in urine during 24 h was 16 (7-47) µg acrylamide

for non-smokers and 74 (38-106) μg acrylamide for smokers. Median intakes (range) of AA were estimated based on 24 h dietary recall as 21 (13-178) μg for non-smokers 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. In a multiple linear regression analysis, the urinary excretion of acrylamide metabolites correlated statistically significant with intake of aspartic acid, protein, starch and coffee. Consumption of citrus fruits correlated negatively with excretion of acrylamide metabolites.

Animal data

Results from animal studies indicate that urinary excretion of metabolites is the principal route of elimination of absorbed acrylamide, with minor amounts of metabolites being excreted via bile in the feces, and as CO_2 in exhaled breath (Barber et al., 2001; Kadry et al., 1999; Sumner et al., 1999, 1992; Dow Chemical Co., 1984; Miller et al., 1982; Hashimoto and Aldridge, 1970).

Fennell et al. (2005) administered 3 mg/kg [1,2,3-^{13}C]-AA by gavage to F344 rats. The low 3 mg/kg dose of AA by gavage to rats resulted in a greater amount of metabolism via GA (41% of the urinary metabolites) compared with a higher dose of 59 mg/kg (28% of the urinary metabolites) (Sumner et al., 2003). The fate of GA was primarily conjugation with GSH, resulting in the excretion of two mercapturic acids. The total amount of AA metabolites recovered by 24 hours after dosing was 50%, similar to that reported by Kadry et al. (1999) and Miller et al. (1982).

In male F344 rats given i.v. (10 mg/kg) or 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 AA. With either route of administration, elimination of radioactivity from tissues was described as biphasic, with half-lives of about 5 hours for the first phase and 8 days for the second phase. The elimination time course of parent AA from tissues followed a single-phase exponential decrease with a half-life of about 2 hours. Calleman (1996) noted that this is a relatively slow elimination half-life for an electrophilic chemical, citing the elimination half-life of acrylonitrile, a related electrophilic chemical, at about 10 minutes in rats. Fecal excretion accounted for 4.8 and 6% of administered radioactivity at 24 hours and 7 days, respectively (Miller et al., 1982). Bile-duct-cannulated rats given single i.v. doses of 10 mg/kg [2,3-^{14}C]-labeled acrylamide excreted about 15% of the administered radioactivity in bile as metabolites within about 6 hours; less than 1% of radioactivity in the bile was in the form of AA. These results are consistent with the existence of enterohepatic circulation of metabolites.

No radiolabeled CO_2 was captured when two rats given [2,3-^{14}C]-labeled acrylamide were placed in metabolism cages designed to trap expired air (Miller et al., 1982). In contrast,

studies with radiolabel in the carbon-1 position suggest that exhalation of CO_2 following cleavage of the amide group is possible but likely represents a minor metabolic and elimination pathway (see Figures 2-1 and 3-1 for carbon numbering and metabolic pathways, respectively). About 6% of an injected dose of 100 mg/kg [1-^{14}C]-labeled acrylamide (Hashimoto and Aldridge, 1970) and about 4% of an injected dose of 2 mg/kg [1,3-^{14}C]-labeled acrylamide (Dow Chemical Co., 1984) were exhaled by rats as CO_2 in 6–8 hours. As noted earlier, however, no exhaled $^{14}CO_2$ was detected following oral administration of 50 mg/kg [1-^{14}C]-labeled acrylamide to male Sprague-Dawley rats (Kadry et al., 1999).

In studies with male F344 rats given single i.v. doses of [1,3-^{14}C]-labeled acrylamide, percentages of the administered dose recovered in excreta, carcass, and cage wash after 72 hours were as follows for four rats exposed to 2 mg/kg: 67% urine; 1.5% feces; 4.2% CO_2; 1.5% skin; 13.1% carcass; and 0.6% cage wash (Dow Chemical Co., 1984). Similar percentages were reported for four rats injected with 100 mg/kg. Other groups of rats were given single i.v. injections of 50 mg/kg [1,3-^{14}C]-labeled acrylamide and were killed in groups of 3–4 after 0, 6, 12, 18, 24, or 48 hours for determination of radioactivity in blood plasma, red blood cells, and selected tissues (testes, epididymis, kidney, and sciatic nerve). The clearance of radioactivity from the plasma and the tissues was consistent with biphasic elimination with an initial rapid phase, followed by a slower phase. Plasma elimination half-times were estimated at 2 hours for the initial phase and 10 hours for the second slower phase. GC/MS analysis indicated that the initial phase was primarily due to clearance of acrylamide, whereas the second phase was due to clearance of radiolabeled metabolites from the plasma.

Tong et al. (2004) estimated the second order rate constants for reaction of AA with human serum albumin and glutathione at 0.0054 and 0.021/mol-second, respectively. These rates were determined under physiological conditions by following the loss of their thiol groups in the presence of excess AA. Based on these in vitro values, the authors concluded that the reactions of AA with these thiols appears to account for most of AA's elimination from the body.

More recently, Doerge et al. (2007) measured 24 hour urinary metabolites, including free AA and GA and their mercapturic acid conjugates (AAMA and GAMA, respectively), using LC/MS/MS in F344 rats and B6C3F(1) mice following a dose of 0.1 mg/kg bw given by intravenous, gavage, and dietary routes of administration. The results were compared with serum/tissue toxicokinetic and adduct data (DNA and hemoglobin) from previous studies in the same laboratory using the identical dosing protocols (Doerge et al., 2005 a,b,c). The goal was to investigate relationships between urinary and circulating biomarkers of exposure, toxicokinetic parameters for AA and GA, and tissue GA-DNA adducts in rodents from single doses of AA. The molar percentage of the total intravenously delivered dose that was recovered as free acrylamide and metabolites in a 24 hour urine collection was 57-74% and 54-57% in male and female rats, respectively; and 62-82% and 60-63% in male and female mice, respectively.

Significant linear correlations were observed between urinary levels of AA with AAMA and GA with GAMA in the current data sets for rats (AA vs. AAMA, r^2 =0.78, p<0.001; GA vs. GAMA, r^2 =0.81, p<0.001) and mice (AA vs. AAMA, r^2 =0.86, p<0.001; GA vs. GAMA, r^2 =0.57, p<0.001) . Concentrations of urinary AA or AAMA correlated significantly with average AUC values for serum AA determined previously in groups of rats (AUC-AA vs. AA, r^2 =0.74, p<0.001; AUC-AA vs. AAMA, r^2 =0.83, p<0.001) and mice (AUC-AA vs. AA, r^2 =0.41, p<0.011; AUC-AA vs. AAMA, r^2 =0.41, p<0.01) similarly dosed with AA. Correlation coefficients for urinary GA and GAMA concentrations versus AUC serum GA and liver GA-DNA adducts were smaller that for the AA and AAMA, but still significant in rats (AUC-GA vs. GA, r^2 =0.53, p<0.001; AUC-GA vs. GAMA, r^2 =0.32, p<0.02) and mice (AUC-GA vs. GA, r^2 =0.34, p<0.022; AUC-GA vs. GAMA, r^2 =0.56, p<0.0001). Significant linear correlations were also observed in rats between urinary concentrations of either GA or GAMA with average GA-DNA adducts (p=0.001 and 0.2, respectively); data not presented in the publication. In mice, a significant linear correlation was observed between urinary concentrations of GA (p=0.03), but not GAMA (p=0.2), with average GA-DNA adducts; data not presented in the publication. In both rats and mice, significant linear correlations were observed between AA or AAMA and average GA-DNA adduct levels (p=0.0005 and 0.004, respectively); data not presented in the publication. Although considerable interindividual variability observed in all urinary measurements weakened the correlation with either average toxicokinetic or biomarker data collected from different groups of animals, overall the results indicate that urinary biomarkers do reflect internal levels of AA and GA, and may be useful (accompanied by appropriate caveats) in estimating levels of exposure and potential risk for adverse effects.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

Two physiologically based toxicokinetic (PBTK) models for acrylamide were available from the literature (Kirman et al., 2003; Young et al., 2007). Kirman et al. (2003) developed a PBTK model that simulates the disposition of AA and its epoxide metabolite, GA, in the rat based upon published kinetic data from the 1980s and early 1990s (Miller et al., 1982; Ramsey et al., 1984; Raymer et al., 1993; Sumner et al., 1992). The Kirman et al. model parameters were recalibrated by EPA using more recent kinetic and hemoglobin adduct data in rats and mice (Sumner et al., 2003, Fennell et al., 2005; Doerge et al., 2005b,c) and human kinetic and hemoglobin adduct data (Fennell et al., 2005, Boettcher et al., 2005). The recalibrated Kirman et al. PBTK model was used in deriving oral and inhalation toxicity values for acylamide's potential noncancer and cancer effects (see Section 5).

Young et al. (2007) developed a PBTK/TD (toxicodynamic) model that simulates AA and GA kinetics in mice, rats, and humans, and adds representation of GA-DNA adduct

formation (considered a toxicodynamic event in the pathway leading to mutagenicity). The Young et al. model parameter values were based on rat and mouse kinetic data generated at the US FDA's National Center for Toxicological Research (NCTR) (Doerge et al., 2005 a,b,c) and from the literature (Barber et al., 2001; Sumner et al., 1992, 2003; Raymer et al., 1993); on published human urinary excretion data (Fuhr et al., 2006; Fennel et al., 2005) and on human hemoglobin adduct data from a dietary exposure (Boettcher et al., 2005). Young et al. use the PBTK model to fit individual animal PK data, and then evaluate the resulting differences in parameter values (and distributions). For the Young et al. (2007) model to be used for specific application in deriving a toxicity value, additional work is needed to determine which parameter values would be the most appropriate to use.

The following discussion provides a general description of each model, and is accompanied by Appendix E and Appendix F that contain tables of text with additional detailed information on model parameters and simulation results.

Kirman et al. (2003) PBTK Model

A diagram of the Kirman et al. (2003) model is presented in Figure 3-2. This model simulates the distribution of AA and GA within five compartments—arterial blood, venous blood, liver, lung, and all other tissues lumped together. The arterial and venous blood compartments are further divided into serum and blood cell subcompartments to model specific data sets (e.g., chemical bound to hemoglobin in red blood cells). Different routes of exposure to AA are represented in the Kirman et al. model including intravenous (i.v.), intraperitoneal (i.p.), oral gavage, oral drinking water, and inhalation. Metabolism of AA and GA are represented only in the liver. Hepatic metabolism of AA proceeds via two pathways: 1) saturable epoxidation by cytochrome P-450 to produce GA; and 2) first-order conjugation with glutathione (GSH) via glutathione S-transferase (GST) to ultimately yield N-acetyl-S-(3-amino-3-oxopropyl)cysteine. Hepatic metabolism of GA proceeds either with: 1) a first-order conjugation with GSH to yield N-acetyl-S-(3-amino-2-hydroxy-3-oxopropyl)cysteine and N-acetyl-S-(carbamoyl-2-hydroxyethyl)cysteine; or 2) with further saturable metabolism by epoxide hydrolase to yield 2,3-dihydroxypropionamide. Based on the reactivity of AA and GA with GSH, and the potential for depletion of hepatic GSH with sufficiently high doses of AA, GSH depletion and resynthesis are also represented in the model structure. Free GA enters into the GA portion of the model from the oxidative metabolism of AA in the liver compartment. The model also represents binding of AA and GA to hemoglobin, or to liver, tissue, or blood macromolecules. The model was originally developed in ACSL, version 11.8.4 (Aegis Technologies Group, Huntsville, AL), and has subsequently been revised in acslXtreme version 2.3.014, as well as inplemented in Excel.

The model parameters values and sources in the original Kirman model are presented in Appendix E, Table E-1. The sources include measured or calculated values for rat physiological parameters from the literature (tissue volumes, blood flows), estimates for the tissue partition coefficients for AA based on a published algorithm or specific chemical properties (e.g., solubility in water and octanol, vapor pressure), estimates for GA tissue partition coefficients from values for AA using a proportionality constant of 3.2 derived from the ratio of structural analogs (acrylonitrile and its epoxide metabolite, cyanoethylene oxide), and estimates of metabolism and tissue binding rates optimized to fit tissue levels of administered [^{14}C]-radiolabeled AA (Ramsey et al., 1984; Miller et al., 1982), or to urinary metabolite levels (Raymer et al. 1993 Sumner et al., 1992; Miller et al., 1982). Once the initial metabolism parameters were defined, these values were held fixed, and the model terms for tissue binding were adjusted to match the tissue-binding data sets, which include the radiolabel time-course data of Miller et al. (1982) and Ramsey et al. (1984). The model terms for metabolism were fine-tuned by refitting simulations of the reparameterized model to the metabolism data sets. Similarly, the model terms for tissue binding were fine-tuned by refitting simulations of the reparameterized model to the tissue binding data sets. This process was repeated until an adequate visual fit was achieved for all data sets using a single set of parameter values.

The original Kirman et al. (2003) model was not parameterized for humans, and the data used to calibrate the model were limited (i.e., urinary metabolite data and AA radiolabel). Additional kinetic and hemoglobin binding data in rats, mice, and humans have subsequently been published (Boettcher et al., 2005; Doerge et al., 2005a,b,c; Fennell et al., 2005; Sumner et al., 2003), and were used by EPA to recalibrate the Kirman et al. (2003) and to improve the utility of the Kirmal et al. model for use in deriving toxicity values, specifically to estimate human equivalent concentrations based on test animal dose-response data, and to conduct a route-to-route extrapolation of the dose-response relationship from an oral-to-an inhalation exposure.

In calibrating a PBTK model, hemoglobin adduct data are considered a better surrogate of serum levels of AA and GA than urinary metabolite data because the formation of hemoglobin adducts is a direct function of the blood concentration of the reactive agents over the time that red cells are exposed in vivo. The use of urinary data as a surrogate for serum levels is based on the assumption that urinary metabolites (and ratios of urinary metabolites) are an accurate reflection of specific metabolic pathways and actual levels in the blood or tissues from those pathways. Uncertainties in this assumption arise if not all of the metabolic pathways that could have a significant effect on disposition are known, and if there are other clearances that may be influencing the levels of urinary metabolites or their ratios. The relative levels of "unrecovered" metabolites are also a source if uncertainty, since fractional recoveries in urine (i.e., the total amount of parent and metabolite recovered in urine compared to the dose) are typically far less

than 100%. Hemoglobin adduct levels, however, provide a direct measure of the total amount of parent acrylamide and glycidamide metabolite 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) x "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 more relevant internal metric to use to calibrate a PBTK model for use in estimating the risk of acrylamide-induced toxicity.

The parameters that were recalibrated in the Kirman et al. model included tissue/blood partition coefficients for both AA and GA, and the balance between P450 metabolism, gluthathione conjugation, and other non-P450 metabolism. The values for the partition coefficients in the Kirman et al. (2003) model were first re-estimated using a methodology (detailed in Appendix E) based on octanol/water partition coefficients and supported by an analysis of the observed versus predicted ratios of 50 chemicals. Additional refinements in the partition coefficients were based on more recent measurements of the volume of distribution of AA and GA in mice and rats (Doerge et al., 2005b,c).

EPA also evaluated an alternative pathway for AA and GA clearance by GSH based on results from Tong et al. (2004) that suggest that the reaction between AA and glutathione may be largely direct, and not requiring catalysis by glutathione transferase enzymes such as glutathione-S-transferase. A variation of the rat PBTK model was developed to represent non-enzymatic binding of AA and GA to glutathione throughout the body. The value of the second order reaction rate constant for AA-glutathione binding was based on Tong et al.'s (2004) results: 0.021/second-mol/L glutathione × 60 seconds/minute × 60 minutes/hour = 75.6/hour-mol/L glutathione or 0.0756/hour-mmol/L glutathione. To implement this model, a similar reaction rate for GA was derived from the GA to AA reaction rate ratios in the liver based on previous simulations. There is also the possibility that the overall binding of AA and GA to glutathione includes a mix of enzyme-mediated and direct binding, for example, with the AA reaction being solely or primarily direct, and the GA reaction being catalyzed by a glutathione-S-transferase. A mixed pathway model, however, was not evaluated pending further data to better resolve this issue.

To estimate the human equivalent concentrations in the derivation of the noncancer and cancer toxicity values, the Kirman et al. PBTK model parameters values were adjusted to simulate an adult human male. The partition coefficients used in the rat model were re-estimated based on human partition coefficient data for other compounds, and information on the octanol/water partition coefficients of acrylamide and the other compounds. The metabolic parameters were then recalibrated to human acrylamide and glycidamide hemoglobin adduct data

collected at reasonably low external exposure levels in a limited number of adult male subjects given a clinical oral exposure (Fennell et al., 2005).

The model parameters values in EPA's recalibrated rat model (and how they were estimated) are presented in Appendix E, Table E-2. The fits to the data used to calibrate the model are also presented in Appendix E, Table E-3 and E-4 and are discussed in greater detail in Appendix E. The model parameters values in EPA's human model (and how they were estimated) are presented in Appendix E, Table E-5.

The specific applications and results of the recalibrated Kirman et al. PBTK model in the derivation of toxicity values are discussed in detail in Section 5 of this Toxicological Review.

Young et al. (2007) PBTK/TD Model

Young et al. (2007) published a PBTK model developed by the US FDA's National Center for Toxicological Research (NCTR) to simulate AA and GA kinetics in mice, rats, and humans, and to add representation of GA-DNA adduct formation. The model was developed in a general purpose PBTK/TD modeling software program called PostNatal (developed at NCTR). PostNatal is a Windows based program that controls up to four PBTK models under one shell with multiple input and output options for various routes (or combinations of routes) of exposure. Each PBTK unit is comprised of 28 organ/tissue/fluids compartments, and each unit can be maintained as an independent unit or be connected through metabolic pathways to simulate complex exposure regimens or to evaluate drug metabolism and disposition in adult mice, rats, dogs, or humans. For the PBTK model for acrylamide, Young et al. represented the kinetics of AA, GA, AA bound to glutathione, and GA bound to glutathione in separate models coupled by input and output terms with urinary excretion represented in each model (see Figure 3-3). AA or GA dosing is represented by the input terms in the AA and GA model, respectively.

Physiological parameter values in the Young et al. model (organ/tissue weights, blood flows) are assigned with values within the PostNatal program based on animal species, gender, and total body weight (specific values and literature sources not specified). The data used to calibrate the Young et al. model for rats and mice are listed in Appendix F, Table F-2. They include AA serum levels in rats from an i.p. acute exposure (Raymer et al., 1993), plasma AA and GA levels, and AA and GA hemoglobin adduct levels following relatively high (50 mg/kg bw) repeat i.p. dosing in rats for 11 days or 2.8mM of AA in drinking water for 47 days (Barber et al. , 2001), urinary excretion profile and AA and GA hemoglobin adduct levels following dosing via i.p. (50 mg/kg bw), gavage (50 mg/kg bw) dermal (150 mg/kg bw) or inhalation (3 ppm for 6 hr) (Sumner et al., 2003); and serum and tissue (liver, lung, muscle, brain) levels of AA and GA, and liver GA-DNA adduct data in rats and mice following relatively low dose dosing via i.v. (AA and GA at 0.1-0.12 mg/kg bw), gavage (AA and GA at 0.12 and 50 mg/kg bw), diet (~0.1 mg/kg bw over 30 minutes), and in drinking water (~ 1 mg/kg bw AA over 42

days) (Doerge et al., 2005a,b,c). 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,b,c) were used to develop partition coefficients. Only those tissues specifically analyzed for AA or GA were partitioned differently from the blood compartment, i.e., assigned a partition coefficient other than 1 (see Table 3-3). Values for the human parameters were calibrated against urinary excretion data (Fuhr et al., 2006; Fennel et al., 2005) and hemoglobin adduct data from a dietary exposure (Boettcher et al., 2005).

Values for the metabolism and elimination of AA or GA, for AA or GA binding to hemoglobin, and for GA-DNA adduct formation were derived by optimizing the fit of the simulation results to individual animal data (i.e., by minimizing the weighted sum of squares of the difference between each data point and its simulated value). All rate constants for the metabolic and elimination processes, the binding and decay of AA or GA to hemoglobin, and the binding of GA to liver macromolecule are represented as first order (i.e., rate constants of min^{-1}). Although Young et al. 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 evaluate the resulting differences among the model parameter values relative to gender and study conditions for insights into the toxicokinetics of AA and GA, and to assess the uncertainty in the model parameter values (see Appendix F, Table F-1 for the results of fitting to the rat data). Although in some cases there are statistically significant differences in the fitted model parameter values for basic physiological functions such as excretion of AA-GSH conjugates in urine (which varies as much as four to six fold for model fits to different studies), the authors argue 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 use in the derivation of a toxicity value, a PBTK model is generally developed with the aim of resolving a single set of parameter values that either fits all of the available data best (i.e., provides the broadest predictive capability) or fits the most relevant data for a specific application (e.g., oral and inhalation data for a route-to-route extrapolation). Evaluating the importance of uncertainty in a parameter value or combination of values also depends upon the choice of the dose metric used in a risk assessment, and how sensitive that metric is to the parameter(s) of interest. For the Young et al. model (2007) to be applicable for use in the development of toxicity values for acrylamide, some additional work will therefore be 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.

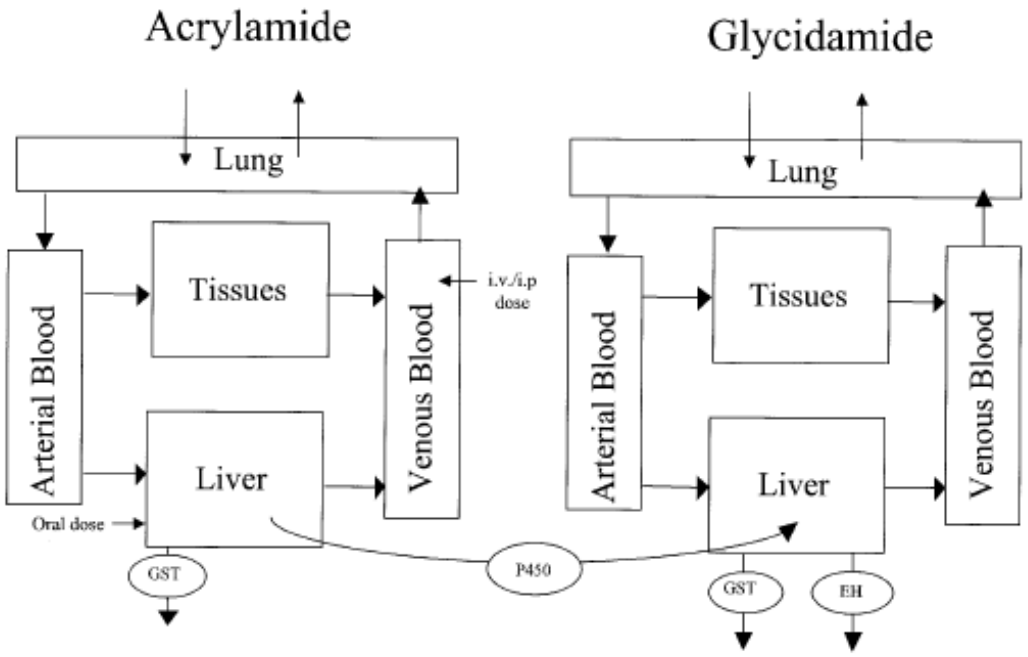

Figure 3-2. Schematic of the Kirman et al. PBTK Model for Acrylamide
(Source: Kirman et al. [2003])

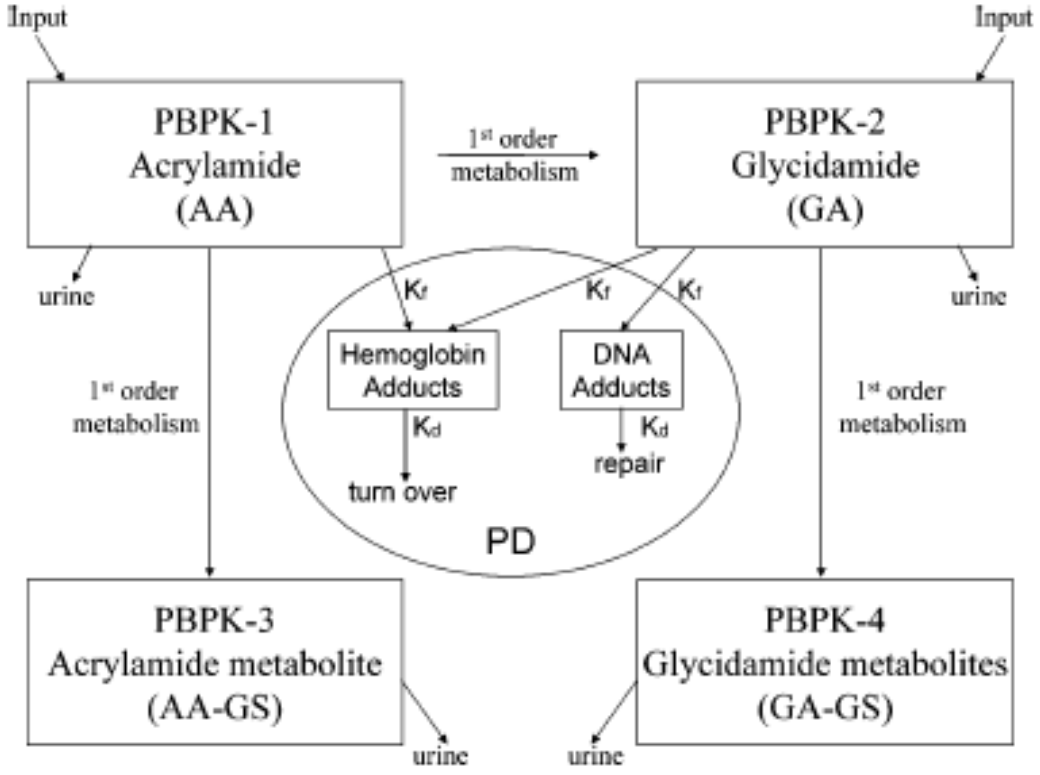

Figure 3-3. Schematic of the Young et al. PBTK Model for Acrylamide
(Source: Young et al. [2007])

4. HAZARD IDENTIFICATION

The importance of assessing the potential health effects from exposure to AA in food has resulted in a unique international collaboration as reflected in international meetings (JIFSAN, 2004, 2002), research programs (U.S. FDA, 2006b), special journal issues (*Mutation Research* vol. 580, issues 1–2, 2005), hazard and exposure assessments (JECFA, 2005; NTP/CERHR, 2004), and internet sites (U.S. FDA, 2006b; FAO/WHO, 2005; JIFSAN, 2005) solely dedicated to providing the research and regulatory community (as well as the private and public sectors) access to the latest information. The discussion here identifies key studies that were used to derive EPA's noncancer and cancer toxicity values and that provide scientific support to the cancer descriptor and the characterization of the noncancer and cancer modes of action.

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

Numerous case reports of occupational exposure to acrylamide involving both inhalation and dermal exposure report neurological impairment in humans from exposure to AA, but levels of exposure are generally not measured (Gjerløff et al., 2001; Mulloy, 1996; Dumitru, 1989; Donovan and Pearson, 1987; Kesson et al., 1977; Mapp et al., 1977; Davenport et al., 1976; Igisu et al., 1975; Takahashi et al., 1971; Fullerton, 1969; Auld and Bedwell, 1967; Garland and Patterson, 1967). Substances like AA that are highly reactive with short half-lives in the blood are more challenging to monitor for estimates of exposure. Acrylamide, however, forms adducts with hemoglobin that persist throughout the life of the adducted red blood cell (estimated at around 120 days), and hemoglobin adducts have been used as biomarker of exposure. There are two cross-sectional health surveillance studies of AA-exposed workers that correlate AA-hemoglobin adduct levels and measures of neurological impairment in acrylamide workers (Hagmar et al., 2001; Calleman et al., 1994).

A quantitative human study on the toxicokinetics of AA was conducted by Fennell et al. (2005) to evaluate metabolism and hemoglobin adduct formation following oral and dermal administration of AA to 24 adult male volunteers. The 24 volunteers were all male Caucasians (with the exception of one Native American), weighing between 71 and 101 kg, and between 26 and 68 years of age. 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 past 6 months. The study was conducted in accordance with the Code of Federal Regulations (CFRs) governing protection of human subjects (21 CFR 50), IRB (21 CFR 56), and retention of data (21 CFR 312) as applicable and consistent with the Declaration of Helsinki. The study used [1,2,3-^{13}C]-acrylamide, and, prior to the conduct of exposures in humans, a low-dose study protocol was evaluated in rats administered 3 mg/kg [1,2,3-^{13}C]-acrylamide by gavage. Subjects

were administered a single oral dose of 0.5, 1.0, or 3.0 mg/kg or a daily dermal dose of 3.0 mg/kg for 3 consecutive days. A comprehensive physical exam was conducted on each individual upon check-in to the clinic, at 24 hours after compound administration, and 7 days after checkout. This exam included medical history, demographic data, neurological examination, 12-lead ECG, vital signs (including oral temperature, respiratory rate, and automated seated pulse and blood pressure), clinical laboratory evaluation (including clinical chemistry, hematology, and complete urinalysis). Each individual also had screens for HIV, hepatitis, and selected drugs of abuse and provided a semen sample to confirm aspermia. Additional ECG, neurological evaluation, abbreviated physical examination, and subjective evaluation were conducted at 4 hours after each AA administration.

No adverse events were reported in the oral phase of the Fennell et al. (2005) study. With the dermal administration, one individual was observed to have a mild contact dermatitis, which is a known response to AA and was part of the informed consent. This individual was seen by a dermatologist who performed a skin biopsy that was consistent with a delayed hypersensitivity reaction. The skin reaction resolved 39 days after the first application of AA and 23 days after the reaction was manifested. An increase in the liver enzyme alanine aminotransferase (ALT) was observed above the upper limit of the reference range (normal) in four of the five individuals who received AA by dermal application, one of whom had a preexisting elevation of this enzyme prior to receiving the dose (data and time of observation not reported). One individual who received dermal AA also had an elevation in serum aspartate transaminase (data and time of observation not reported). The elevated liver function tests returned to within or near the reference range at subsequent determinations and were judged to be not clinically significant by the study physician. When administered to the skin, AA may cause a moderate increase in ALT levels. Serum prolactin, testosterone, and luteinizing hormone did not differ between subjects who received AA at these levels and those who received placebo (data not reported). All blood parameters and hormone levels were within the normal range. There were no neurological or cardiovascular findings in the study participants at either 24 hours or 7 days postexposure.

The human cancer study data are limited, although the recent discovery of AA in foods has prompted a number of studies to evaluate a potential association between dietary acrylamide intake and cancer. To date, no association has been established between increased levels of acrylamide in the diet and increased risk for a variety of cancer types. Most of the available epidemiology studies on increased risk of cancer from AA in food have been conducted by Mucci and colleagues, including three case-control studies for increased risk of cancers of the large bowel, bladder, kidneys, renal cell, or breast (Mucci et al., 2005, 2004, 2003) and one prospective study for colorectal cancers (Mucci et al., 2006). In another large case-control study, Pelucchi et al. (2006) evaluated the relation between dietary AA intake and cancers of the

oral cavity and pharynx, esophagus, large bowel, rectum, larynx, breast, ovary, and prostate. None of these studies report a significant increased incidence of cancer associated with increased intake of AA in food at the levels of intake observed.

One study (of lesser utility) examined the incidence of breast cancer in later life relative to diet composition during the 3- to 5-year-old early development stage (Michels et al., 2006). The authors report an increased risk related to early consumption of French fries, a food considered to have relatively high levels of AA. This limited study, however, makes no mention of AA, and the study methods are lacking.

Two cohort mortality studies evaluated increased risk for cancer in AA workers (Marsh et al., 1999; Collins et al., 1989; Sobel et al., 1986,).

No human studies were identified that assessed the potential for adverse reproductive or developmental effects from exposure to acrylamide.

An important factor in evaluating epidemiology studies that relate dietary intake to effects concerns the characterization of the variability in acrylamide internal dose relative to differences in diet composition and consumption rates. Hagmar et al. (2005) observed relatively narrow interindividual variation in AA adduct levels, and suggests that estimates of individual dietary AA intake will need to be very precise to be useful in cancer epidemiology. Hagmar et al. (2005) evaluated variation in dietary exposure to AA relative to measurement of AA hemoglobin adduct levels (as a biomarker of exposure) in blood samples from the Malmö Diet and Cancer Cohort (n = 28,098). The blood donors were well characterized with regard to their food habits, and 142 individuals were selected to obtain the highest possible variation in the adduct levels from AA (i.e., none, random, or high intake of coffee, fried potatoes, crispbreads, and snacks, food items estimated to have high levels of AA). The median hemoglobin adduct level in the randomly selected group of nonsmokers was compatible with earlier studies (0.031 nmol/g). The variation in the average internal dose, measured as hemoglobin adducts, was somewhat smaller than estimated for daily intake by food consumption questionnaires in other studies. Among 70 nonsmokers, the AA adduct levels varied by a factor of 5 (range: 0.02–0.1 nmol/g), with considerable overlap in AA-adduct levels among the different dietary groups. There was a significant difference between men with high dietary exposure to AA compared to men with low dietary exposure ($p = 0.04$). No such difference was found for women. As expected, smokers had a higher level (range: 0.03–0.43 nmol/g) of AA adducts. Smoking women with high dietary exposure to AA had significantly higher AA adduct levels compared to smoking women with low dietary exposure ($p = 0.01$), however, no significant difference was found in smoking men.

Cohort mortality studies

Collins et al. (1989) conducted a cohort mortality study of all male workers (8854, of which 2293 were exposed to AA) who had been hired between January 1, 1925 and January 31, 1973 at four American Cyanamid factories, three in the United States (Fortier, LA [1295 workers]; Warners, NJ [7153 workers]; and Kalamazoo, MI [60 workers]) and one in the Netherlands (Botlek [346 workers]). Estimations of AA exposure were based on available monitoring data and worker knowledge of past jobs and processes. Industrial hygiene monitoring was in place at all four plants in 1977. Acrylamide levels monitored at that time were typically considered to be representative of levels during the entire period of plant operation. Workers were classified as unexposed when cumulative AA exposure was less than 0.001 mg/m^3-years. Exposure groups were divided into three categories of cumulative exposure: 0.001 to 0.030, 0.030 to 0.30, and greater than 0.30 mg/m^3-years. Smoking history records were available for approximately 35% of the total cohort, 76% of whom were smokers. Smoking status of the other workers was unknown. 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 AA-exposed workers (including cancer of the digestive or respiratory systems, bone, skin, reproductive organs, bladder, kidney, eye, central nervous system, thyroid, or lymphatic system). All causes of both exposed and nonexposed workers were significantly ($p < 0.05$) lower than expected (SMRs = 0.81 and 0.91, respectively; 95% confidence intervals [CI] were not reported). 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 AA.

In the latest update report for the three facilities in the United States, Marsh et al. (1999) reported that, for the 1925–1994 study period (during which 3282 deaths occurred among the 8508 people included in the cohort), excess and deficit overall mortality risks were found for several tissue sites of interest; however, none of these were statistically significant or associated with exposure to AA. Table 4-1 lists all of the observed deaths and SMRs for selected causes. Cited SMRs of interest included: brain and other central nervous system (SMR 0.65, 95% CI 0.36–1.09); thyroid gland (SMR 2.11, 95% CI 0.44–6.17); testis and other male genital organs (SMR 0.28, 95% CI 0.01–1.59); and cancer of the respiratory system (SMR 1.10, 95% CI 0.99–1.22). Table 4-2 lists the SMRs from observed deaths for selected cancer sites (esophagus, rectum, pancreas, and kidney) for all U.S. workers who died between 1950 and 1994, according to the following exposure parameters and categories: duration of employment (categories of <1 year, 1–14 years, and ≥15 years), time since first employment (<20, 20–29, and ≥30 years), duration of exposure (unexposed, 0.001–4.999, 5–19, and ≥20 years), cumulative exposure (<0.001, 0.001–0.029, 0.03–0.29, and ≥0.30 mg/m^3-years), and estimated mean exposure

concentrations (unexposed, 0.001–0.019, 0.02–0.29, and ≥0.3 mg/m^3). In these exploratory exposure-response analyses of esophageal, rectal, pancreatic, and kidney cancers a statistically significantly elevated SMR was found for pancreatic cancer in workers with the highest cumulative AA exposure category (≥0.30 mg/m^3-years), but the risks for the four exposure categories did not increase monotonically from the lowest to highest category.

SMRs for the lowest to highest cumulative exposure categories (mg/m^3-years, SMR, followed by 95% CI, and number of pancreatic cancer deaths) were as follows: <0.001, 0.80 (0.54–1.14, 30 deaths); 0.001–0.029, 2.77 (0.57–8.09, 3 deaths); 0.03–0.29, 0.73, (0.09–2.64, 2 deaths); and ≥0.30, 2.26 (1.03–4.29, 9 deaths). In contrast, risk of pancreatic cancer showed a monotonic increase with exposure-duration category, but none of the SMRs were statistically significantly elevated in any of the four exposure categories.

SMRs for the lowest to highest duration categories (years, SMR, followed by 95% CI, and number of pancreatic cancer deaths) were as follows: unexposed, 0.80 (0.54–1.14, 30 deaths); 0.001–0.4999, 1.46 (0.47–3.41, 5 deaths); 0.5–19, 1.79 (0.58–4.17, 5 deaths); and ≥20, 2.42 (0.66–6.19, 4 deaths). A relative risk modeling analysis showed patterns of relative risks that were similar to those observed in the exploratory exposure-response analysis of SMRs. Marsh et al. (1999) concluded that their study provides "little evidence for a causal relation between exposure to AA and mortality from any cancer sites." Limitations of the study are the large proportion of short-term workers in the cohort, incomplete smoking data, and somewhat limited follow-up duration (about 37% of the cohort had died through 1994). Strengths of the study include the relatively large size of the cohort and the quantitative measures of exposure that were made; with continued follow-up, important information will be gathered.

Table 4-1. Observed deaths and SMRs for selected causes by follow up period for all workers (compared with the general US population)

	1925–1983			1984–1994			1925–1994		
Cause of death (ICDA–8)[a]	**Obs**	**SMR**	**95% CI**	**Obs**	**SMR**	**95% CI**	**Obs**	**SMR**	**95% CI**
All causes (000–999):	2167	0.91[b]	0.87–0.95	1115	0.76[b]	0.72–0.81	3282	0.85[b]	0.82–0.88
All malignant neoplasms (140–209)	496	1.06	0.96–1.15	357	0.89[c]	0.80–0.99	853	0.98	0.92–1.05
Buccal cavity and pharynx (140–149)	13	0.83	0.44–1.42	8	0.96	0.41–1.89	21	0.88	0.54–1.34
Digestive organs and peritoneum (150–159)	141	1.07	0.90–1.26	85	0.89	0.71–1.10	226	0.99	0.87–1.13
Esophagus (150)	16	1.15	0.66–1.87	15	1.30	0.73–2.14	31	1.22	0.83–1.73
Stomach (151)	35	1.34	0.94–1.87	12	0.95	0.49–1.66	47	1.22	0.89–1.62
Large intestine (153)	38	0.94	0.67–1.29	28	0.78	0.52–1.13	66	0.87	0.67–1.10
Rectum (154)	16	1.20	0.69–1.95	8	1.26	0.55–2.49	24	1.22	0.78–1.82
Liver (155, 156)	5	0.51	0.16–1.20	5	0.58	0.19–1.35	10	0.55	0.26–1.00
Pancreas (157)	27	1.09	0.72–1.59	17	0.91	0.53–1.46	44	1.01	0.74–1.36
Respiratory system (160–163)	202	1.25[b]	1.08–1.44	139	0.94	0.79–1.11	341	1.10	0.99–1.22
Larynx (161)	8	1.10	0.48–2.18	6	1.25	0.46–2.71	14	1.16	0.63–1.95
Lung (162, 163)	194	1.27[b]	1.10–1.46	133	0.94	0.78–1.11	327	1.11	0.99–1.24
Bone (170)	2	0.88	0.11–3.18	0	–	0.00–6.19	2	0.70	0.08–2.52
Skin (172, 173)	4	0.48	0.13–1.23	6	0.89	0.33–1.93	10	0.66	0.31–1.22
Prostate (185)	29	0.96	0.64–1.38	38	0.82	0.58–1.13	67	0.88	0.68–1.11
Testis and other male genital organs (186–187)	0	–	0.00–1.23	1	1.92	0.05–10.70	1	0.28	0.01–1.59
Bladder (188)	13	1.06	0.56–1.81	14	1.38	0.75–2.31	27	1.20	0.79–1.75
Kidney (189)	12	1.06	0.55–1.86	10	1.11	0.53–2.04	22	1.08	0.68–1.64
Brain and other central nervous system (191, 192)	5	0.36[c]	0.12–0.85	9	1.15	0.53–2.19	14	0.65	0.36–1.09
Thyroid gland (193)	2	2.32	0.28–8.37	1	1.80	0.04–10.01	3	2.11	0.44–6.17
All lymphopoietic tissue (200–209)	39	0.88	0.62–1.20	21	0.60[c]	0.37–0.92	60	0.76[c]	0.58–0.97
Lymphosarcoma and reticulosarcoma (200)	6	0.70	0.26–1.53	0	–	0.00–2.35	6	0.59	0.22–1.29
Hodgkin's disease (201)	8	1.39	0.60–2.74	0	–	0.00–4.04	8	1.20	0.52–2.37
Leukemia and aleukemia (204–207)	14	0.78	0.43–1.31	9	0.68	0.31–1.29	23	0.74	0.47–1.10
Other lymphatic tissue (202, 203, 208)	11	0.92	0.46–1.66	11	0.65	0.32–1.16	22	0.76	0.48–1.16
Benign neoplasms (210–239)	8	1.24	0.54–2.44	2	0.60	0.07–2.15	10	1.02	0.49–1.87
Diabetes mellitus (250)	26	0.77	0.50–1.12	15	0.53[b]	0.30–0.87	41	0.66[b]	0.47–0.89
Diseases of the circulatory system (390–458)	1019	0.90[b]	0.85–0.96	434	0.61[b]	0.56–0.67	1453	0.79[b]	0.75–0.83
Nonmalignant respiratory disease (460–519)	105	0.75[b]	0.62–0.91	74	0.53[b]	0.42–0.67	179	0.64[b]	0.55–0.74
Cirrhosis of the liver (571)	68	1.08	0.84–1.37	12	0.54[c]	0.28–0.94	80	0.94	0.74–1.17
All external causes of death (800–998)	199	0.70[b]	0.61–0.81	43	0.65[b]	0.47–0.87	242	0.69[b]	0.61–0.79
Unknown causes (999.9)	101			70			171		
People (n)	8508			5942			8508		
Person-years	228,816			58,916			287,731		

[a]Monson life table program ICD–8 categories, labels and codes for U.S. plants for 1925–89; corresponding rates for 1990–1994 from the mortality and population data system (MPDS)maintained at the University of Pittsburgh.
[b]$p \leq 0.01$.
[c]$p \leq 0.05$.

Source: Marsh et al. (1999).

Table 4-2. Observed deaths and SMRs for selected cancer sites by duration of employment, time since first employment, and measures of exposure to acrylamide, all U.S. workers, 1950–1994 (compared with the local male populations)

	Esophagus			Rectum			Pancreas			Kidney		
	Obs	**SMR**	**95% CI**	**Obs**	**SMR**	**95% CI**	**Obs**	**SMR**	**95% CI**	**Obs**	**SMR**	**95% CI**
Duration of employment (years)												
<1	12	0.84	0.43–1.46	5	0.51	0.16–1.18	17	0.87	0.51–1.39	8	0.79	0.34–1.55
1–14	9	0.95	0.43–1.80	12	1.37	0.71–2.39	15	0.95	0.53–1.57	7	0.86	0.35–1.78
≥15	10	1.60	0.77–2.94	5	0.86	0.28–2.02	12	1.19	0.61–2.08	7	1.36	0.55–2.79
Time since first employment (years)												
<20	3	0.69	0.14–2.01	3	0.71	0.15–2.07	4	0.66	0.18–1.68	2	0.58	0.01–2.09
20–29	6	0.80	0.29–1.73	5	0.85	0.28–1.98	11	1.08	0.54–1.92	3	0.54	0.11–1.58
≥30	22	1.21	0.76–1.83	14	0.98	0.53–1.64	29	1.00	0.67–1.44	17	1.18	0.69–1.89
Duration of exposure (years)												
Unexposed	24	0.96	0.61–1.42	17	0.82	0.48–1.32	30	0.80	0.54–1.14	16	0.84	0.48–1.37
0.001–4.999	4	1.63	0.45–4.18	3	1.92	0.40–5.61	5	1.46	0.47–3.41	2	0.99	0.12–3.59
5–19	3	1.80	0.37–5.26	1	0.71	0.02–3.96	5	1.79	0.58–4.17	3	1.88	0.39–5.48
≥20	0	–	0.00–4.10	1	1.20	0.03–6.70	4	2.42	0.66–6.19	1	1.18	0.03–6.56
Cumulative exposure (mg/m^3-years)												
<0.001	24	0.96	0.61–1.42	17	0.82	0.48–1.32	30	0.80	0.54–1.14	16	0.84	0.48–1.37
0.001–0.029	2	2.58	0.31–9.30	1	2.31	0.06–12.9	3	2.77	0.57–8.09	1	1.45	0.04–8.08
0.03–0.29	3	1.70	0.35–4.97	2	1.73	0.21–6.23	2	0.73	0.09–2.64	2	1.17	0.14–4.23
≥0.30	2	0.82	0.10–2.98	2	0.92	0.11–3.31	9	2.26[a]	1.03–4.29	3	1.49	0.31–4.35
Mean intensity of exposure (mg/m^3)												
Unexposed	24	0.96	0.61–1.42	17	0.82	0.48–1.32	30	0.80	0.54–1.14	16	0.84	0.48–1.37
0.001–0.019	2	1.37	0.17–4.95	2	2.12	0.26–7.65	4	1.69	0.46–4.32	1	0.66	0.02–3.66
0.02–0.29	3	1.53	0.32–4.47	0	–	0.00–2.03	5	1.50	0.49–3.49	3	1.71	0.35–5.01
≥0.30	2	1.26	0.15–4.53	3	2.89	0.60–8.43	5	2.31	0.75–5.40	2	1.68	0.20–6.08

[a]$p < 0.05$.
Source: Marsh et al. (1999).

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. The cohort was identified from annual and monthly census lists generated between 1955 and 1979. Analysis and review of air monitoring data and job classifications resulted in estimates of personal 8-hour time-weighted average AA 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. Fourteen of the 371 workers had been exposed to organic dyes in another area of the facility for 5 or more years but moved to the AA areas when organic dye processes were discontinued. SMRs, calculated for categories in which at least two deaths were observed, were based on mortality of white males in the United States.

A total of 29 deaths from all causes was observed among the cohort up until 1982, compared to 38 expected. Incidences of tumors of the central nervous system, thyroid gland, and endocrine organs, as well as mesotheliomas, were of particular interest within the cohort in view of a report of increased tumor incidences at these sites in AA-exposed rats (Johnson et al., 1986); however, no statistically significantly increased incidences of cancer-related deaths were observed. Mortality from cancer among the entire cohort was slightly elevated (11 vs. 7.9 expected) but was lower than expected when the workers with previous exposure to the organic dyes were excluded (4 deaths vs. 6.5 expected). This study is limited by small cohort size, exposure to other chemicals (e.g., acrylonitrile), relatively short duration of employment for many of the workers (276 were employed for 4 years or less, 167 of whom had less than 1 year of employment at the facility), limited follow-up duration, and the inability to detect small increases in risk among site-specific cancers.

Swaen et al. (2007) provide an update of the Sobel study cohort (of 371 acrylamide workers) and expand the cohort to include employees hired since 1979. A total of 696 acrylamide workers were followed from 1955 through 2001 to ascertain the long-term health effects of occupational exposure to acrylamide among production and polymerization workers and the cause of death. Exposure to acrylamide was retrospectively assessed based on personal samples from the 1970s onwards and area samples over the whole study period. The study reports fewer of the acrylamide workers died (n = 141) compared to an expected number of 172.1 (SMR 81.9, 95% CI 69.0 to 96.6). No cause-specific SMR for any of the investigated types of cancer was exposure related. Similar to the earlier Marsh et al. (1999) results, the authors report more total pancreatic cancer deaths (n=5) than expected (n=2.3) (SMR 222.2, 95% CI 72.1 to 518.5), however, 3 of the 5 were in the low dose group, with no apparent dose-response relationship with acrylamide exposure, and thus questionable support for an acrylamide related carcinogenicity. Although these studies provide no good evidence of a cancer risk from occupational exposure to acrylamide at production facilities, additional studies are needed to further evaluate the potential carcinogenicity in humans from exposure to acrylamide.

Case-control studies

No statistically significant associations were found between high consumption of foods with high (300–1200 μg/kg) or moderate (30–299 μg/kg) AA concentrations and an increased risk of large bowel, kidney, or bladder cancer in a reanalysis (Mucci et al., 2003) of an existing population-based case-control study (Augustsson et al., 1999). Augustsson et al. (1999) identified the existing population to study the relation between heterocyclic amines in fried foods and cancer of the large bowel and urinary tract. Individuals in this study were born in Sweden between 1918 and 1942 and resided in Stockholm for at least 1 month between November 1992 and December 1994. Cases were identified from a national cancer registry. Controls were selected from a national population registry and matched by age and gender to cases. Questionnaires concerning dietary habits in the 5 years previous to the study were mailed to 692 controls and 875, 391, and 186 cases of cancer of the large bowel, bladder, and kidney, respectively. Based on completed questionnaires, the final sample size was 538 controls, 591 large bowel cancer cases, 263 bladder cancer cases, and 133 kidney cancer cases. In an unconditional logistic regression analysis, odds ratios (ORs) were calculated for frequency and amounts consumed of 14 food types with high (e.g., potato crisps, French fried potatoes) or moderate (e.g., various types of breakfast cereals and breads) levels of AA vs. each type of cancer. No statistically significantly elevated ORs were found for frequent consumption of any of these food types and risks for large bowel, bladder, or kidney cancer. A summary measure of dietary AA intake was estimated for each individual, based on the results of the questionnaire and median concentrations of AA in foods determined by the Swedish National Food Administration. Quartiles of the summary dietary AA measure were based on distribution in the control group and were modeled as categorical variables with the lowest quartile as the referent group. Tests for trend were calculated using likelihood ratio tests, where the categorical medians of each quartile were modeled as covariates. In regression analyses that adjusted for age and gender or several additional potential confounding variables (e.g., smoking, alcohol intake, and fruit and vegetable intake), no statistically significant trends for increasing ORs with increasing AA exposure measure were found for the three types of cancers. Strengths of this study include the population basis of the design, the moderately high participation rate, the large number of cases, and the estimation of individual dietary exposures to AA. Limitations of the study to detect increased cancer risks include the relatively low dietary intake of the study population compared with the intake of AA in rat bioassays demonstrating cancer and the restriction of the cases to large bowel, kidney, and bladder cancers. Other limitations include the relevance a 5-year recall questionnaire would have to a lifetime exposure estimate for individuals born between 1918 and 1942. There may also have been considerable changes in food processing and the types of food in the diet over that time period, e.g., potato crisp and French fry intake may

have been considerably different pre-World War II, and breads and cereal products have changed considerably over time.

In the renal cancer cell study, Mucci et al. (2004) reanalyzed data from a large population-based Swedish case-control study of renal cell cancer. Again, food frequency data were linked with national food databases on AA content, and daily AA intake was estimated for participants. The risk of renal cell cancer was evaluated for intake of food items with elevated AA levels and for total daily AA dose. Adjusting for potential confounders, there was no evidence that food items with elevated AA, including coffee (OR [highest vs. lowest quartile] = 0.7; 95% CI = 0.4–1.1), crispbreads (OR [highest vs. lowest quartile] = 1.0; 95% CI = 0.6–1.6), and fried potatoes (OR [highest vs. lowest quartile] = 1.1; 95% CI = 0.7–1.7), were associated with a higher risk of renal cell cancer risk. There was also no association between estimated daily AA intake through diet and cancer risk (OR [highest vs. lowest quartile] = 1.1; 95% CI = 0.7–1.8; $p = 0.8$ for trend). The authors state that the results of this study were in line with the previous studies examining dietary AA, suggesting that there is no association between dietary AA and risk of renal cell cancer.

In the breast cancer evaluation, Mucci et al. (2005) assessed AA intake of more than 43,000 women, including 667 breast cancer cases, who were enrolled in the Swedish Women's Lifestyle and Health Cohort. Acrylamide intake was determined from food frequency questionnaires reported by the women in 1991, and the women's health status was tracked via national health registers until the end of 2002. The average daily acrylamide intake among the participants was estimated at 25.9 µg/day, with less than 1.5% of the women consuming more than 1 µg/kg-day of AA. The foods that contributed the most to AA intake were coffee (54% of AA dose), fried potatoes (12% of dose), and crispbreads (9% of dose). Mucci et al. (2005) compared women in the study who had the lowest daily AA intake with women whose intake was higher and reported no significant increased risk of breast cancer in the higher intake group.

A different research group reported similar findings for a broad spectrum of cancers. Pelucchi et al. (2006) evaluated data from an integrated network of Italian and Swiss hospital-based case-control studies to investigate the relation between dietary AA intake and cancers of the oral cavity and pharynx (749 cases, 1772 controls), esophagus (395 cases, 1066 controls), large bowel (1394 cases of colon cancer, 886 cases of rectal cancer, 4765 controls), larynx (527 cases, 1297 controls), breast (2900 cases, 3122 controls), ovary (1031 cases, 2411 controls), and prostate (1294 cases, 1451 controls). All the studies included incident, histologically confirmed cancer cases and controls admitted to the same network of hospitals for acute nonneoplastic conditions. Odds ratios were derived from multivariate logistic regression models, adjusted for energy intake and other major covariates of interest. The ORs for the highest vs. the lowest quintile of AA intake were 1.12 (95% CI = 0.76–1.66) for cancer of the oral cavity/pharynx, 1.10 (95% CI = 0.65–1.86) for esophageal, 0.97 (95% CI = 0.80–1.18) for colorectal, 1.23 (95% CI =

0.80–1.90) for laryngeal, 1.06 (95% CI = 0.88–1.28) for breast, 0.97 (95% CI = 0.73–1.31) for ovarian, and 0.92 (95% CI = 0.69–1.23) for prostate. None of the risk trends were significant. The authors concluded that this uniquely large and comprehensive data set did not show any consistent association between intake of AA and the risk of breast and several other common cancers.

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. The case-control study is a nested study that included 582 women with breast cancer and 1569 controls free of breast cancer, selected from participants in two prospective cohort studies, the Nurses' Health Study and the Nurses' Health Study II. The cohorts in the two prospective studies consisted of 121,700 and 116,678 female registered nurses, respectively, born between 1921–1965. For both cohorts, biennial self-administered questionnaires provided updated information on demographic, anthropometric, and lifestyle factors and on newly diagnosed diseases, including breast cancer. Pathology reports confirmed a breast cancer diagnosis, and the current study was restricted to cases of invasive breast cancer. Information concerning childhood diet of the nurses at ages 3–5 years was obtained from the mothers of the participants with a 30-item food-frequency self-administered questionnaire. The median year of birth of the mothers was 1914 for case mothers and 1913 for control mothers. The median year of birth for the cases is not reported but is calculated from the data in the report to be around 1939. The date of the questionnaire is not stated in the report, but 1993 is when the cases were identified.

Frequencies of intake of the individual foods were converted into servings/day (e.g., number of glasses of milk per day) or servings/week depending on the food, and used as continuous variables. For 718 nurses, complete data on the frequencies of food intake were available, but for 1433 participants data were missing or the mother did not remember the frequency of intake of one or more food items. On average mothers marked the "don't remember" option for 8.5% of the food items and left 3.8% of food items blank. Overall, the proportion of missingness (blanks and don't remembers) ranged from 4.5% for milk to 21% for cheese. Odds ratios were obtained using unconditional logistic regression models. The association between food consumption and breast cancer was estimated for each individual food item, combinations of foods, and nutrients. Of the 582 breast cancer cases and 1569 controls, 63% were premenopausal, 27% were postmenopausal, and 10% were of uncertain menopausal status.

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. A caveat here is the time frame of the 3- to 5-year-olds, which for at least half of the cases would be in the early 1940s, when restaurants and diets were considerably different from today.

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 AA content in the foods being evaluated, and the ability of mothers to accurately recall preschool diets from 30–50 years ago is questionable. The researchers do attempt to assess the validity of the diet questionnaire protocol by administering a questionnaire to mothers of participants in a similar longitudinal study population (the Fels Longitudinal Study) for whom 7-day diet records were kept by the mothers when the participants were 3–6 years old. These participants were born between 1929–1950, and the questionnaire was administered in 1997. The mothers in this validation study ranged in age from 60–93 years old. The sample size of completed questionnaires was small (n = 29). Spearman correlations of mean daily consumption of foods reported by the mothers on the 7-day diet records and on the recall questionnaire were 0.46 ($p = 0.2$) for whole milk, 0.37 ($p = 0.07$) for broccoli, and 0.36 ($p = 0.07$) for French fries. Since these mothers took records during the years of interest for the Fels cohort (in contrast to the mothers in the Nurses' Health Study cohort), the above correlations can be considered an upper bound, suggesting high uncertainty in the accuracy of the recall results.

Prospective studies for cancer

Mucci et al. (2006) conducted a prospective study to evaluate an association between AA in food and risk of colon and rectal cancers using prospective data from the Swedish Mammography Cohort. The cohort comprised 61,467 women at baseline between 1987 and 1990. Through 2003, the cohort contributed 823,072 person-years, and 504 cases of colon and 237 of rectal cancer occurred. Mean intake of AA through diet was 24.6 µg/day (Q25–70 = 18.7–29.9). Coffee (44%), fried potato products (16%), crispbreads (15%), and other breads (12%) were the greatest contributors. After adjusting for potential confounders, the authors report no association between estimated AA intake and colorectal cancer. Comparing extreme quintiles, the adjusted relative risks (95% CI; p for trend) were for colorectal cancer 0.9 (0.7–1.3; $p = 0.80$), colon cancer 0.9 (0.6–1.4; $p = 0.83$), and rectal cancer 1.0 (0.6–1.8; $p = 0.77$). Intake of specific food items with elevated AA (e.g., coffee, crispbreads, and fried potato products) was not associated with cancer risk.

Cross-sectional neurological evaluations

He et al. (1989) studied 71 workers (45 males and 26 females) between 17 and 41 years of age who were exposed to AA 8 hours/day, 6 days/week for 1 to 18 months at a factory in China. A referent group consisted of 33 male and 18 female unexposed workers (17 to 35 years of age) from the same town. Production of AA was initiated in May 1984, and subjects were tested in October 1985. Atmospheric concentrations of AA reached 5.56–9.02 mg/m^3 between March and June 1985 during an exceptional increase in production, and decreased to an average of 0.0324 mg/m^3 after July 1985. The workers were evaluated in October 1985. An AA level of 410 mg/L was measured in the water in which three of the workers washed their hands. Clinical and laboratory examinations included personal interviews 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. Laboratory analysis included routine blood and urine tests, liver function (serum glutamate pyruvate transaminase and the thymol turbidity test for increased globulin components in sera), serum hepatitis B surface antigen, serum β-glucuronidase, and immunoglobulins. Sixty-nine of the exposed workers and 48 of the referent workers were subjected to electroneuromyographic examinations that included measurements of electrical activity in abductor pollicis brevis and abductor digiti minimi muscles of the hand, maximal motor nerve conduction velocity in the lower arm and leg, maximal sensory nerve conduction velocity in the lower arm, and the H-reflex and Achilles tendon reflex. Statistical methods employed included the chi-square test to analyze symptoms and clinical signs and the Student's t-test to assess electroneuromyographic parameters. The level of statistical significance was $p < 0.05$.

The prevalence of a variety of symptoms reported by the exposed and referent groups is shown in Table 4-3. Compared to the referent group, significantly greater percentages of the AA-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 AA and that other symptoms appeared following 3 to 10 months of occupational exposure. Additional statistically significant signs included greater percentages of exposed workers exhibiting erythema of the hands, sensory impairments (vibration, pain, and touch sensation), diminished reflexes in biceps, knee, and ankle, loss of reflexes in the knee and ankle, and intention tremor. Results from visual acuity and visual field testing were normal.

Table 4-3. Neurological symptoms self-reported by acrylamide workers and nonexposed workers

Symptoms	Acrylamide group (n = 71)		Reference group (n = 51)	
	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

[a]$p < 0.05$.
[b]$p < 0.01$ (chi-square test).

Source: He et al. (1989).

Electrical activity, monitored in both the abductor pollicis brevis and abductor digiti minimi muscles of the hand of 69 exposed workers, revealed 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 the group of 48 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 were seen in both the abductor pollicis brevis and abductor digiti minimi muscles of the exposed group. Twenty-seven of the 69 exposed subjects had neuropathologic signs (e.g., impairment of distal sensation or reflexes). When these 27 were excluded from the exposed group, the remaining 42 subjects (i.e., with no observed neuropathologic signs) still demonstrated a statistically significant effect of AA exposure on motor unit potentials (with the exception of mean amplitude in the abductor pollicis brevis muscle). The H-reflex was nonresponsive in 18 of the 27 exposed subjects with neuropathologic signs and was significantly longer in mean latency among the 9 subjects in which a reflex was detected. Seventeen of the 27 exposed subjects with neuropathologic signs, and 4 of the 42 exposed subjects without neuropathologic signs were nonresponsive to the Achilles tendon reflex test. Among the remaining exposed subjects with (n = 10) or without (n = 38) neuropathologic signs, considered separately or combined (n = 48), observed Achilles reflexes were significantly longer in mean latency compared with referent values. Sensory action potentials in the wrist (both median and ulnar nerves) and sural nerve of the 27 exposed subjects with neuropathologic signs, as well as the entire group of 69 exposed subjects, were significantly lower in mean amplitude than those of

the referents. Similar measurements in the elbow revealed a significantly lower mean amplitude in the 27 exposed subjects with neuropathologic signs. Assessment of nerve conduction velocity, electrocardiography, electroencephalography, and laboratory test results revealed no statistically significant exposure-related effects.

This study associated abnormalities in nervous activity with occupational exposure to AA. The results suggest that some measures of abnormal electrical activity may be used to identify early stages of AA-induced neurotoxicity. However, exposure scenarios were poorly characterized. Dermal exposure was likely a major source of exposure for at least some of the exposed workers, as evidenced by numerous reports of peeling of the skin and excessive sweating of the hands. But inhalation exposure was also likely, based on measurable concentrations of airborne AA. The study does not include information concerning dose-response relationships or hemoglobin adduct levels in the group of exposed workers. Nor were adjustments made for confounding factors such as smoking and exposure to other chemicals.

Calleman et al. (1994) performed a cross-sectional analysis of hemoglobin adduct formation and neurological effects in a group of 41 factory workers (34 males and 7 females, aged 18 to 42 years) 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 AA in a factory in China. Other reports on this population include those by Bergmark et al. (1993) who detected glycidamide adducts of hemoglobin in AA-exposed workers indicating that the transformation of AA to GA occurs in humans, and by Deng et al. (1993). Acrylamide mean exposure concentrations, measured during the summer of 1991, were 1.07 and 3.27 mg/m^3 in the synthesis and polymerization rooms, respectively. Exposure concentrations measured during the time of collection of biomarker data (September 1991) were lower, averaging 0.61 and 0.58 mg/m^3 in the synthesis and polymerization rooms, respectively. The exposed group included 13 synthesis workers, 12 polymerization workers, 5 packaging workers, and 6 ambulatory workers, classified according to their primary work location. The remaining four workers were either exposed for less than 6 months (two subjects) or had not been exposed to AA during the 4 months preceding the study. Blood sampling and medical and neurological examinations were performed approximately 1 hour after a work shift. The beginning of a work shift marked the beginning of 24-hour urine sampling. For vibration sensitivity testing, a referent group consisted of 105 unexposed healthy adults (51 males and 54 females aged 20–60 years). A historical control of 80 persons was used as referent for electroneuromyography tests. A group of 10 nonexposed male workers from the same city as the exposed group was used as a referent group for biomarkers of exposure and signs and symptoms of neurotoxicity.

Information regarding demographic factors, smoking and drinking habits, height and weight, occupational history, past illnesses, current symptoms, and reproductive history were collected by questionnaire. Vibration sensitivity thresholds were measured in fingers and toes

using the Vibratron II instrument (Deng et al., 1993). Physical and neurological examinations and electroneuromyographic (ENMG) testing were similar to those described by He et al. (1989). A neurotoxicity index, with a maximal score of 50, was used to express severity of peripheral neuropathy (Table 4-4); the information used to derive the score was collected by questionnaire. The prevalence of specific symptoms was also assessed individually. Biomarkers of exposure to AA that were reported in the study included free AA in plasma, mercapturic acids in urine, and the hemoglobin adduct formed by the reaction of AA with the N-terminal valine of hemoglobin (AAVal).

Table 4-4. Scoring system for the neurotoxicity index

Endpoint	Points[a]
Numbness of extremities	1
Cramping pain	1
Loss of position sensation	2
Loss of pain sensation	0, 1, 2, or 3[b]
Loss of touch sensation	0, 1, 2, or 3[b]
Loss of vibration sensation[c]	
According to tuning fork	1
Vibration threshold in big toe	0, 1, or 2
Vibration threshold in index finger	0, 1, or 2
Clumsiness of hands	4
Difficulty grasping	4
Unsteady gait	4
Decrease or loss of ankle reflexes	3 or 5
Muscular atrophy	6
Electroneuromyographic abnormalities[d]	0.5 per abnormality (maximum 6)
Maximum total score	50

[a]Points were intended to reflect weight given to these observations by a clinical physician diagnosing a peripheral neuropathy.
[b]Workers who had lost their pain or touch sensation were assigned 1 to 3 points depending on the extent of loss: fingers, hands, or forearms.
[c]The ratio between the vibration threshold of an individual and that of the corresponding control group with regard to age was used for scoring vibration sensitivity using the Vibratron instrument. One point was given if this ratio was 1.5–2.5 for fingers or 1.5–4.0 for toes and 2 points if it was 2.5–5.0 for fingers or 4.0–8.0 for toes.
[d]Abnormalities consisted of measured alterations in electrical activity of selected muscles and nerves.

Source: Calleman et al. (1994).

Statistical analyses included the chi-square test to analyze symptoms and clinical signs and the Student's t-test to assess ENMG parameters. Variance analysis and the *Q*-test were used in the comparison of vibration thresholds between the reference group and the exposed group. Univariate and multivariate linear regression analysis was used to estimate correlation coefficients and levels of statistical significance for biomarkers of exposure. The level of statistical significance was $p < 0.05$.

Significant differences in vibration threshold were observed among three age subgroups of referents (<31 years of age, 31–40 years of age, and >40 years of age). Comparisons of vibration threshold between AA-exposed workers and referents within these age groupings

showed a significant increase in the exposed workers. Comparison of the results of ENMG measurements between the exposed workers and the referent group revealed a 10 to 20% decrease in conduction velocity in the peroneal and sural nerves and 25 to 36% increase in latency in median, ulnar, and peroneal nerves within the exposed group.

The prevalence of symptoms and signs of adverse health effects in the AA-exposed workers (n = 41) that were not reported in the referent group (n = 10) included statistically significant incidences of numbness (71%), fatigue (71%), sweating of hands and feet (68%), skin peeling (59%), loss of pain sensation (54%), loss of touch sensation (46%), dizziness (44%), anorexia (41%), loss of vibration sensation (41%), and nausea (39%). Other signs and symptoms that were observed only in the exposed group but were not found to be statistically different from referents included loss of ankle reflexes (29%), headache (27%), unsteady gait (22%), loss of knee jerk (20%), unsteady Romberg sign (20%), and loss of triceps and biceps reflexes (10%).

Group mean biomarker levels and neurotoxicity indices are presented in Table 4-5 for controls and the work locations of packaging, polymerization, ambulatory, and synthesis. The average neurotoxicity index scores, as well as the averages of the hemoglobin adduct levels of AA, decreased with physical distance from the synthesis room where the monomer itself was handled. This relationship was not reflected by measured free plasma AA, urinary mercapturic acid, or hemoglobin adduct levels of acrylonitrile or by results of hand or foot vibration sensitivity measurements or estimates of accumulated in vivo doses of AA. Statistically significant correlations were reported between each of the biomarkers of exposure and the calculated neurotoxicity indices, with the exception of free plasma AA concentrations.

Table 4-5. Group means ± SD of biomarkers in different categories of workers

	Free AA[a] (μmol/L)	Merc. ac.[b] (μmol/24 hours)	AAVal[c] (nmol/g)	ANVal[d] (nmol/g)	$AccD_{AA}$[e] (mM/hour)	NIn[f]
Controls	0.92	3 ± 1.8	0.0 ± 0.0	0.23 ± 0.18	0.0 ± 0.0	0.0 ± 0.0
Packaging	2.2	93 ± 72	3.9 ± 2.5	19.1 ± 5.7	8.1 ± 6.6	8.9 ± 9.1
Polymerization	1.3	58 ± 75	7.7 ± 3.4	19.1 ± 12.9	27.0 ± 23.9	10.0 ± 5.8
Ambulatory	2.0	53 ± 35	9.5 ± 7.3	16.3 ± 3.7	37.6 ± 21.9	11.3 ± 9.8
Synthesis	1.8 ± 0.8	64 ± 46	13.4 ± 9.8	19.5 ± 7.6	68.3 ± 64.2	19.2 ± 10.6

[a]Free plasma acrylamide.
[b]Urinary mercapturic acid.
[c]Hemoglobin adduct between N-terminal valine and acrylamide.
[d]Hemoglobin adduct between N-terminal valine and acrylonitrile.
[e]Predicted cumulative in vivo acrylamide dose (based on rates of acrylamide-hemoglobin adduct formation in human globin hydrolysates and mean acrylamide exposure concentrations measured in areas of polymerization and synthesis by station sampling) (see Section 3.1 and Bergmark et al. [1993] for additional information).
[f]Neurotoxicity index.

Source: Calleman et al. (1994).

A principal finding of the study of Calleman et al. (1994) was the strong correlation between hemoglobin adduct levels of acrylamide and neurological impairment (Table 4-6), as assessed by a combined index of self-reported symptoms and clinically assessed effects. No significant correlation was found between free plasma AA levels and neurotoxicity index, but significant correlations were found between neurotoxicity index and the other markers of exposure indicated in Table 4-5. The data provide a description of the relationship between an internal measure of dose (hemoglobin adducts) from repeated exposure to AA (1 month–11.5 years; mean = 3 years) and an index of neurological impairment. 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 exposure was considered to have been the major exposure route.

Table 4-6. Correlation coefficients (linear regression) for relationships between biomarkers and neurotoxicity index

X variable	*Y* variable	Correlation coefficient	*p*-Value
Free AA[a]	NIn[f]	0.15	0.31
Merc. ac.[b]	NIn	0.42	<0.01
AAVal[c]	NIn	0.67	<0.001
ANVal[d]	NIn	0.69	<0.001
$AccD_{AA}$[e]	NIn	0.60	<0.001

[a]Free plasma acrylamide.
[b]Urinary mercapturic acid.
[c]Hemoglobin adduct between N-terminal valine and acrylamide.
[d]Hemoglobin adduct between N-terminal valine and acrylonitrile.
[e]Predicted cumulative in vivo acrylamide dose (based on rates of acrylamide-hemoglobin adduct formation in human globin hydrolysates and mean acrylamide exposure concentrations measured in areas of polymerization and synthesis by station sampling) (see Section 3.1 and Bergmark et al. [1993] for additional information).
[f]Neurotoxicity index.

Source: Calleman et al. (1994).

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 as well as inhalation exposure. The workers were exposed to the grouting agent for 55 days (August 4 through September 30, 1997), after which exposure was stopped due to the development of neurological symptoms in cows that drank water from a creek that contained leakage water from the tunnel. One week after grouting stopped, 210 workers (of 242 total workers) agreed to participate in the study. 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 AA and NMA; further analysis suggested that the air contained a 50:50 mixture of these compounds. Workers were classified by exposure level. The levels were designated as "high" (103 subjects who had injected the grouting agent), "some" (89 subjects), or "none" (18 subjects without obvious exposure), based on self-reported exposure. 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 AA 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.

An important caveat in interpreting the hemoglobin adduct data relative to neurotoxic responses to AA in the Hagmar et al. (2001) study is that both AA and NMA form the same

N-(2-carbamoylethyl)valine adduct in hemoglobin. Fennell et al. (2003) measured levels of this adduct following separate exposure to equimolar doses of AA and NMA to rats and reported formation of 21 ± 1.7 pmol/mg globin from AA and 41 ± 4.9 pmol/mg from NMA (mean ± SD, n = 4). Since the levels of adduct formation were not comparable and there is no way to distinguish whether the N-(2-carbamoylethyl)valine arose from reaction of hemoglobin with AA or with NMA, conclusions about AA exposure (with adducts as the surrogate for internal exposure) vs. responses are confounded by not being able to reliably distinguish the AA internal dose from the NMA internal dose in humans.

Hemoglobin adduct levels for 18 nonsmoking unexposed reference subjects 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.

Clear relationships (statistically significant trend tests) were found between increasing levels of hemoglobin adducts and increased incidences of self-reported symptoms of peripheral neurological impairment and irritation of the eyes. Statistically significant positive correlations ($p < 0.05$) between prevalence of 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. This symptom is consistent with peripheral nervous impairment and was noted with the highest frequency among the reported symptoms in this study. Irritant symptoms and symptoms of 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. Table 4-7 summarizes the symptoms showing the greatest increases in incidences with increasing hemoglobin adduct levels.

Table 4-7. Incidences of symptoms in 210 tunnel workers classified into exposure groups based on levels of hemoglobin adducts of acrylamide

Symptoms with trend test *p*-value <0.001	Hemoglobin adducts of acrylamide (nmol/g globin)[a]			
	<0.08	**0.08–0.29**	**0.30–1.00**	**1.00–17.7**
Numbness/tingling in feet or legs	2/47 (4)	10/89 (11)	9/36 (25)	14/38 (37)
Leg cramps	3/47 (6)	6/89 (7)	2/36 (6)	10/38 (26)
Eye irritation	6/47 (14)	19/87 (23)	17/36 (47)	29/38 (76)
Nose irritation	6/47 (14)	17/89 (21)	13/36 (36)	20/38 (53)
Throat irritation	4/47 (10)	19/89 (23)	17/36 (47)	28/38 (47)
Coughing	4/47 (10)	9/89 (11)	11/36 (31)	19/38 (50)
Headache	6/47 (14)	27/89 (33)	11/36 (31)	24/38 (63)

[a]Percentages of workers reporting symptoms are noted in parentheses.

Source: Hagmar et al. (2001).

The principal findings of the study of Hagmar et al. (2001) are the positive correlations between measures of exposure (hemoglobin adducts) and self-reported symptoms of neurological impairment. Pairwise comparisons (Fisher's Exact test performed by Syracuse Research Corporation) between the group of subjects with adduct levels <0.08 nmol/g globin and each of the three groups with higher adduct levels (0.08–0.29, 0.30–1.00, and >1.00 nmol/g globin) show statistically significantly ($p < 0.05$) increased prevalence of numbness or tingling in the feet or legs for the two higher exposure groups, but not in the group with lower adduct levels (0.08–0.29 nmol/g globin). This analysis indicates that an adduct level in the range of 0.08–0.29 nmol/g globin was the NOAEL, and 0.30–1.00 nmol/g globin was the LOAEL, for self-reported symptoms of AA-induced peripheral neuropathy. Limitations of this study, with respect to describing dose-response relationships for chronic exposure to AA, are the relatively short period (2 months) of occupational exposure to AA, the possible confounding contribution of NMA to the noted effects, and the fact that both AA and NMA form the same N-terminal valine hemoglobin adduct (Fennell et al., 2003) that was used as an internal measure of dose.

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 AA 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 mean age of the subjects was 30 years and the mean length of service 24 months; no significant differences were seen for these variables between exposed and unexposed groups.

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 AA-related abnormalities (inclusive) 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 to 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 with a Vibratron II vibration sensation tester that was not available in the earlier investigation. 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.

The studies of Myers and Macun (1991) and Bachmann et al. (1992) show an association between occupational exposure to AA above the NIOSH REL of 0.3 mg/m^3 and signs and symptoms of mild neuropathy. However, in the absence of more reliable measures of exposure (e.g., hemoglobin adduct levels), meaningful effect levels were not established.

Case reports

Numerous case reports have been published in which exposure to AA, predominantly in occupational settings, has been associated with observed cutaneous and neurological effects ranging from dermal effects, such as peeling of skin in fingertips, to numerous signs of impaired neurological performance in peripheral and central nervous systems (Gjerløff et al., 2001; Mulloy, 1996; Dumitru, 1989; Donovan and Pearson, 1987; Kesson et al., 1977; Mapp et al., 1977; Davenport et al., 1976; Igisu et al., 1975; Takahashi et al., 1971; Fullerton, 1969; Auld and Bedwell, 1967; Garland and Patterson, 1967). Although these reports provide supportive

evidence of AA-induced neurotoxicity, they lack information regarding primary exposure routes and exposure-response relationships.

4.2. SUBCHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Oral Exposure

The standard bioassay database for subchronic and chronic oral exposures to acrylamide consists of one 90-day drinking water study in F344 rats (Burek et al., 1980) that demonstrated neurotoxicity and two 2-year drinking water studies in F344 rats with the main effects being neurotoxicity and cancer (Friedman et al., 1995; Johnson et al., 1986, 1984).

4.2.1.1. *Subchronic Studies*

Neurotoxic effects

Burek et al. (1980) administered AA to groups of 6-week-old male (23–29/group) and female (10/group) F344 rats in the drinking water for up to 93 days at concentrations designed to result in AA 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 microscopic examination of the sciatic nerve on days 25 and 111 posttreatment. 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. Three of these rats were processed for electron microscopic examination of the sciatic nerve.

All rats were observed daily (during the 5 day workweek) 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) that had been 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 less than 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 the sciatic nerve was 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.

Significantly lower body weights were reported in male and female rats of the 20 mg/kg-day group 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. At the 20 mg/kg-day dose level, treatment-related effects on organ weights included 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. Female rats of the 20 mg/kg-day dose level 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, as well as significantly higher relative brain and liver weights.

Significantly increased instances of hindlimb foot splay were observed in 20 mg/kg-day 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 20 mg/kg-day groups showed cleared signs of improvements continuing to day 111 with only slight posterior weakness and curling of the toes. By day 144, these high dose treated 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 20 mg/kg-day females. Significant decreases in packed cell volume, total erythrocyte count, and hemoglobin concentrations in 20 mg/kg-day males and females and 5 mg/kg-day females were noted. Results of urinalysis did not reveal any AA-induced abnormalities. By day 144 posttreatment, the 20 mg/kg-day 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 20 mg/kg-day treatment 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 at the 20 mg/kg-day treatment level 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.

Results of sciatic nerve examinations using light and electron microscopy are summarized in Table 4-8. Light microscopic examination of the sciatic nerve sections (stained with hematoxylin and eosin) revealed severe degeneration in the 20 mg/kg-day group 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 20 mg/kg-day 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 alteration 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.

Table 4-8. Light and electron microscopic data for left sciatic nerves from rats exposed to acrylamide in drinking water for 90 days

	Dose (mg/kg-day)					
Endpoint	**0**	**0.05**	**0.2**	**1**	**5**	**20**
Electron microscopy						
Number of rats (only males were examined)	3	3	3	3	3	3
Total fields examined	450	450	350	453	443	435
Axolemma invaginations	36	24	27	30	33	8
Axolemma invaginations with cell organelles and/or dense bodies	32	15	17	78	109	48
Schwann cells without axons and/or with degenerating myelin	0	0	0	0	7	183
Incidence of fields with any alteration	68/450	39/450	44/350	108/453	149/443	239/435
Light microscopy						
(10 rats/sex/dose were examined)						
Moderate to severe degeneration						
Female	0/10	0/10	0/10	0/10	0/10	10/10
Male	0/10	0/10	0/10	0/10	0/10	10/10
Equivocal to very slight degeneration						
Female	0/10	0/10	0/10	0/10	6/10	0/10
Male	0/10	0/10	0/10	0/10	9/10	0/10

Source: Burek et al. (1980).

Electron microscopic 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 20 mg/kg-day rats (other treatment groups were not subjected to 7-day interim sacrifice). After 33 days of treatment, 20 mg/kg-day 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 at the 20 mg/kg-day dose level. 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 or without 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 20 mg/kg-day group were partially or completely reversed by day 144 posttreatment.

In summary, the 90-day toxicity study of F344 rats exposed to AA in the drinking water (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 or without 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..

4.2.1.2. *Chronic Studies*

Johnson et al. (1986, 1984) study

Johnson et al. (1986, 1984) conducted a chronic toxicity and carcinogenicity study in which groups of F344 rats (90/sex/treatment group) were administered AA in the drinking water at concentrations calculated to provide AA 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. Rats were observed twice daily on workdays for clinical signs and examined monthly for palpable masses. Individual body weights were recorded monthly and fasting body weights were measured at scheduled necropsy. Based on body weight and water consumption data from a subgroup of 20 rats/treatment group, recorded weekly for the first 3 months and monthly (water consumption measured for 1 week each month) thereafter, concentrations of AA in the drinking water were adjusted to maintain target doses for the remaining rats of each treatment group. During the final 6 months of treatment, mean group weights of all rats, rather than those of the subgroup, were used in calculating the concentrations of AA required to maintain target treatment levels.

Blood and urine were collected randomly from 10 rats/sex/group at 3 months and just prior to 6-, 12-, 18-, and 24-month scheduled necropsies. Hematological parameters investigated included packed cell volume, hemoglobin, total erythrocytes, leukocyte count, platelet count, and red cell indices. Stained blood smear examinations and differential leukocyte counts were conducted. Urine was analyzed for specific gravity, pH, protein, glucose, blood, ketones, bilirubin, and urobilinogen. During necropsy, blood serum was collected and analyzed for concentrations of glutamic-pyruvate transaminase, alkaline phosphatase, blood urea nitrogen, total protein, albumin, glucose, and cholinesterase.

Complete postmortem gross pathologic examinations were performed on all rats in the study. Organ-to-body weight ratios were calculated for brain, heart, liver, kidneys, and testes.

Representative sections from all major organs and tissues were stained with hematoxylin and eosin and subjected to histopathologic examination. Light microscopic examinations were performed on sections 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 that had been stained with hematoxylin and eosin.

Cumulative mortality data were analyzed by the Gehan-Wilcoxon test. Analysis of variance and Dunnett's *t* test were used to analyze body weight data, clinical chemistry, hematology, urine specific gravity, and organ weight. Cumulative incidence of microscopic pathologic findings was analyzed by Fisher's Exact probability test. For observations with a control incidence of at least 6%, a Bonferonni correction for multiple treatment-control comparisons was applied. In the absence of a positive Fisher's Exact test for a microscopic lesion, the Cochran-Armitage test for linear trend was performed. Supplemental mortality-adjusted tests of Peto, and the analogous extension of the Cochran-Armitage test, were performed when deemed appropriate. The level of significance chosen for all tests was $p \leq 0.05$.

Additional groups of rats (18/sex/group) were added to the study for independent assessment of neurohistopathologic effects (the results of this portion of the study were reported by Johnson et al., 1985). Three rats/sex were sacrificed at each scheduled interim examination (3, 6, 12, and 18 months) and at terminal sacrifice (24 months). An additional three rats/sex/dose were placed on study to provide for adequate number of rats at the 24-month sacrifice. All survivors were sacrificed at 24 months. Both light and electron microscopic examinations were performed on nerve tissue samples taken from the same regions as those described above. As in the Burek et al. (1980) study, preparations for light microscopy included the use of LFB/PAS reaction for myelin staining and Bodian's stain to elucidate more subtle axonal changes.

Nonneoplastic results—primarily neurotoxicity

Incidence data were presented only for mortality and tibial nerve degeneration (at terminal necropsy). Other nonneoplastic results were typically described according to statistical comparison with controls, but the report did not include incidence or mean data.

Based on water consumption data, AA doses varied from 94 to 105% of target levels. Cumulative mortality data showed no apparent dose-related effect before 21 months of treatment, after which the 2.0 mg/kg-day group (especially females) exhibited increasing mortality that was significantly higher than controls after 24 months of treatment (approximately 32% in 2.0 mg/kg-day females vs. 20% in control females and 41% in 2.0 mg/kg-day males vs. 26% in control males). Beginning on treatment day 89, mean body weight of 2.0 mg/kg-day males was significantly lower (about 2%) than controls. By the end of the study, the difference had increased to approximately 4%. No consistent significant treatment-related body weight effects were seen in 2.0 mg/kg-day females or rats of either sex from lower dose groups. There

were no treatment-related effects on food or water consumption. Clinical observations, hematology, clinical chemistry, and urinalysis did not reveal any indications of treatment-related effects in any treatment group. On study day 210, some male and female rats from all dose groups exhibited excessive lacrimation and enlarged salivary glands consistent with sialodacryoadenitis virus infection. Both males and females appeared to be equally affected, and the symptoms resolved within about 10 days.

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. Although the report indicated that 12-month assessment revealed increases in both incidence and degree of degeneration in the 2.0 mg/kg-day group, particularly the males, the actual data were not presented, precluding an independent analysis of the findings. Incidences of nerve degeneration increased in controls and treated groups alike throughout the remainder of the treatment period. Table 4-9 summarizes the light microscopic findings in tibial nerve sections of the groups of rats from the main study that were treated for 2 years. There were no indications of significant effects on incidence of very slight or slight degeneration in control or treated males or females. There was a statistically significant trend towards increased moderate and severe degeneration in tibial nerves of male rats up to the 2.0 mg/kg-day dose level, although the increase for the pooled moderate-to-severe data at the high dose was not statistically different from controls. There was a statistically significant increase in pooled incidence of slight-to-moderate degeneration in tibial nerves for female rats at 2.0 mg/kg-day.

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 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.

In summary, the most significant noncancer chronic effects observed in F344 rats exposed to AA in the drinking water for 2 years (Johnson et al., 1986, 1985, 1984) 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. A NOAEL for these neurological effects was identified at 0.5 mg/kg-day, and a LOAEL was identified at the 2.0 mg/kg-day dose level.

Table 4-9. Light microscopic data for tibial nerves from F344 rats exposed to acrylamide in drinking water for 2 years

	Dose (mg/kg-day)				
Endpoint	**0**	**0.01**	**0.1**	**0.5**	**2**
Males					
Number of rats examined	60	60	60	60	60
Within normal limits	2	3	4	3	4
Degeneration					
Very slight	30	29	23	25	19
Slight	19	22	21	19	21
Moderate	8	5	12	13	12
Severe	1	1	0	0	4
Moderate + severe	9	6	12	13	16[a,b]
Females					
Number of rats examined	60	60	60	60	61
Within normal limits	12	10	10	11	8
Degeneration					
Very slight	45	43	45	42	37
Slight	3	7	5	7	13
Moderate	0	0	0	0	3
Slight + moderate	3	7	5	7	16[c,d]

[a]The data for moderate and severe degeneration were pooled due to low incidence.
[b]Indicates a linear trend by the Mantel-Haenszel extension of the Cochran-Armitage test ($p < 0.05$) for pooled moderate and severe degeneration. Note no statistical significance for the high dose group.
[c]The data for slight and moderate degeneration were pooled due to low incidence.
[d]Statistically different from control group, mortality adjusted via Mantel-Haenszel procedures ($p < 0.05$).

Source: Johnson et al. (1986).

Neoplastic results—tumors at multiple sites

Until the last few months of treatment, observations of palpable masses were infrequent. The authors noted that rats dosed at 2.0 mg/kg-day appeared to have slightly increased incidences of palpable masses during the last 4 months of treatment, most of which were subsequently identified as tumors originating from the skin or subcutaneous tissues and glands, particularly the mammary gland. Study results provide evidence of carcinogenicity from chronic high dose exposure to AA, as presented in Table 4-10. Upon histopathological examination at the end of 2 years, male F344 rats exposed to 2.0 mg/kg-day of AA in water had developed statistically significantly increased incidences of thyroid (follicular cell) adenomas (no carcinomas), mesotheliomas of the tunica vaginalis testis (i.e., scrotal sac), and benign adrenal pheochromocytoma. Female F344 rats exposed to 2.0 mg/kg-day for 2 years developed statistically significantly increased incidences of mammary gland benign tumors (adenoma, fibroadenoma, or fibroma), central nervous system (CNS) 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. Statistically significant increases in tunica vaginalis testicular mesotheliomas were also observed in male rats exposed to 0.5 mg/kg-day of AA in water. No other significant increases were observed at other sites for males or females at AA doses less than or equal to 0.5 mg/kg-day.

Table 4-10. Incidences of selected tumors in male and female F344 rats exposed to acrylamide in drinking water for 2 years

	Dose (mg/kg-day)				
Tumor type	**0**	**0.01**	**0.1**	**0.5**	**2.0**
Males					
CNS tumors or glial proliferation suggestive of early tumor	5/60	2/60	0/60	3/60	8/60
Thyroid (follicular cell) adenoma (no carcinomas found)	1/60	0/58	2/59	1/59	7/59[a]
Tunica vaginalis testis mesothelioma	3/60	0/60	7/60	11/60[a]	10/60[a]
Squamous cell carcinoma or papilloma, oral cavity	6/60	7/60	1/60	5/60	6/60
Pheochromocytomas, benign (adrenal)	3/60	7/59	7/60	5/60	10/60[a]
Females					
Mammary gland adenocarcinoma	2/60	1/60	1/60	2/58	6/61
Mammary gland benign tumors (adenoma, fibroadenoma, or fibroma)	10/60	11/60	9/60	19/58	23/61[a]
CNS tumors of glial origin	1/60	2/59	1/60	1/60	9/61[a]
Thyroid (follicular cell) adenoma or adenocarcinoma	1/58	0/59	1/59	1/58	5/60[a]
Squamous cell carcinoma, oral cavity	0/60	0/60	0/60	2/60	1/61
Squamous papilloma, oral cavity	0/60	3/60	2/60	1/60	7/61[a]
Uterus adenocarcinoma	1/60	2/60	1/60	0/59	5/60[a]
Clitoral adenoma, benign	0/2	1/3	3/4	2/4	5/5[a]
Pituitary gland adenoma	25/59	30/60	32/60	27/60	32/60[a]

[a]Significantly different from control, $p < 0.05$, after Mantel-Haenszel mortality adjustment.

Source: Johnson et al. (1986).

In summary, chronic exposure of male and female F344 rats to the highest dose of 2.0 mg/kg-day of AA in water (Johnson et al., 1986) resulted in increased incidences for tumors at multiple sites in both sexes. Chronic exposure to the next lowest dose of 0.5 mg/kg-day resulted in a significant increase only in male testicular sac mesotheliomas. No significant increases over controls were observed in female tumors at the 0.5 mg/kg-day dose or in male or female tumors at doses lower than 0.5 mg/kg-day.

Friedman et al. (1995) study

A second cancer bioassay in F344 rats exposed to acrylamide in drinking water (Friedman et al., 1995; Tegeris Laboratories, 1989) included 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 (see Table 4-11). Ambiguities in the Johnson

et al. (1986) study (e.g., abnormally high background for CNS and oral cavity tumors in the control males and possible confounding from a sialodacryoadenitis virus infection) also prompted the design and conduct of this second study.

An additional group of 25 rats/sex was observed during the course of this study for signs of viral infections (to address concerns about sialodacryoadenitis virus infection in the first bioassay). Control rats were divided into two separate groups to more accurately assess the variability of low-incidence background tumors.

Table 4-11. Dosing parameters of groups of rats given acrylamide in drinking water for 106–108 weeks in the carcinogenicity study

	Males		Females	
Group	Number of rats	Dose (mg/kg-day)	Number of rats	Dose (mg/kg-day)
1	102	0	50	0
2	102	0	50	0
3	204	0.1	–	–
4	102	0.5	100	1.0
5	75	2.0	100	3.0

Sources: Friedman et al. (1995); Tegeris Laboratories (1989).

Water consumption was measured weekly throughout the study. Body weight and food consumption were recorded for each animal prior to the start of treatment, weekly for the initial 16 weeks of treatment, and every 4 weeks thereafter. All animals were observed twice daily for mortality, morbidity, and obvious clinical signs of toxicity. Physical examinations were performed weekly for the first 16 weeks, every 4 weeks for the ensuing 24 weeks, and biweekly for the remainder of the study. Examinations for palpable masses were initiated in study month 6 but the frequency of these examinations was not included in the study report.

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 stained with hematoxylin and eosin for histopathologic examination. Initially, 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.

Body weight, food consumption, and water consumption were analyzed by one-way analysis of variance; Dunnett's t-test was used to determine if means of treated groups were significantly different from controls. Statistical evaluations included comparisons of all groups relative to each control group, as well as to pooled controls. Pairwise t-tests were used to compare the mean absolute organ weights (and mean percentage relative organ weights) between the pooled control groups and each treated group by sex and organ. Two-sided trend tests were performed to determine whether the mean weights increased or decreased with increasing dose. Statistical analysis of survival included the Kaplan-Meier method, the log rank test, and a test for dose-related trend in survival. Tumor incidence data were also analyzed using lifetime tumor rates that were not time adjusted, utilizing the Cochran-Armitage trend test. Tarone's method of analysis was used to assess the lethality of mesotheliomas of the tunica vaginalis testis. For all tumor types, the interval-based method of Peto and the logistic score test were used. Results of statistical tests were generally considered significant at the $p < 0.05$ level.

Nonneoplastic results—primarily neurotoxicity

Cumulative mortality data were depicted graphically, and statistical significance was not reported. There were only minor dose-related increases in cumulative mortality observed among the male rat groups during the first 60 weeks of treatment, after which mortality increased in high dose males compared with all other groups, increasing by the end of the study to 75% vs. 53% and 44% in control groups 1 and 2, respectively. Differences in mortality among the male control groups were greater than differences among either control groups and the low- or mid-dose-treated males at study end. There were only minor differences in female rat mortality within the first 23 months; however, by study end, mortality rates in controls 1 and 2 and the 1.0 and 3.0 mg/kg-day treatment groups were 40, 28, 35, and 49%, respectively.

Group mean body weights for control and treated groups were depicted graphically. No significant differences were seen among experimental groups regarding food or water consumption. Mean body weights of 2.0 mg/kg-day male rats were consistently decreased from those of control groups starting at week 8 and were significantly decreased from week 40 (398 grams vs. 408 grams in controls, approximately 2.5% lower) to study end (375 grams vs. 412 grams in controls, approximately 9% lower). Body weights of 0.1 and 0.5 mg/kg-day males did not differ significantly from controls at any time during the study. Mean body weights of 3.0 mg/kg-day females were significantly lower than controls from week 3 to study end, although the data in the graphical depiction indicated that the difference was greatest near study end and did not exceed 8%. Slight but significantly lower mean body weight was observed in 1 mg/kg-day females from weeks 8 to 32. However, this treatment group did not exhibit significant differences in mean body weight at other time points. The study authors did not provide data

concerning organ weights but stated that slight differences (significant in some cases) between group mean organ weights generally reflected group differences in mean final body weight.

At the level of behavioral and clinical observation performed in this bioassay protocol, no clinical signs of neurotoxicity were reported in any treated rats. Table 4-12 summarizes the light microscopic findings in sciatic nerve sections of selected rats of each sex and treatment level. Sciatic nerve degeneration was characterized by vacuolated nerve fibers of minimal-to-mild severity. The authors did not include results of statistical analysis of increased incidences of sciatic nerve degeneration among high-dose male and female rats, relative to controls. However, application of Fisher's Exact test shows significantly increased incidences of sciatic nerve degeneration among both male and female high-dose rats.

Table 4-12. Light microscopic data for sciatic nerves from F344 rats exposed to acrylamide in drinking water for 2 years

	Dose (mg/kg-day)						
Endpoint	**0**	**0**	**0.1**	**0.5**	**1**	**2**	**3**
Males							
Number examined	83	88	65	38	–	49	–
Degeneration[a]	30	29	21	13		26	
	(36%)	(33%)	(32%)	(34%)		(53%)[b]	
Females							
Number examined	37	43	–	–	20	–	86
Degeneration[a]	7	12			2		38
	(19%)	(28%)			(10%)		(44%)[b]

[a]Number of sciatic nerves (% of examined nerves) that exhibited light microscopic evidence of degeneration.
[b]Statistically different from control groups according to Fisher's Exact test ($p < 0.05$) performed by Syracuse Research Corporation.

Sources: Friedman et al. (1995); Tegeris Laboratories (1989).

The authors stated that palpable masses in male rats, located primarily in the inguinal area and most likely associated with inflammation of the preputial gland, were observed beginning in the first 12 months of the study. The incidences of these masses were similar in all dose groups during the second year of treatment. Although no dose-related differences were seen in the percentage of rats with masses at individual locations, the total percentage of rats with palpable masses was increased in the high-dose group, compared with either control group or the pooled controls (specific data not presented).

To summarize, the noncancer effects, the Friedman et al. (1995) study observed peripheral nerve degeneration based on light microscopic examination (electron microscopy was not conducted) in F344 rats exposed to AA in drinking water for 2 years. A NOAEL of 1 mg/kg-day was identified in female rats (0.5 mg/kg-day in male rats) with a LOAEL of 2 mg/kg-day for male rats.

Neoplastic results—tumors at multiple sites

Incidences of selected neoplastic lesions in male and female rats are presented in Tables 4-13 and 4-14, respectively. Histopathologic examination revealed significantly increased incidences of male thyroid gland (follicular cell) adenoma (and adenoma or carcinoma combined) and tunica vaginalis mesothelioma in the 2.0 mg/kg-day group. Females exposed to 1.0 and 3.0 mg/kg-day developed a significantly increased incidence of mammary gland fibroadenomas or combined fibroadenomas and carcinomas. Only the high-dose (3.0 mg/kg-day) females exhibited a significantly increased incidence of thyroid gland follicular cell neoplasms (adenomas or carcinomas combined).

Table 4-13. Incidences of tumors in male F344 rats exposed to acrylamide in drinking water for 2 years

	Dose (mg/kg-day)				
	0	**0**	**0.1**	**0.5**	**2.0**
Number of animals/group	102	102	204	102	75
Tissue/lesion					
Brain (glial origin)[a]					
Astrocytoma	1/102	0/102	0/98	0/50	2/75
Oligodendroglioma	0/102	1/102	1/98	1/50	0/75
Spinal cord (glial origin)					
Astrocytoma	0/82	0/90	1/68	0/37	1/51
Reproductive organs and accessory tissues					
Tunica vaginalis testis mesothelioma	4/102	4/102	9/204	8/102	13/75[b]
Thyroid gland (follicular cell)					
Adenoma	2/100	0/102[c]	9/203	5/101	15/75[b,d]
Carcinoma	1/100	2/102	3/203	0/101	3/75
Adenoma or carcinoma (combined)	3/100	2/102[c]	12/203	5/101	17/75[b,e]

[a]Does not include two rats with "malignant reticulosis" of the brain, one dosed male and one control male. The male 0.1 mg/kg-day group had only 98/204 brains and 68/204 spinal cords examined. The male 0.5 mg/kg-day had only 50/102 brains and 37/102 spinal cords examined. All male brains of high-dose rats and all male control brains (both subgroups) were examined, but only 82/102 and 90/102 control spinal cords and 51/75 high dose spinal cords were examined. (Footnote from Rice, 2005).
[b]Significantly different from control, $p < 0.05$.
[c]The data reported in Table 4 in Friedman et al. (1995) list 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) list no follicular cell adenomas in this group. The corrected number for adenomas (0) and the total number of combined adenomas and carcinomas (2) in the second control group are used in this table and this assessment.
[d]Twelve rats had a single follicular cell adenoma and three rats had multiple follicular cell adenomas.
[e]A single rat had both an adenoma and a carcinoma.

Source: Friedman et al. (1995).

Table 4-14. Incidences of tumors in female F344 rats exposed to acrylamide in drinking water for 2 years

	Dose (mg/kg-day)			
	0	**0**	**1.0**	**3.0**
Number of animals/group	50	50	100	100
Tissue/lesion				
Brain (glial origin)[a]				
Astrocytoma	0/50	0/50	2/100	2/100
Oligodendroglioma	0/50	0/50	0/100	0/100
Spinal cord (glial origin)				
Astrocytoma	0/45	0/44	0/21	1/90
Mammary gland				
Fibroadenoma	5/46	4/50	20/94[b]	26/95[b]
Adenocarcinoma	2/46	0/50	2/94	4/95
Adenoma or carcinoma (combined)	7/46	4/50	21/94[b]	30/95[b]
Thyroid gland (follicular cell)				
Adenoma	0/50	0/50	7/100	16/100[c]
Carcinoma	1/50	1/50	3/100	7/100
Adenoma or carcinoma (combined)	1/50	1/50	10/100	23/100[b]

[a]Does not include five dosed female rats with "malignant reticulosis" of the brain. All female brains were examined, but only 45/50, 44/50, 21/100, and 90/100 spinal cords in control 1, control 2, low-, and high-dose females, respectively, were examined. (Footnote from Rice, 2005).
[b]Significantly different from control, $p < 0.001$ as reported by Friedman et al. (1995).
[c]Statistically different from control groups according to Fisher's Exact test ($p < 0.05$) performed by Syracuse Research Corporation.

Source: Friedman et al. (1995).

These findings confirm the results of the earlier Johnson et al. (1986) drinking water bioassay with F344 rats; i.e., significantly increased incidences of thyroid follicular cell tumors in males and females, tunica vaginalis testis mesotheliomas in males, and mammary gland tumors in females. Results of the study of Johnson et al. (1986) that were not reported as being replicated in the study of Friedman et al. (1995) include the statistically significantly increased incidences of adrenal pheochromocytomas in males, CNS tumors of glial origin in females, oral cavity tumors in females, and clitoral or uterus tumors in females.

In a review of the Friedman et al. (1995) study data, Rice (2005) noted that, although glial tumors of brain and spinal cord were reported not to be increased, not all of the brains and spinal cords in the test animals were examined, and seven cases of a morphologically distinctive category of primary brain tumor described as "malignant reticulosis" were reported but were excluded from the authors' analysis. Rice (2005) comments that it is unusual to exclude brain tumors of this kind from the results of a bioassay. The neoplasms diagnosed as "malignant reticulosis" are of uncertain origin but have some features in common with anaplastic astrocytomas. Both astrocytomas and neoplasms consistent with a descriptive designation of "malignant reticulosis" are also induced in rats by the structurally closely related compound, acrylonitrile (IARC, 1994b) and by the simple epoxide carcinogen, ethylene oxide (IARC, 1999). Rice (2005) concluded that the primary brain tumors were underreported in the Friedman

et al (1995) study and provided the following details from his review of the study records (also see footnotes in Tables 4-13 and 4-14):

> . . . tabulated data in the study report does not include seven rats with "malignant reticulosis" of the brain, including five dosed females, one dosed male and one control male. The male 0.1 mg/kg-day group had only 98/204 brains and 68/204 spinal cords examined. The male 0.5 mg/kg-day had only 50/102 brains and 37/102 spinal cords examined. All male brains of high-dose rats and all male control brains (both subgroups) were examined, but only 82/102 and 90/102 control spinal cords and 51–75 high dose spinal cords were examined. All female brains were examined, but only 45/50, 44/50, 21/100 and 90/100 spinal cords in control, control, low- and high-dose females, respectively were examined.

EPA agrees that the brain tumor incidence rates and analyses should have been more fully documented in the Friedman et al. (1995) report tables and discussion, and concurs with the Rice (2005) conclusion that the CNS tumors be considered one of the tumor types replicated in the Friedman et al. (1995) study, even though the incomplete brain and spinal cord tumor data set precludes a quantitative analysis of CNS tumor incidence in the characterization of dose-response.

Iatropoulos et al. (1998) reevaluated reproductive tissue from the 38 male rats originally diagnosed with tunica vaginalis mesotheliomas and arrived at a different diagnosis than the original analysis (which considered all of the mesotheliomas to be malignant as reported in Friedman et al. [1995] and Tegeris Laboratories [1989]). Using criteria specified by McConnell et al. (1992), tissue blocks and slides were reevaluated and reclassified into one of three types of mesothelial lesions: (1) focal mesothelial hyperplasia, (2) benign mesothelioma, and (3) malignant mesothelioma. Proliferating cells from the mesothelial lesions were stained for proliferating cell nuclear antigen to assess the fraction of cells that were replicating. In addition, for each rat, the extent of Leydig cell neoplastic proliferation was assessed as occupying 25, 50, 75, or 100% of the testes. The evaluations were reported to have been conducted in a blinded fashion. The reevaluation assessed that not all of the previously diagnosed mesotheliomas were malignant (see Table 4-15). All rats reevaluated as having malignant mesotheliomas were assessed as having 75 or 100% of the testes occupied by Leydig cell neoplasia. In contrast, rats reevaluated as having focal mesothelial hyperplasia or benign mesothelioma showed either no Leydig cell neoplasia or 25 or 50% of the testes occupied by Leydig cell neoplasia. The comparison suggests that the extent of Leydig cell neoplasia and the development of malignant mesotheliomas may have been linked.

Table 4-15. Reevaluation and comparison of mesothelial lesions and extent of Leydig cell neoplasia in male F344 rats exposed to acrylamide in drinking water for 2 years

Dose (mg/kg-day)	Rat no.	Diagnosis[a]	Evidence of metastasis or invasion	Leydig cell neoplasia[b]
Control Group 1	126	No mesothelial tissue was present	Metastasis to mesentery	L+++
	134	Benign mesothelioma, focal		L+
	170	Malignant mesothelioma		L+++
	179	Benign mesothelioma, focal	Metastasis to seminal vesicles	L++
Control Group 2	257	Malignant mesothelioma	Metastasis to peritoneal cavity	L++++
	301	Focal mesothelial hyperplasia		L+
	335	Focal mesothelial hyperplasia		L+
	353	Malignant mesothelioma	Invasion through the serosa	L+++
0.1	432	No mesothelial change		–
	457	Malignant mesothelioma	Metastasis to neighboring skeletal muscle	L++++
	473	Malignant mesothelioma	Metastasis to mesentery	L+++
	484	Malignant mesothelioma	Invasion through the serosa	L++++
	514	Focal mesothelial hyperplasia		L+
	564	Malignant mesothelioma	Metastasis to mesentery	L+++
	594	Focal mesothelial hyperplasia		L+
	603	Malignant mesothelioma	Metastasis to hepatic serosa	L+++
	606	Focal mesothelial hyperplasia		L+
0.5	729	Malignant mesothelioma	Metastasis to mesentery, splenic serosa	L+++
	732	Malignant mesothelioma	Metastasis to splenic serosa	L+++
	756	Benign mesothelioma, focal		L+
	758	Benign mesothelioma, focal		–
	762	Malignant mesothelioma	Metastasis to neighboring skeletal muscle, splenic serosa	L++++
	767	Focal mesothelial hyperplasia		–
	780	Benign mesothelioma, focal		L++
	783	Benign mesothelioma, focal		L++
2.0	810	Benign mesothelioma, focal		L+
	813	Malignant mesothelioma	Metastasis to urinary bladder	L+++
	814	Benign mesothelioma, focal		L+
	816	Malignant mesothelioma	Invasion through the serosa	L+++
	821	Focal mesothelial hyperplasia		–
	824	Focal mesothelial hyperplasia		L+
	832	Malignant mesothelioma	Metastasis to seminal vesicles, epididymis	L+++
	841	Benign mesothelioma, focal		L++
	844	Malignant mesothelioma	Metastasis to neighboring skeletal muscle, mesentery	L+++
	847	Benign mesothelioma, focal		L++
	850	Benign mesothelioma, focal		L++
	868	Malignant mesothelioma	Metastasis to mesentery	L+++
	878	Benign mesothelioma		L++

[a]Rats previously diagnosed as having mesothelioma of the tunica vaginalis testis (Friedman et al., 1995).
[b]Leydig cell neoplasms occupying 25% (+), 50% (++), 75% (+++), or 100% (++++) of testes; – denotes no neoplasm.

Source: Iatropoulos et al. (1998).

4.2.2. Inhalation Exposure

Information on the response to subchoronic or chronic exposure to inhaled AA in animals is limited to three subchronic studies in cats, dogs, and rats from the mid-1950s (Hazleton

Laboratories, 1954, 1953) with demonstration of neurotoxicity dependent on dose and species tested. No chronic animal inhalation studies for exposure to AA were identified.

4.2.2.1. *Subchronic Studies*

Exposure of four cats to acrylamide vapors at a mean analytical concentration of 1.65 ppm (4.8 mg/m^3), 6 hours/day, 5 days/week for 3 months, resulted in no apparent clinical signs or adverse effects on body weight (Hazleton Laboratories, 1954). Results of periodic blood studies (hematocrit, hemoglobin, sedimentation rates, and white blood counts) and plasma pseudocholinesterase activity levels were within normal limits.

Exposure of dogs and rats to an aerosol of AA dust at a concentration of 15.6 mg/m^3, 6 hours/day, 5 days/week for up to 12 exposures, resulted in progressive signs of neurotoxicity and death (Hazleton Laboratories, 1953). Simultaneously exposed guinea pigs showed no neurotoxic signs.

4.2.2.2. *Chronic Studies*

No chronic inhalation animal studies were identified.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

There is a large database for reproductive effects from oral exposure to AA, and the reproductive section begins with a discussion of the recent expert panel review of the database (NTP/CERHR, 2004).

There were no inhalation studies found in the literature that measured reproductive or developmental in animals exposed to AA.

4.3.1. Reproductive Toxicity Studies

An NTP-sponsored expert panel (NTP/CERHR, 2004) conducted a comprehensive review of reproductive and developmental toxicity studies for a variety of exposure routes: by drinking water in rats or mice (NTP, 1993; Smith et al., 1986; Zenick et al., 1986), by gavage in rats (Sublet et al., 1989; Working et al., 1987b), by i.p. injection in mice (Holland et al., 1999; Nagao, 1994; Ehling and Neuhäuser-Klaus, 1992; Dobrzynska et al., 1990; Shelby et al., 1987, 1986), and by dermal application in mice (Gutierrez-Espeleta, 1992). The NTP/CERHR (2004) report summarized that the lowest effective doses of acrylamide reported were 30 ppm in drinking water in rats (a cumulative dose of about 200 mg/kg by the time of mating) (Smith et al., 1986), 6.78 mg/kg-day in drinking water in mice (a cumulative dose of 949 mg/kg over the 20-week exposure period) (NTP, 1993), 15 mg/kg-day for 5 days by gavage in rats (Sublet et al., 1989), 75 mg/kg i.p. in mice (single dose) (Ehling and Neuhäuser-Klaus, 1992), and 25 mg/kg-day for 5 days applied dermally to mice (Gutierrez-Espeleta, 1992). The panel concluded that

the dominant lethal data provide firm in vivo postmetabolic evidence of genotoxicity in mammals and that AA was effective via all routes in all species at comparable doses. The report notes that the stage effect was consistent but that the dominant lethal test does not effectively assess damage in spermatogonial stem cells. The panel cautioned against assigning stage-specific effects in these studies based on the kinetics of spermatogenesis, given that some chemical agents (including, perhaps, AA) may alter the kinetics of spermatogenesis. In the case of AA, the dominant lethal studies most likely indicate an effect on the ability of epididymal spermatozoa and spermatids to fertilize an oocyte, along with potential pre- and postimplantation genetic effects. Although the anti-fertilization effect may be due to nongenetic actions, the doses needed to elicit the anti-fertilization effects were generally higher than that needed to elicit the postimplantation genetic effects, and thus the anti-fertilization effects are of limited utility for predicting human risk.

The following discussion presents details of the oral studies, including two-generation/dominant lethal studies (Tyl et al., 2000a; Chapin et al., 1995) and dominant lethal studies (Tyl et al., 2000b; Sublet et al., 1989; Working et al., 1987; Smith et al., 1986; Zenick et al., 1986). The results for other reproductive function endpoints are also discussed (Sakamoto et al., 1988; Sakamoto and Hashimoto, 1986; Zenick et al., 1986).

Tyl et al. (2000a) two-generation/dominant lethal study

Tyl et al. (2000a) performed a two-generation reproduction and dominant lethal study of AA in F344 rats. Groups of F0 weanlings (30/sex/group) were exposed to AA in the drinking water at concentrations that would provide dose levels of 0, 0.5, 2.0, or 5.0 mg/kg-day during a prebreeding period of 10 weeks. The breeding period consisted of 14 days of cohabitation, during which males and females were paired one-to-one. During mating, gestation, and the first week of lactation, female rats of each treatment group were given the same concentration of AA in the drinking water as that to which they had been exposed during the final week prior to mating; during the cohabitation mating period, males were exposed to AA based on the body weights of the corresponding females during mating to avoid overexposure of the females. As soon as each successful mating was confirmed, each pair was separated. Mated females were weighed on GDs 0, 6, 13, and 20. Dams and litters were weighed on postnatal days (PNDs) 1, 4, 7, 14, 21, and 28. Pups were weaned on PND 28. Following mating, F0 males were maintained on their respective AA doses until 2 days prior to being mated with naive unexposed females in the dominant lethal portion of the study, after which impregnated females were separated from the males and sacrificed on GD 14. Gross examinations were performed and number of ovarian corpora lutea and number and distribution of total uterine implantation sites, resorption sites, and live and dead implants were determined.

Thirty F1 male and 30 F1 female rats of each dose group were selected to be continued on AA (in the same manner as their parents) to produce F2 pups. The prebreeding treatment period for F1 rats was 11 weeks. All F0 and F1 parental rats in all treatment groups were subjected to gross necropsy. In addition, 30 male and 30 female F1 parental rats each from control and high-dose groups were subjected to histologic examination of major reproductive tissues and representative target neurological tissues (peripheral nerves, brain, and spinal cord). Sciatic and tibial nerve sections from six high-dose male and three control male F1 adults and spinal cord sections from three high-dose and two control female F1 adults were stained with Bodian's method for additional histologic examination. Selected F1 and F2 weanling rats were subjected to the same histologic examinations as were the F1 parental rats. The study report does not indicate that tissues from F0 rats were histologically examined.

Results for quantitative continuous variables were analyzed using Levene's test for equal variances, ANOVA, and t-tests. Nonparametric data were statistically evaluated by using the Kruskal-Wallis test, followed by the Mann-Whitney U-test for pairwise comparisons. Fisher's Exact test was used to compare frequency data. For all statistical tests, the level of significance was $p < 0.05$.

F0 males in all three treatment groups showed statistically significantly reduced mean body weight compared with controls (~4–6% decreased), starting after 4–6 weeks and continuing through 13 weeks when exposure ceased. Body weights in 2.0 and 5.0 mg/kg-day F1 males showed similar depressions of body weight throughout their 13 weeks of exposure. Body weights in F0 females were statistically significantly lower than controls during the latter 4 weeks of the prebreeding period in the 2.0 and 5.0 mg/kg-day groups (~4–6% decreased), at the end of gestation in the 5.0 mg/kg-day group (~9% decreased), and most of the lactation period in the 5.0 mg/kg-day group (~4–6% decreased). Body weights in F1 females were statistically significantly lower than controls during the latter 8 weeks of prebreeding in the 2.0 and 5.0 mg/kg-day groups (~5% decreased), at the end of gestation in the 2.0 (~3% decreased) and 5.0 mg/kg-day groups (~12% decreased), and during the middle 3 weeks of lactation in the 5.0 mg/kg-day group (~4–6% decreased). In F2 offspring, statistically significant changes in body weight were restricted to the 5.0 mg/kg-day group at PND 14 (~7% decreased). The depressions in body weight, although not large, provide evidence of mild systemic toxicity, most consistently in 2.0 and 5.0 mg/kg-day F0 and F1 adult males.

Increased incidences of rats with foot splay occurred in F0 exposure groups relative to controls. Incidences for foot splay were 3/30, 10/30, 7/30, and 10/30 for control through 5.0 mg/kg-day F0 males and 1/30, 2/30, 6/30, and 6/30 for F0 females. Fisher's Exact test (performed by Syracuse Research Corporation) indicated that incidences were statistically significantly ($p < 0.05$) elevated in the low- and high-dose male groups; incidences in the mid- and high-dose female groups were marginally ($p = 0.51$) elevated compared with controls. No

foot splay was observed in F1 males or F1 females in any groups. Head tilt was displayed by some F0 and F1 males and F1 females, but the incidences of this sign of neurotoxicity were not statistically significantly different from controls, except for a marginally significant ($p = 0.056$) elevation in the 5.0 mg/kg-day F1 males (0/30, 0/30, 0/30, and 4/30).

Gross examinations of all F0 rats, all F1 pups that died during lactation, and selected F1 weanlings yielded no treatment-related findings. Histopathologic examination of reproductive and nervous system tissues of the F1 weanlings revealed no signs of treatment-related adverse effects. Histopathology of selected nervous system tissues from control and 5.0 mg/kg-day F1 adults and all necropsied F2 weanlings showed no exposure-related lesions with conventional staining (hematoxylin and eosin). However, when peripheral nerve sections (from sciatic and tibial nerves) were examined with Bodian's stain, minimal to mild axonal fragmentation and/or swelling was observed in 6/6 F1 5.0 mg/kg-day males compared with 0/3 control F1 males (female tissues were not examined). Spinal cord sections from 3 high-dose females and 2 control females, stained by the same method, showed no lesions (male tissues were not examined). Tissues from F0 rats and F1 rats in lower exposure groups were not examined histologically.

Acrylamide treatment did not significantly affect F0 or F1 reproductive parameters involving success of mating and impregnation or gestation length, but 5.0 mg/kg-day induced statistically significantly decreased numbers of implantations/dam and live pups/litter on PND 0, and increased postimplantation loss in the F0 and F1 generations (Table 4-16). F1 and F2 pup survival between PNDs 0 to 4 was unaffected by treatment, with the exception that, in the 5.0 mg/kg-day group, three one-pup F1 litters and three one-pup F2 litters did not survive.

No effects on F1 pup body weights were seen on PNDs 1, 4, or 7. However, measurements made on PNDs 14, 21, and 28 (when rat pups had begun to drink and feed themselves) revealed significantly reduced pup weight (8–11% lower than controls) in 5.0 mg/kg-day males. Significantly reduced mean F2 pup body weight (approximately 8%) was seen only in 5.0 mg/kg-day pups and only on PND 14.

Table 4-16. Changes in reproductive parameters in F344 rats exposed to acrylamide in drinking water for two generations

Parameters	Dose (mg/kg-day)			
	0	0.5	2.0	5.0
F0 parents/F1 mating (30 pairs/group)				
No. males impregnating	17	24	22	21
No. females pregnant	20	24	26	18
No. implantations/dam	10.4 ± 2.5	10.0 ± 3.6	10.2 ± 2.2	6.8 ± 3.1[a]
No. live pups/litter (PND 0)	9.8 ± 3.1	9.8 ± 3.5	9.7 ± 2.4	4.5 ± 2.6[a]
Postimplantation loss (%)	7.9 ± 18.5	2.1 ± 4.7	5.7 ± 9.1	34.4 ± 25.9[a]
F1 parents/F2 mating (30 pairs/group)				
No. males impregnating	23	25	25	27
No. females pregnant	23	25	27	23
No. implantations/dam	11.3 ± 1.5	10.0 ± 3.4	10.5 ± 2.1	6.8 ± 3.3[a]
No. live pups/litter (PND 0)	10.8 ± 1.5	10.0 ± 2.9	9.6 ± 2.4	5.1 ± 3.2[a]
Postimplantation loss (%)	4.4 ± 7.6	3.3 ± 7.9	9.1 ± 14.4	23.1 ± 28.2

[a]Significantly ($p < 0.05$) different from control value. Values are group means ± SD.

Source: Tyl et al. (2000a).

Dominant lethal results

In the dominant lethal mutation protocol in which exposed male rats were mated with nonexposed female rats, exposure did not adversely affect fertility or mating indices or the number of corpora lutea (Table 4-17). However, the total number of implants/litter and the percentages of pre- and postimplantation loss were statistically significantly different from controls in nonexposed females mated to treated 5.0 mg/kg-day F0 males.

In summary, the two-generation reproductive toxicity/dominant lethal mutation study with F344 rats exposed to AA in drinking water (Tyl et al., 2000a) identified 5.0 mg/kg-day as the LOAEL and 2.0 mg/kg-day as the NOAEL for effects on reproduction in the F0 and F1 generations (decreased implantations/dam and decreased number of live pups/litter). The same NOAEL and LOAEL were identified for dominant lethal mutation effects (decreased live implants/litter and increased pre- and postimplantation loss) when exposed males were bred with nonexposed females. Decreased body weights (4–6% compared with control values) were observed most consistently in F1 males at doses ≥2.0 mg/kg-day (not at 0.5 mg/kg-day), and signs of neurotoxicity (increased incidences of foot splay) were observed in F0 males in the low- and high-dose groups (0.5 and 5.0 mg/kg-day) and in F0 females in the 2.0 and 5.0 mg/kg-day groups.

Table 4-17. Results of the dominant lethal mutation assay in F344 rats

Parameter	Acrylamide dose (mg/kg-day) in the drinking water			
	0.0	**0.5**	**2.0**	**5.0**
No. males paired	30	30	30	30
No. females paired	60	60	60	60
No. fecund males[a]	29 (96.7%)	30 (100.0%)	30 (100.0%)	30 (100.0%)
No. fertile males[b]	28 (93.3%)	29 (96.7%)	29 (96.7%)	29 (96.7%)
No. plug- or sperm-positive females	57 (95.0%)	56 (93.3%)	59 (98.3%)	57 (95.0%)
No. pregnant females	52 (91.2%)	50 (89.3%)	57 (96.6%)	52 (91.2%)
Mating index[c]	52/60 (86.7%)	50/60 (93.3%)	57/60 (95.0%)	52/60 (86.7%)
No. corpora lutea/dam	11.8 ± 2.1[d]	11.5 ± 1.1	11.8 ± 1.1	11.4 ± 1.2
No. total implants/litter	10.0 ± 2.3	9.9 ± 2.5	10.2 ± 2.2	8.6 ± 2.7[f]
Percent preimplantation loss	14.3 ± 19.6	14.3 ± 21.2	13.5 ± 18.4	24.9 ± 22.7[f]
Live implants/litter	9.4 ± 2.2	9.5 ± 2.5	9.6 ± 2.3	7.5 ± 2.6[g]
Nonlive implants/litter	0.6 ± 0.7	0.4 ± 0.7[e]	0.6 ± 0.7	1.1 ± 1.0[e]
Percent postimplantation loss	6.2 ± 7.0	3.7 ± 6.8[e]	6.1 ± 6.9	14.2 ± 17.1[f]

[a]Number of males that produced at least one plug- or sperm-positive female.
[b]Number of males that produced at least one pregnant female.
[c]Ratio of pregnant females to paired females.
[d]Mean ± SD.
[e]$p < 0.05$.
[f]$p < 0.01$.
[g]$p < 0.001$.

Source: Tyl et al. (2000a).

Chapin et al. (1995) two-generation/dominant lethal/grip strength study

Chapin et al. (1995) conducted a two-generation continuous breeding reproductive toxicity study in CD-1 mice that included an assessment of grip strength in F0 and F1 adult mice. Male and female CD-1 mice (20/sex/treatment group) were individually housed and administered AA in the drinking water at concentrations of 3, 10, or 30 ppm for 7 days, followed by continuous dosing during 14 weeks of cohabitation as mating pairs. At test concentrations of 3, 10, and 30 ppm, the authors estimated AA doses of 0.81, 3.19, and 7.22 mg/kg-day for both male and female F0 mice, based on water consumption data of F0 females. A control group consisted of 40 mating pairs. Mice were monitored for clinical signs, but the frequency of observations was not specified. Body weights of F0 mice were recorded following the delivery of each litter produced during the cohabitation period, at necropsy, and at other unspecified time points. Pups from each litter were counted, sexed, weighed, and killed. Reproductive indices measured included fertility (number of pairs delivering at least 1 litter), number of litters/pair, and number of live pups/litter, sex ratio, day of delivery, and pup birth weight. Parental food and water consumption were measured for 1 week both immediately prior to (study week 1) and following (study week 16) the cohabitation period (study weeks 2–15). Forelimb and hindlimb grip strength were assessed in 10 male and 10 female F0 mice/group during study weeks 0, 3, 6, 9, 12, and 17.

At the end of the 14-week cohabitation period, the F0 pairs were separated and dosed for an additional 6 weeks, during which time pregnant dams were allowed to deliver and wean F1 litters. The F1 pups were culled to two/sex/litter and maintained on the same dosing regimen as their parents. Upon reaching 74 days of age, F1 females were mated to nonsibling males of the same treatment group for up to 1 week then separated and continued on their respective AA treatment levels until delivery of the F2 generation. Reproductive variables evaluated for the F1 parental mice were the same as those for the F0 generation. Grip strength was measured in F1 parental mice at weeks 3, 5, 7, 10, and 16 (necropsy week) of treatment. At necropsy, body and selected organ weights were recorded. Microscopic examinations were performed on sural and gastrocnemius nerves of both sexes of F1 mice, testes and epididymides of F1 males, and visible gross lesions.

During the 6-week separation period following 98 days of F0 cohabitation, selected control and exposed F0 males were cohabited with three untreated females for up to 4 days in order to evaluate dominant lethal effects in the males. Pregnant females were subjected to necropsy on GD 16. Uteri were examined for number of live, dead, and resorbed implants.

Following the 6-week separation period, crossover mating tests of control and high-dose male and female F0 mice were performed, which resulted in pairings of control males with control females, control males with high-dose females, and high-dose males with control females. The pairs were allowed to mate for 1 week, during which time AA treatment was suspended. Treatment then continued throughout gestation and delivery. Reproductive indices measured included fertility (number of pairs delivering at least 1 litter), number of litters/pair, and number of live pups/litter, sex ratio, day of delivery, and pup birth weight. Estrous cyclicity in parental females was assessed for 12 days following delivery. At necropsy, body and selected organ weights were determined for all F0 mice. Sperm quality was assessed in male F0 mice.

Grip strength measurements were performed by testing forelimb first, then hindlimb. The results of three such trials were averaged for each animal tested. Grip strength values were compared by ANOVA. In the dominant lethal tests, all data from females mated to a given male were pooled. Differences in results between treated and control groups were considered significant at the level of $p < 0.05$.

Acrylamide treatment did not affect body weight or food consumption in F0 males or F0 females, but water consumption was erratic in males. In F1 mice selected for mating, exposure-related effects on body weight were not found, except for an 8% decrease in body weight, compared with controls, in 30-ppm females. The authors estimated AA doses to be approximately 0.86, 2.9, and 7.7 mg/kg-day, based on water consumption during the week following mating. To compare with other AA toxicity studies, approximate average doses for the groups in this study are taken to be 0, 0.8, 3.1, and 7.5 mg/kg-day.

No treatment-related effects were observed regarding proportion of F0 fertile pairs, percentage of cohabiting F0 pairs with litters, average number of F1 litters/pair, proportion of live F1 pups born, sex ratio, or mean live F1 pup weight. A slight, but statistically significant, decrease in aggregate mean number of live F1 pups was observed at 30 ppm (12.2 ± 0.5, n = 18, vs. 13.6 ± 0.5, n = 39, for controls). This 10% change was due to significantly reduced numbers of live pups in the second and third litters of high-dose mice but not in the first, fourth, or fifth litters.

Acrylamide treatment had no adverse effect on postnatal survival or body weight gain prior to weaning in F1 mice selected for mating. No treatment-related effects were seen regarding the numbers of impregnated F1 females or percentage of F1 females that delivered offspring. The mean number of live F2 pups was significantly decreased in the 30-ppm group (7.9 ± 1.0 live pups/litter vs. 14.8 ± 0.5 in controls) in the absence of a significant treatment-related alteration in live pup birth weight. Postpartum dam body weight was significantly lower (11%) in 30-ppm F1 dams (34.1 ± 0.9 grams vs. 37.7 ± 0.9 grams in controls).

Dominant lethal results

When exposed F0 male mice were mated with nonexposed females, dominant lethal effects were observed at the 30-ppm exposure level. Significantly increased early resorptions, total postimplantation loss, and decreased number of live fetuses were observed in the 30-ppm group (see Table 4-18). Percentages of impregnated females were 83, 83, 81, and 77 for the control through 30-ppm groups, respectively, indicating no effects on male fertility.

Table 4-18. Results of dominant lethality testing in male Swiss CD-1 mice exposed to acrylamide in the drinking water

	Acrylamide concentration (ppm)			
	0	**3**	**10**	**30**
Number of males tested	20	20	19	20
Early resorptions	0.86 ± 0.1[a]	0.78 ± 0.26	1.04 ± 0.17	1.74 ± 0.17[b,c]
Dead fetuses	0.03 ± 0.02	0.06 ± 0.03	0.04 ± 0.02	0.09 ± 0.06
Total implantation loss	0.98 ± 0.12	0.99 ± 0.28	1.14 ± 0.16	1.95 ± 0.17[b,c]
Live fetuses	12.5 ± 0.3	12.5 ± 0.2	12.5 ± 0.4	11.5 ± 0.4[b]

[a]Mean ± standard error of the mean (SEM); number/litter/male.
[b]Significantly different from controls ($p < 0.05$).
[c]Dose-related trend ($p < 0.05$).

Source: Chapin et al. (1995).

The crossover mating tests of control males with control females, control males with high-dose females, and high-dose males with control females resulted in averages of 11.4, 11.5, and 9.4 pups/litter, respectively. The study authors found no statistically significant differences in litter sizes among the different groups but suggested that the smaller average litter size in the

group of high-dose males mated with control females (9.4 pups/litter, compared with 11.4 and 11.5 pups/litter in the other two groups) indicated that the dominant lethal effect was related to toxicity in males rather than females. However, the study report did not include additional details of the results (incidence data or variation from mean values).

Necropsy results of all F0 mice did not reveal any signs of treatment-related adverse effects on body weight or absolute or relative weights of liver, kidneys/adrenals, right ovary, right testis or cauda epididymis, prostate, or seminal vesicles. Sperm analysis revealed no treatment-related effects on epididymal sperm concentration, motility, frequency of abnormal forms, or total spermatid heads/testis. However, the mean number of spermatids/gram testis was statistically significantly ($p < 0.05$) lower in the 10- and 30-ppm F0 males (11.1 ± 0.4, 10.6 ± 0.4, 9.8 ± 0.8, and 10.0 ± 0.5 spermatids/gram testis in controls through 30 ppm). No AA-related effect on estrous cyclicity was seen in females (data were not shown).

Gross necropsy of F1 parental mice did not reveal treatment-related effects on male terminal body weight or weight of liver, kidneys/adrenals, right testis, epididymis, or seminal vesicles. Acrylamide treatment did not adversely affect female terminal body weight, absolute or relative liver weight, or right ovary weight. Absolute kidney and adrenal weight (combined) of 10- and 30-ppm females was significantly lower than controls (550.4 ± 8.5 mg, 540 ± 12.2 mg, 503.7 ± 11.7 mg, and 519 ± 22.9 mg for controls, low-, mid-, and high-dose groups, respectively). Relative liver weight was significantly increased (12 and 6%) in mid- and high-dose females, respectively. The authors reported a dose-related decrease in absolute mean prostate weight that was statistically significant in the 30-ppm male F1 group (controls 34.6 ± 1.9 mg; high dose 29.7 ± 1.7 mg), but mean weights of other treatment groups were not specified. Relative prostate weights were not significantly different from controls. No significant effects were seen regarding sperm quality or estrous cycle length. Upon histopathologic examination, testicular degeneration was noted in 1/10 mid- and high-dose males but was not observed in males of low-dose or control groups. Acrylamide treatment did not increase the incidence of grossly visible lesions or histopathologic findings in examined nerve tissues of male or female F1 parental mice.

Grip strength results

Absolute grip strength increased over time in control and exposed F0 groups during 17 weeks of exposure, and was reported to not be adversely affected by exposure. However, 30-ppm male and female F0 mice showed statistically significantly smaller increases over time, relative to controls (see Table 4-19). Statistically significantly reduced forelimb absolute grip strength was observed in 10- and 30-ppm F1 males (compared with controls) following 10 weeks of AA treatment. However, the biological significance of this finding is uncertain since the

authors found no treatment-related effects on grip strength in F1 males or females following 3, 5, 7, or 16 weeks of treatment.

Table 4-19. Effects of acrylamide in drinking water on grip strength of mice

	Acrylamide concentration (ppm)			
	0	**3**	**10**	**30**
F0 relative grip strength increase (%)[a]				
Males				
Forelimb	43.4 ± 18.3	39.6 ± 10.4	2.4 ± 11.7	6.9 ± 5.5[c,d]
Hindlimb	108.9 ± 12.2	66.4 ± 14.1	89.8 ± 11.8	67.6 ± 9.2[c,d]
Females				
Forelimb	37.3 ± 13.8	44.3 ± 12.6	3.2 ± 5.4[c]	1.4 ± 7.3[c,d]
Hindlimb	112.4 ± 28.6	126.0 ± 14.8	94.8 ± 15.8	72.6 ± 12.1
F1 absolute grip strength (grams)[b]				
Males				
Forelimb	96.4 ± 4.1	94.8 ± 4.4	81.4 ± 4.8[c]	84.5 ± 2.6[c,d]
Hindlimb	118.2 ± 4.0	123.5 ± 5.5	122.8 ± 5.9	115.6 ± 2.2
Females				
Forelimb	79.6 ± 2.7	74.7 ± 5.0	76.7 ± 4.8	80.0 ± 4.3
Hindlimb	103.1 ± 3.6	126.0 ± 14.8	102.7 ± 6.3	102.2 ± 4.1

[a]Percentage increase in grip strength during growth after 17 weeks of treatment (mean ± SEM, n = 10).
[b]Grip strength measured at F1 parental treatment week 10 (mean ± SEM, n = 10).
[c]Significantly different from controls ($p < 0.05$).
[d]Dose-related trend ($p < 0.05$).

Source: Chapin et al. (1995).

In summary, the results presented by Chapin et al. (1995) identified 30 ppm acrylamide in drinking water (7.5 mg/kg-day) as a LOAEL and 10 ppm (3.1 mg/kg-day) as a NOAEL for reproductive toxicity effects (e.g., increased early resorptions, total postimplantation loss; decreased number of live fetuses, decreased number of live F1 and F2 pups/litter) that appear to be male-mediated in Swiss CD-1 mice. No clear and consistent exposure-related effects on fertility, gross necropsy, organ or body weights, or histology of testicular or nervous system tissues were found. Mild changes in grip strength were noted in F0 and F1 male and female mice of the 30-ppm exposure groups and in F0 female and F1 male mice of the 10-ppm exposure groups.

Additional oral exposure dominant lethal studies

In a study designed to assess dominant lethal effects of AA, groups of male Long-Evans rats (10–11/group) were administered AA in the drinking water at concentrations of 0, 15, 30, or 60 ppm for a total of 80 days (Smith et al., 1986). Based on twice weekly recording of body weights and water consumption, the authors calculated the AA doses in the 15-, 30-, and 60-ppm exposure groups to be 1.5, 2.8, and 5.8 mg/kg-day, respectively. During the final 8 days of treatment, each male rat was paired nightly with two virgin untreated females until each male had impregnated two females or until the end of the treatment period. Sperm-positive female

rats were sacrificed on GD 14 and examined for numbers of corpora lutea and for living and dead fetal implants. Fertility rates and percentages of pre- and postimplantation losses were calculated. Following the completion of the mating period, six males of each group were sacrificed for histologic analysis of sperm. Segments of sacral, sciatic, and tibial nerves were excised, fixed, and stained with hematoxylin and eosin or toluidine blue for histopathologic examination. The remaining treated males were sacrificed 12 weeks after the end of treatment for assessment of reciprocal translocations in spermatocytes. Data on fertility rates were analyzed using chi-square statistics. Effects on pre- and postimplantation loss were analyzed using Kruskal-Wallis ANOVA with Mann-Whitney U-test for post hoc comparisons.

There were no statistically significant differences among controls and treated rats regarding body weights or water consumption. As shown in Table 4-20, fertility rates did not differ significantly among the groups. A significant elevation in preimplantation loss occurred only in females that had been mated with high-dose males. Postimplantation loss was statistically significantly higher in females mated with mid- or high-dose males relative to low-dose or control males. At the high dose, the percentage was more than 6 times higher than that of controls. None of the treated males exhibited hindlimb splaying, a characteristic sign of AA-induced neurotoxicity. No significant pathological lesions were seen in preparations of the sciatic nerve. The NOAEL in this study is 15 ppm (1.5 mg/kg-day) and the LOAEL is 30 ppm (2.8 mg/kg-day) for male-mediated reproductive effects (increased postimplantation loss). No histological changes were found in sacral, sciatic, and tibial nerves, and no evidence of hindlimb splaying was found in rats exposed to AA concentrations as high as 60 ppm (5.8 mg/kg-day).

Table 4-20. Fertility rates and pregnancy outcomes in Long-Evans rats following 72-day oral exposure of males to acrylamide in the drinking water

Number of males/group	Exposure level (ppm)	Dose (mg/kg-day)	Fertility (%)[a]	Preimplantation loss (%)[b]	Postimplantation loss (%)[c]
9	0	0	87	10.4 ± 1.8	5.7 ± 1.6
9	15	1.5	76	9.3 ± 2.3	7.2 ± 1.6
10	30	2.8	95	12.2 ± 1.4	13.3 ± 2.1[e]
11	60	5.8	80	25.1 ± 4.0[d]	36.7 ± 5.6[d]

[a](Number pregnant/number mated) × 100.
[b]([(Number corpora lutea – number implants]/[number corpora lutea]) × 100.
[c]([Number implants – number fetuses]/[number implants]) × 100.
[d]Significantly different from control, low-, and mid-dose groups, $p \leq 0.01$.
[e]Significantly different from control, low-, and high-dose groups, $p \leq 0.01$.

Source: Smith et al. (1986).

Several additional studies have demonstrated reversible dominant lethal effects and reversible effects on male fertility in animals orally exposed to AA for short time periods. Working et al. (1987) observed reversible male-mediated reproductive effects (dominant lethal

effects: increased implantation losses) in F344 rats exposed to 30 mg/kg-day for 5 days. Sublet et al. (1989) observed dominant lethal effects (increased implantation losses) and effects on male impregnation success in Long-Evans male rats exposed to oral doses as low as 15 mg/kg-day for 5 days. In this study, males were gavaged with 0, 5, 15, 30, 45, or 60 mg/kg AA for 5 days prior to mating. Reduced fertility and increased preimplantation loss were found in all dose groups except 5 mg/kg at week 1 posttreatment. Increased postimplantation loss was seen at weeks 2 and 3 in the 15, 30, 45, and 60 mg/kg groups. In sperm samples collected from the 45 mg/kg group, the percentage of motile sperm was modestly decreased to a statistically significant degree (58% vs. 73% in controls) at week 3 but not at weeks 2 or 4. Sublet et al. (1989) concluded that altered motility of sperm may have contributed to, "but can not completely account for, the poorer reproductive performance of these males." Similarly, Tyl et al. (2000b) observed significantly decreased fertility and increased postimplantation losses following mating of untreated female rats with males that had been administered AA at oral gavage doses of 15, 30, 45, or 60 mg/kg-day for 5 days prior to mating. No statistically significant effects were seen regarding motility or concentration of epididymal sperm from AA-treated males, although sperm beat cross frequency (in cycles/second), a measure of sperm motion and swimming pattern, was significantly increased in the 60 mg/kg-day group. Clinical signs of neurotoxicity, including unsteady movement and lethargy, were observed at the 45 and 60 mg/kg-day dose levels. High-dose males exhibited significantly lower hindlimb grip strength than controls, in the absence of microscopic evidence of sciatic nerve lesions.

Glycidamide as the putative toxin for dominant lethal effects

To determine the relative potencies between AA and GA for dominant lethal effects, Adler et al. (2000) administered 1-aminobenzotriazole (ABT), an inhibitor of CYP450 metabolism, to reduce the levels of the epoxide glycidamide. Male mice were pretreated with ABT (i.p. at 3 × 50 mg/kg) on 3 consecutive days followed by AA treatment (i.p. at 125 mg/kg) on day 4. Parallel groups of animals were treated with AA (i.p. at 125 mg/kg), ABT (i.p. at 3 × 50 mg/kg) or with the solvent double-distilled water. The experiment was repeated once with slightly varied mating parameters. The authors state that results of both experiments showed that ABT inhibited or significantly reduced the AA-induced dominant lethal effects supporting the hypothesis that the AA metabolite GA is the ultimate clastogen in mouse spermatids. In the NTP/CERHR (2004) review, however, the panel noted that the dominant lethals were decreased 2 weeks after treatment, but that, during the first week after treatment ABT did not decrease the dominant lethal effect of AA, suggesting either that AA itself has dominant lethal effects or that ABT requires more than 1 week to completely prevent metabolism to GA. A lack of a good explanation for the delay before effect and other weaknesses in the results/argument (including a decrease in the rate of dominant lethals in their study compared with other studies in mice, lack

of direct confirmatory evidence that ABT actually affected AA metabolism, and evidence that ABT was also spermatotoxic and did not effectively antagonize the spermatotoxic effect of AA treatment) prompted the panel to conclude that this study alone does not provide compelling evidence for the effect of ABT treatment in support of the hypothesis that GA is the ultimate clastogen in mouse spermatids.

More definitive support for GA as the primary toxin for dominant lethal effects comes from a recent study by Ghanayem et al. (2005a), who compared germ-cell mutagenicity in male CYP2E1-null and wild-type mice treated with AA. CYP2E1-null and wild-type male mice were treated by i.p. injection with 0, 12.5, 25, or 50 mg AA in 5 mL saline/kg-day for 5 consecutive days. At defined times after exposure, males were mated to untreated B6C3F1 females. Females were killed in late gestation, and uterine contents were examined. Dose-related increases in resorption moles (chromosomally aberrant embryos) and decreases in the numbers of pregnant females and the proportion of living fetuses were seen in females mated to AA-treated wild-type mice. No changes in any fertility parameters were seen in females mated to AA-treated CYP2E1-null mice. The authors state that their results constitute the first unequivocal demonstration that AA-induced germ cell mutations in male mice require CYP2E1-mediated epoxidation of AA. A further study by Ghanayem et al. (2005b) demonstrated the absence of AA-induced genotoxicity in somatic cells in CYP2E1-null mice compared with wild-type mice treated with AA. These results support further evaluation of CYP2E1 polymorphisms in human populations as a major determinant of variability in, and susceptibility to, AA genotoxicity in the human population. The results also provide insight into results from previous investigations of AA‘s germ cell activity in mice where stronger effects were observed after repeated administration of low doses compared with a single high dose. The differences may be due to nonlinearities in AA metabolism (and thus internal levels and distribution of GA) for different dose rates and durations.

Other reproductive function studies

Zenick et al. (1986) reproductive function study

Zenick et al. (1986) examined the potential effects of AA on male and female reproductive function in Long-Evans rats. Male reproductive function was assessed in rats that were given 0, 50, 100, or 200 ppm of AA in the drinking water (average AA intakes of 0, 4.6, 7.9, and 11.9 mg/kg-day)[3] for 10 weeks. During a 3-week pretreatment period, males were allowed to mate several times with ovariectomized, hormonally primed females. Body weights of males were recorded at least once per week, and water consumption was monitored daily throughout the study. During the treatment period, males were observed for clinical signs of toxicity (frequency of observations was not reported) and mated with untreated primed females

on a weekly basis. Copulatory behavior (mount frequency, number of mounts and intromissions, and ejaculation latency) with primed females was recorded during the mating session in which a baseline was established (1 week prior to the start of AA treatment) and on alternating weeks during treatment. At baseline and at treatment week 9, mated females were sacrificed and ejaculate was removed from the genital tract for measurements of total sperm count, percent motility, sperm morphology, and seminal plug weight. During treatment week 10, each control and mid-dose (100 ppm) male was housed with an untreated estrous female overnight in order to assess the reproductive success of AA-treated males. Following the sacrifice of dams on GD 17, the number of fetuses and implantation sites were recorded. All treated males that survived the treatment period were sacrificed during the following week and assessed for selected organ weights (liver, brain, kidney, adrenals, spleen, heart, and reproductive organs). Histologic examinations were performed on one testis and one epididymis per rat; the other testis and epididymis were used for spermatid and sperm counting. The level of significance was $p \leq 0.05$ for results of statistical analyses.

During treatment week 5, one 200-ppm male was found dead and two others were sacrificed moribund. All other 200 ppm high-dose males were sacrificed during week 6 (i.e., this dose group was terminated due to high mortality). No mortality was observed in any other treatment groups. Throughout treatment, until death or sacrifice at week 6, the high-dose group exhibited significantly lower mean body weight and water consumption than controls. Body weight and water consumption in the mid-dose group were consistently, but not statistically significantly, lower than controls. There were no statistically significant treatment-related effects on body or organ weights or sperm parameters in 50- or 100-ppm males following 10 weeks of treatment.

Hindlimb splaying was observed in the 200-ppm males by treatment week 4 and less severely in 100-ppm males at week 8. Clinical signs of neurotoxicity were not seen in the 50-ppm group. Prior to the appearance of clinical signs of neurotoxicity, biweekly assessments of copulatory behavior (data plotted graphically as square root or logarithmic transformations) revealed statistically significantly increased numbers of mounts in the 100- and 200-ppm groups relative to controls. At week 9, a nonsignificant increase in number of mounts was noted in low-dose males. At treatment weeks 4 and 9, high- and mid-dose males, respectively, exhibited statistically significant increases in the number of intromissions compared with controls. No statistically significant treatment-related changes were seen in mount or ejaculation latency, although the authors noted that only 4/12 200-ppm and 11/15 100-ppm males ejaculated within a 30-minute period on the final weeks of assessment (weeks 6 and 9, respectively).

Results of sperm analysis through week 9 of treatment and male fertility testing following 10 weeks of treatment are shown in Table 4-21. Mean sperm count was statistically significantly

[3] Calculated from graphically presented data on body weight and water consumption.

lower in mid-dose males compared with controls, but the authors indicated that vaginal leakage may have influenced total sample recovery, particularly in light of the fact that no adverse effects on sperm parameters were seen in low- and mid-dose males examined histologically after 10 weeks of treatment. Sperm motility and morphology evaluations could not be performed in the mid-dose group because sperm was recovered from the uterus of only 1 of the 11 females in which ejaculation had been observed. Low-dose treatment had no statistically significant effect on sperm parameters assessed. Statistically significant findings of fertility testing (performed only on controls and mid-dose males) included a decreased number of pregnant females and increased postimplantation loss in the mid-dose males.

Table 4-21. Results of sperm analysis (baseline and week 9) and male fertility testing (following 10 weeks of treatment) of Long-Evans rats exposed to acrylamide in the drinking water

	Acrylamide concentration (ppm)		
Parameter	**0 (n = 15)**	**50 (n = 15)**	**100 (n = 11)[a]**
Sperm count (× 10^6)			
Baseline	46 ± 12[b]	45 ± 19	43 ± 14
Week 9	56 ± 18	36 ± 23	14 ± 20[c]
Sperm motility (%)			
Baseline	43 ± 9.1	39 ± 9.2	41 ± 6.3
Week 9	41 ± 11.3	46 ± 11.2	[d]
Sperm morphology (% normal)			
Baseline	96 ± 2.7	96 ± 2.3	95 ± 1.8
Week 9	94 ± 3.6	96 ± 2.0	[d]
Seminal plug weight (mg)			
Baseline	115 ± 20	100 ± 38	111 ± 20
Week 9	118 ± 42	117 ± 27	146 ± 49
Females sperm positive/females mated	14/14	—	15/15
Females pregnant/females mated (%)	11/14 (79%)	—	5/15 (33%)[f]
Postimplantation loss (%)[e]	8.0 ± 1.1		31.7 ± 3.8[f]

[a]Four males failed to ejaculate in a 30-minute trial.
[b]Mean ± SD.
[c]Significantly different from control, $p < 0.05$.
[d]Sperm recovered from the uterus of only 1 female.
[e]Postimplantation loss = ([number of implants – number of fetuses]/[number of implants]) × 100
[f]$p < 0.01$.
Note: The 200 ppm male dose group was terminated at week 6 due to high mortality.

Source: Zenick et al. (1986).

In a female reproduction assessment phase, Zenick et al. (1986) exposed regular-estrous female Long-Evans rats (15/group) to AA in the drinking water at concentrations of 0, 25, 50, or 100 ppm for 2 weeks prior to mating and throughout gestation and lactation. The study authors did not specify the intake levels of AA for the various exposure groups; however, dam body weights were recorded at least once per week and water consumption was monitored daily throughout the study. Based on graphically presented weekly mean body weight and water

consumption data, time-weighted average AA doses were approximately 3.4, 5.6, and 11.1 mg/kg-day during the 2-week prebreeding period; 5.3, 9.5, and 17.2 mg/kg-day during 3 weeks of gestation; and 6.5, 11.3, and 15.4 mg/kg-day during 3 weeks of lactation for the 25-, 50-, and 100-ppm treatment groups, respectively. Overall average doses for females were calculated to be 5.1, 8.8, and 14.6 mg/kg-day.

During treatment week 3, untreated males were placed with the females at night for up to 7 nights. Presence of sperm in the vagina or a copulatory plug marked day 1 of gestation. Dams were observed for clinical signs of toxicity, but the frequency of clinical observations was not reported. Rat pups were sexed and weighed at birth (weighed weekly thereafter). Litters were culled to four/sex on lactation day 4 and to two/sex at weaning. Terminal sacrifice was performed on PND 42.

High-dose dams exhibited hindlimb splaying as early as gestation week 2. The mean body weight of this treatment group was statistically significantly lower than that of controls by the end of the prebreeding treatment period and was more than 10 and 20% lower than controls at some time points during gestation and lactation, respectively. Slightly, but significantly lower mean body weight (approximately 6% lower) was seen in mid-dose dams but only during lactation. The body weight effects were at least partially reflected in decreased water consumption.

No statistically significant effects were seen regarding mating efficiency, live litter size, or 4- or 21-day pup survival in any treatment group. Comparisons of body weights between pups of treated dams and pups of control dams revealed slightly (but statistically significantly) lower mean pup birth weights in male and female pups of high-dose dams. Significantly depressed mean body weights were seen in male and female pups of mid- and high-dose dams during lactation and postweaning periods (approximately 30–35% and 10% lower, respectively). The study authors stated that statistical analysis revealed an association between cumulative AA dose to dams and effects on pup body weight, but no significant associations between pup body weights and dam body weights or water consumption.

In summary, the Zenick et al. (1986) study supports a LOAEL of 100 ppm of AA in drinking water (7.9 mg/kg-day) for 10 weeks, based on male-mediated reproductive effects (decreased percentage impregnation of nonexposed females and increased postimplantation loss) in Long-Evans rats. No NOAEL was identified, as reproductive performance was not assessed in the 50-ppm exposure group. Increased numbers of mounts and incidence of hindlimb splaying were observed in the 100- and 200-ppm (7.9 and 11.9 mg/kg-day) exposure groups. Effects on female reproductive performance were only observed as depressed body weights in offspring of 50- and 100-ppm dams, accompanied by decreased dam body weight. No effects on mating efficiency, liver litter size, or pup survival were observed. For female-mediated reproductive

effects (decreased pup body weight), this study supports a LOAEL of 50 ppm (8.8 mg/kg-day) and a NOAEL of 25 ppm (5.1 mg/kg-day).

Sakamoto and Hashimoto (1986) reproductive function study

Sakamoto and Hashimoto (1986) conducted a crossover study in ddY mice. In the assessment of male reproductive effects, groups of males (14 controls and 14 at the high dose, 9/group at the other dose levels) were administered AA at levels of 0, 0.3, 0.6, 0.9, or 1.2 mM in the drinking water for 4 weeks, resulting in doses of approximately 0, 3.3, 9.0, 13.3, and 16.3 mg/kg-day, respectively, based on body weight and water consumption data provided by the authors. Half of the treated males were allowed to mate with untreated females (one male per three females) for a period of 8 days. All of the dams in each group (only half of the high-dose group) were sacrificed on GD 13 and examined for numbers of implantations and resorptions. After the remaining dams of the high-dose pairings were allowed to deliver, the number and body weights of offspring were recorded. Offspring were observed for 4 weeks for any signs of abnormal behavior and body weight gain. The remaining treated males were sacrificed immediately following the dosing period, after which weights of liver, testis, and seminal vesicle were recorded. Sperm counts and sperm morphology were assessed from epididymal samples.

The high-dose males exhibited slight signs of hindlimb weakness during or following exposure. As shown in Table 4-22, results of examinations after 13 days of gestation revealed significantly decreased fertility at the highest exposure level, significantly reduced numbers of fetuses/dam, and increased numbers of resorptions at the two highest exposure levels relative to controls. Significant decreases in both fertility and number of offspring were seen among dams allowed to deliver. There were no significant treatment-related effects regarding pup body weights or selected organ weights. Sperm analysis revealed significantly reduced numbers of sperm and increased percentages of abnormal sperm in high-dose males.

The study design of the assessment of reproductive effects in treated females was similar to that of the treated males, but treatment was limited to a single exposure level (24 females administered AA at a level of 1.2 mM in the drinking water, resulting in an AA dose of approximately 18.7 mg/kg-day that was based on body weight and water consumption data provided by the authors). The only effect noted in this part of the study was a slight but statistically significant increase in the number of resorptions/dam (1.9 ± 1.5, n = 24) as compared with controls (0.2 ± 0.3, n = 18) at day 13 of gestation.

Table 4-22. Reproductive effects following exposure of male ddY mice to acrylamide in drinking water for 4 weeks and subsequent mating with untreated females

Effects observed following 13 days of gestation				
Treatment (mM)	**Calculated dose (mg/kg-day)**	**Fertility rate[a]**	**Number of fetuses/dam**	**Number of resorptions/dam**
0	0	8/9	11.3 ± 1.4[b]	0.3 ± 0.4
0.3	3.3	9/12	11.2 ± 2.5	0.7 ± 0.7
0.6	9.0	11/12	10.4 ± 3.9	1.3 ± 2.9
0.9	13.3	10/12	7.8 ± 3.7[d]	2.9 ± 3.4
1.2	16.3	2/9[c]	2.5 ± 1.5[d]	3.0 ± 0.0[d]
Effects observed on the day of delivery				
Treatment (mM)	**Calculated dose (mg/kg-day)**	**Fertility rate**	**Number of offspring/dam**	**Offspring body weight (grams)**
0	0	12/15	11.1 ± 1.2	1.75 ± 0.12
1.2	16.3	3/15[c]	3.7 ± 1.2[d]	1.81 ± 0.16
Effects on sperm count and morphology				
Treatment (mM)	**Calculated dose (mg/kg-day)**		**Sperm count ($\times 10^5$/mg epididymis)**	**Percentage abnormal sperm**
0	0		35.8 ± 4.3	3.65 ± 0.73
0.3	3.3		43.7 ± 6.3	4.37 ± 2.54
0.6	9.0		47.7 ± 4.2[d]	4.22 ± 0.88
0.9	13.3		49.9 ± 7.1[d]	4.21 ± 2.80
1.2	16.3		23.1 ± 2.8[d]	8.12 ± 2.32[d]

[a]Number of fertile females/number of mated females.
[b]Mean ± SD.
[c]$p < 0.05$ vs. control by Fisher's Exact test.
[d]$p < 0.05$ by one-way ANOVA followed by Duncan's multiple-comparison procedure.

Source: Sakamoto and Hashimoto (1986).

The results identify 0.6 mM acrylamide (9.0 mg/kg-day for 4 weeks) as a NOAEL and 0.9 mM (13.3 mg/kg-day) as a LOAEL for male-mediated reproductive effects (decreased number of fetuses/dam) in ddY mice (Sakamoto and Hashimoto, 1986). At a higher exposure level, 1.2 mM (16.3 mg/kg-day), more severe effects were observed, including decreased fertility, increased resorptions, and sperm alterations. In female mice exposed to 1.2 mM (18.7 mg/kg-day) for 4 weeks and mated with nonexposed mice, no clearly adverse reproductive effects were observed.

Sakamoto et al. (1988) histology of testicular lesions

Sakamoto et al. (1988) administered AA (95% purity) to ddY mice as a single oral dose (presumably gavage) of 100 or 150 mg/kg at age 30 days (prepubertal) or 60 days (adult). Animals were killed 1, 2, 3, 5, 7, or 10 days after dosing. Testes were fixed in Bouin's fluid for 1 hour, cut, and then further fixed in formalin. Sections were stained with periodic acid-Schiff stain and hematoxylin and eosin. Four animals were used for each treatment condition and evaluation time point. The 150 mg/kg dose was lethal to 50% of the 30-day-old and 65% of the

60-day-old mice. In the prepubertal mice, body weight was significantly decreased at 1 and 5 days after dosing with 150 mg/kg acrylamide. The numeric values for mean body weight at 2 and 3 days after dosing were similar to the 1- and 5-day values, but the larger standard deviation prevented identification of statistical significance. In the adult mice, body weight was significantly reduced 1, 2, and 3 days after dosing with 150 mg/kg acrylamide. There were no significant alterations in testicular weight at either dose of AA. There were no deaths and no significant effects on body weight at 100 mg/kg acrylamide in either age group. Histologic abnormalities in the testes of prepubertal animals treated with 150 mg/kg acrylamide appeared in spermatids, particularly round spermatids (Golgi and cap phase) 1 day after treatment. Nuclear vacuolization and swelling were the most common lesions in the spermatids. Degeneration of spermatocytes and spermatogonia was also noted. By the second day after treatment, spermatid degeneration was more prominent. On day 3, multinucleated giant cells were frequent. By days 7–10, clearing of the histologic abnormalities was evident. The description of the pattern of histologic alteration was similar after treatment with 100 mg/kg and in adult animals. Overall, spermatogonia, spermatocytes, Sertoli cells, and Leydig cells appeared more resistant to AA-induced cell death than did spermatids.

Several additional studies have demonstrated reversible dominant lethal effects and reversible effects on male fertility in animals orally exposed to AA for short time periods. Working et al. (1987) observed reversible male-mediated reproductive effects (dominant lethal effects: increased implantation losses) in male F344 rats exposed to 30 mg/kg-day for 5 days. Sublet et al. (1989) observed dominant lethal effects (increased implantation losses) and effects on male impregnation success in Long-Evans male rats exposed to oral doses as low as 15 mg/kg-day for 5 days. In this study, males were gavaged with 0, 5, 15, 30, 45, or 60 mg/kg AA for 5 days prior to mating. Reduced fertility and increased preimplantation loss were found in all dose groups except 5 mg/kg at week 1 posttreatment. Increased postimplantation loss was seen at weeks 2 and 3 in the 15, 30, 45, and 60 mg/kg groups. In sperm samples collected from the 45 mg/kg group, the percentage of motile sperm was modestly decreased to a statistically significant degree (58% vs. 73% in controls) at week 3 but not at weeks 2 or 4. Sublet et al. (1989) concluded that altered motility of sperm may have contributed to, "but can not completely account for, the poorer reproductive performance of these males." Similarly, Tyl et al. (2000b) observed significantly decreased fertility and increased postimplantation losses following mating of untreated female rats with males that had been administered AA at oral gavage doses of 15, 30, 45, or 60 mg/kg-day for 5 days prior to mating. No statistically significant effects were seen regarding motility or concentration of epididymal sperm from AA-treated males, although sperm beat cross frequency was significantly increased in the 60 mg/kg-day group. Clinical signs of neurotoxicity, including unsteady movement and lethargy, were observed at the 45 and 60

mg/kg-day dose levels. High-dose males exhibited significantly lower hindlimb grip strength than controls, in the absence of microscopic evidence of sciatic nerve lesions.

In a summary paper, Bishop et al. (1997) reported tests of female "total reproductive capacity" involving 29 chemicals tested over a 10-year period. Female mice were treated with a single i.p. dose of AA (purity not stated) in Hanks' balanced salt solution (HBSS) at 0 or 125 mg/kg. The female mice were F1 hybrid SEC × C57BL6 and the males were F1 hybrid C3H/R1 × C57BL10. The following day, females were paired with males for approximately 1 year. When litters were produced, pups were removed, counted, and killed. The number of litters produced over either 347 or 366 days (the design changed during the course of these studies, and the specific length for the AA study was not given) and the total number of offspring produced was used to assess total reproductive capacity. There were no significant differences between the AA- and vehicle-treated females in number of offspring/female (AA 142.6, control 146.2) or number of litters/female (AA 14.3, control 14.6). The paper lists 34 breeding pairs; it is assumed (but not stated) that this number refers to the AA-treated animals. In a separate table describing vehicle groups used for the 29 chemicals, the HBSS group with 146.2 offspring/female and 14.6 litters/female contained seven animals. (It was not stated that controls were run concurrently. Neither standard error nor standard deviation were given.) Because this is a summary of a large number of studies, the specifics of the AA study are neither available nor presented, which represents a weakness, and it is difficult to ascertain the specifics of the AA experiment or whether there were any characteristics that might flag the results as unusual or give grounds for caution, another weakness in the AA portion of this study. The lack of specifics and details moderate the conclusions that can be reached concerning AA's lack of effect on female reproductive function.

4.3.2. Developmental Toxicity Studies

Developmental effects associated with oral exposure to AA are restricted to body weight decreases in rats (Wise et al., 1995; Field et al., 1990; Zenick et al., 1986) and mice (Field et al., 1990) and neurobehavioral changes (e.g., decreased auditory startle response) in the offspring of female Sprague-Dawley rats exposed to 5 and 15 mg/kg-day, respectively, on GDs 6–10 (Wise et al., 1995). No exposure-related fetal malformations or variations (gross, visceral, or skeletal) were found in Sprague-Dawley rats exposed to doses up to 15 mg/kg-day on GDs 6–20 or in CD-1 mice exposed to doses up to 45 mg/kg-day on GDs 6–17 (Field et al., 1990). These doses decreased the maternal weight gain. No signs of hindlimb foot splay or other gross signs of peripheral or central neuropathy were noted in suckling offspring of female Wistar rats that were given gavage doses of 25 mg/kg-day during the postnatal lactation period (Friedman et al., 1999a). The results of these studies are summarized in Table 4-31, and discussed below, except for the Zenick et al. (1986) study, which has been discussed previously in Section 4.3.1. It is

worth noting that many of the adverse effects discussed in the mutagenicity and heritable germ cell sections can also be considered adverse developmental effects (e.g., dominant lethality, heritable translocations, specific locus mutations, abnormal conceptus).

Field et al. (1990) developmental toxicity study—gestational exposure

Field et al. (1990) administered AA (in distilled water) to groups of timed-mated Sprague-Dawley rat dams (29–30/group) in oral gavage doses of 0, 2.5, 7.5, or 15 mg/kg-day on GDs 6–20 and to groups of timed-mated CD-1 mice (30/group) at doses of 0, 3, 15, or 45 mg/kg-day on GDs 6–17. Body weights were recorded on GD 0 and daily during treatment. Dosed animals were observed daily for clinical signs of toxicity and sacrificed on the last treatment day. Maternal body, liver, and intact uterus weights were recorded. Uteri were examined for number of implant sites and resorptions. Live fetuses were counted, weighed, and examined for external and visceral abnormalities, as well as skeletal variations and abnormalities.

Treatment-related effects are summarized in Table 4-23. Hindlimb splaying was observed only in mice of the highest dose group (45 mg/kg-day). Statistically significant adverse effects, relative to respective controls, included reduced maternal body weight gain during treatment at high dose in both species, reduced weight gain corrected for gravid uterine weight in rat dams of the 7.5 and 15 mg/kg-day groups (approximately 12 and 18% lower, respectively), and reduced male and female fetal weights in the high-dose group of mice (approximately 15% lower than controls). Acrylamide treatment did not adversely affect maternal liver weight in rats or mice, percentages of pregnant rats or mice at sacrifice, number of implantations in either species, or incidences of external, visceral, or skeletal malformations in rat or mouse fetuses. The percentage of resorptions/litter did not differ significantly among treated and control rats and mice, although a significantly increased percentage of litters with resorptions was seen in mid-, but not high-dose mice. In rats, 15 mg/kg-day is the LOAEL and 7.5 mg/kg-day is the NOAEL for maternal toxicity displayed as decreased weight gain. The highest dose level, 15 mg/kg-day, is a NOAEL for fetal developmental effects (e.g., external, visceral, or skeletal malformations or variations were not increased). In mice, 15 mg/kg-day is the NOAEL and 45 mg/kg-day the LOAEL for maternal toxicity (decreased weight gain). The highest dose level, 45 mg/kg-day, is a NOAEL for developmental effects in mouse fetuses.

Table 4-23. Maternal and fetal effects in Sprague-Dawley rats and CD-1 mice following oral (gavage) administration of acrylamide to pregnant dams

Effects in rats	Dose (mg/kg-day)			
	0	**2.5**	**7.5**	**15**
Number (%) dams pregnant at sacrifice	23 (85)	26 (96)	26 (90)	24 (89)
Maternal weight gain (g)[a]				
Gestation period	151.1 ± 4.1	152.0 ± 4.2	143.4 ± 4.0	139.2 ± 3.8
Treatment period	107.7 ± 4.0	111.0 ± 3.5	100.2 ± 3.6	96.3 ± 3.2[c]
Corrected weight gain[b]	78.6 ± 2.3	75.8 ± 3.2	69.4 ± 2.7[c]	64.3 ± 3.7[c]
Effects in mice	**Dose (mg/kg-day)**			
	0	**3**	**15**	**45**
Number (%) dams pregnant at sacrifice	28 (93)	26 (87)	29 (100)	25 (89)
Maternal weight gain (g)[a]				
Gestation period	23.6 ± 0.7	24.6 ± 0.8	21.5 ± 1.1	19.9 ± 0.7[c]
Treatment period	21.2 ± 0.7	22.1 ± 0.7	19.5 ± 1.0	17.7 ± 0.8[c]
Corrected weight gain[b]	4.7 ± 0.4	5.2 ± 0.4	5.0 ± 0.4	3.8 ± 0.4
Gravid uterine weight (g)	18.8 ± 0.6	19.4 ± 0.5	16.5 ± 0.8[c]	16.1 ± 0.7[c]
Number of litters	28	26	29	25
% resorptions/litter	3.5 ± 1.1	5.5 ± 1.5	11.7 ± 3.9	3.4 ± 1.6
% litters with resorptions	32.1	46.2	58.6[c]	24.0
Mean male fetal body weight (g)/litter	1.05 ± 0.02	1.03 ± 0.02	1.02 ± 0.01	0.89 ± 0.02[c]
Mean female fetal body weight (g)/litter	1.01 ± 0.02	0.97 ± 0.02	0.99 ± 0.01	0.86 ± 0.02[c]

[a]Includes all dams pregnant at sacrifice, mean ± SEM.
[b]Weight gain during gestation minus gravid uterine weight.
[c]Significantly different from controls; $p < 0.05$.

Source: Field et al. (1990).

Wise et al. (1995) developmental neurotoxicity study—gestational exposure

Wise et al. (1995) investigated developmental neurotoxicity in pups of Sprague-Dawley rat dams (12/group) that had been administered AA (in deionized water) at doses of 0, 5, 10, 15, or 20 mg/kg-day from GD 6 to lactation day 10. Dams were observed daily for clinical signs. Dam body weights were recorded periodically throughout gestation and lactation. All F1 pups were counted, sexed, examined for external abnormalities, and weighed at birth. On PND 3, each litter was reduced to five pups/sex. An additional four pups/sex/litter were retained for behavioral assessment. Open-field behavior was tested on a single F1 rat/sex/litter on PNDs 13, 17, and 21 (the same animals were used for each session) and on PND 59 (F1 rats that had been previously assessed for auditory startle habituation). Auditory startle habituation was tested on PND 22 (naive F1 rats) and PND 59 (F1 rats previously subjected to open-field testing). Short-term learning was assessed using a passive avoidance paradigm in previously untested F1 rats on PNDs 24 and 59, and long-term retention was assessed in these rats 1 week later. The level of significance was $p < 0.05$ for results of statistical analyses.

Postsacrifice examinations were performed on one F1 pup/sex/litter following interim sacrifice on PND 11 and on one F1 rat/sex/litter that had been used for passive avoidance testing (sacrificed during postnatal week 11). Following sacrifice, body and brain weights were recorded. Nervous tissues (brain, spinal cord, and unspecified peripheral nerve) were processed

and stained with hematoxylin eosin. Histologic examinations were performed on these tissues only from F1 rats of the control and 15 mg/kg-day treatment groups. All other F1 rats were euthanized and discarded without further examination following completion of designated testing.

Hindlimb splaying was observed in all F0 dams of the two highest dose levels (15 and 20 mg/kg-day) during the first few days of lactation. No clinical signs of neurotoxicity were seen in F0 dams of lower dose groups. Statistically significant decreases in average maternal weight gain between GDs 6 and 20 were observed in 15 and 20 mg/kg-day groups (14 and 26% below controls, respectively). No adverse effects on maternal body weight gain during gestation were seen at lower dose levels. All F0 and F1 rats of the 20 mg/kg-day dose group were euthanized between GD 24 and PND 4, due to high pup mortality (33% by PND 3) that was likely the result of obvious maternal toxicity in this dose group. Between PNDs 4 and 21, pup mortality (13%) was also seen in the 15 mg/kg-day dose group but not in other groups. Visceral examination of dead pups did not reveal a cause of death. During the lactational dosing period (PNDs 0–10), F0 dams of the 10 and 15 mg/kg-day dose groups exhibited statistically significant decreased average weight gain (45 and 90% lower than controls, respectively). No adverse effect on maternal weight gain during lactation was seen in the 5 mg/kg-day group.

The study authors noted statistically significant, dose-related decreases in average pup weights during the preweaning period. The effect was slight and transient in the 5 mg/kg-day group (5–9% below controls and statistically significant only in female pups), moderate in the 10 mg/kg-day group (9–23% lower than controls), and still more severe in the 15 mg/kg-day group. During the postweaning period, male and female F1 rats of the 15 mg/kg-day group continued to exhibit significantly decreased average body weight (23 and 15% lower than control at postnatal week 9). Body weight gain in F1 males (but not F1 females) was also significantly depressed in the 15 mg/kg-day group. The average body weight of F1 males of the 10 mg/kg-day group was significantly less than controls (6% lower) at postnatal week 9, but overall weight gain in this group was similar to that of controls during this period. No adverse effects on postweaning F1 body weights were seen in the 5 mg/kg-day group. No deaths or adverse clinical signs were seen in any group of F1 rats during the postweaning period.

No significant treatment-related effects were seen concerning open-field activity of F1 rats tested on PNDs 13 or 17. When tested on PND 21, the only statistically significant effect observed was that of increased overall average horizontal activity among female (but not male) pups of the 15 mg/kg-day group. This effect was not seen in any groups that were tested as adults. A decrease in the overall average peak amplitude of the auditory startle habituation test was seen only in male and female F1 rats of the 15 mg/kg-day group tested on PND 22 and in female F1 rats tested as adults. No apparent treatment-related effects were seen regarding performance in passive avoidance testing.

The results indicate that 5 mg/kg-day is the NOAEL and 10 mg/kg-day is the LOAEL for maternal toxicity (decreased weight gain) in Sprague-Dawley rats (Wise et al., 1995). Higher doses (15 and 20 mg/kg-day) produced hindlimb splaying and more severe effects on maternal weight gain. The lowest dose, 5 mg/kg-day, is a developmental LOAEL for decreased body weights in the offspring during the preweaning period. Neurodevelopmental effects in the offspring (increased overall average horizontal activity and decreased auditory startle response) were observed at 15 mg/kg-day but not at 5 or 10 mg/kg-day. Histologic examination of brain, spinal cord, or peripheral nerve tissue samples collected on PND 11 and postnatal week 11 revealed no changes, relative to controls, in 15 mg/kg-day offspring.

Husain et al. (1987) developmental neurotoxicity study—lactational and postnatal exposure

Husain et al. (1987) assessed the potential for AA-induced neurotoxic effects on levels of catecholamines (noradrenaline, dopamine, and 5-hydroxytryptamine) and activity of selected enzymes in the brain of the developing rat. Two separate protocols were used in the study. In one protocol, pups (number was not reported) were exposed during lactation via their nursing mothers, which were administered AA orally at a dose level of 25 mg/kg-day (in 0.15 M NaCl) throughout lactation. Brain levels of the catecholamines and enzymes of interest were measured in selected pups that were serially sacrificed at 2, 4, 8, 15, 30, 60, and 90 days of age. The second protocol involved the oral administration of AA (25 mg/kg-day) for 5 consecutive days to rats of 12, 15, 21, or 60 days of age, followed by analysis of catecholamine levels in various brain regions. Vehicle controls were included in both protocols. The level of significance was $p < 0.05$ for results of statistical analyses.

No treatment-related effects on body or brain weights were seen in rats that had been exposed via their mothers. Between the ages of 2 and 15 days, statistically significantly decreased levels of noradrenaline, dopamine, and 5-hydroxytryptamine were observed in the whole brains of offspring (5-hydroxytryptamine levels were also decreased in 30 day-old offspring) but not at later time points. Compared with age-matched controls, the brain activity of monoamine oxidase was significantly increased and that of acetylcholine esterase was significantly decreased in offspring sacrificed at 2–30 days of age but not in 60- and 90-day-old rats. Twelve-, 15-, and 21-day-old (but not 60-day-old) rats, treated according to the second protocol, exhibited significantly decreased concentrations of noradrenaline in pons medulla and basal ganglia, relative to age-matched controls. Noradrenaline was significantly decreased in the mid-brain of all tested age groups. Other significant treatment-related alterations in brain catecholamines included decreased levels of dopamine in cerebellum and midbrain at all ages tested and in pons medulla of 12-, 15-, and 21-day-old rats and decreased levels of 5-hydroxytryptamine in pons medulla, hypothalamus, and cerebral cortex at all ages tested. The study authors stated that decreased levels of catecholamines were associated with "progressive

development of behavioral disorders leading to complete hindlimb paralysis," but the report does not describe any specific observations of behavior in the rats. Thus, the report provides evidence of neurochemical changes in the male offspring of rats exposed to 25 mg/kg-day during 21 days of lactation but does not provide clear information that the male offspring had behavioral disorders including hindlimb paralysis.

Friedman et al. (1999a) developmental neurotoxicity study—lactational exposure

Friedman et al. (1999a) administered AA to female Wistar rats (15/group) with litters at oral gavage doses of 0 or 25 mg/kg-day in saline throughout the lactation period (PNDs 0–21). Dams were weighed on PNDs 0, 4, 7, 14, and 21. Maternal food and water consumption were measured for the intervals of PNDs 0–4, 4–7, 7–14, 14–21, and 0–21. Clinical observations were made at least twice daily during the dosing period. On PNDs 7, 14, and 21, dams were evaluated by an extensive functional observational battery that included observations of home cage and open field behavior, clinical signs during handling, and sensory and neuromuscular assessment (tail pinch response, hindlimb foot splay and grip strength, approach response, pupil response, startle response, and pupil size). All live pups were individually counted, sexed, weighed, and examined grossly at birth. Pups were examined at least twice daily for mortality and morbidity.

At weaning on PND 21, maternal rats were weighed and sacrificed. Thoracic and abdominal cavities and organs were examined, uterine implantation sites counted, and brain and one sciatic nerve were fixed. Histopathologic examinations were performed on the sciatic nerve preparation of each maternal rat, but details on tissue preparation and staining were not provided. Female offspring were subjected to gross external examination and sacrificed on PND 21. Brain, pituitary, and one sciatic nerve from one female pup of each litter were retained in fixative. Male pups were weighed individually on PND 21 and weekly thereafter until PND 91. Ten male pups/group were selected for grip strength measurements (forelimb and hindlimb) on PNDs 30, 60, and 90. Any selected male rat not available for grip strength assessment was replaced by another male from the same litter, if possible. On PNDs 30, 60, and 91 (following grip strength testing), one male rat/litter was sacrificed (when possible), and brain, pituitary, and one sciatic nerve were retained in fixative. On PND 91, all remaining male pups were subjected to external examination at terminal sacrifice.

For statistical analysis of results, the unit of comparison was the maternal female or the litter. Statistical analysis of the data included Bartlett's test for homogeneity of variances, general linear models procedures for ANOVA, the Kruskal-Wallis test, chi-square test, and a test for statistical outliers. Differences in results between treated and control groups were considered significant at the level of $p < 0.05$.

Mean maternal body weight was similar between controls and treated groups just prior to the beginning of dosing. Significantly lower body weight among AA-treated dams (relative to controls) was noted as early as PND 4. Between PNDs 14 and 21, both controls and treated dams exhibited weight loss, although the weight loss of treated dams was significantly greater than that of controls. For the entire treatment period (PNDs 0–21), treated dams exhibited a mean weight loss of 14 grams, whereas a net mean weight gain of 47 grams was seen in controls. Clinical signs of toxicity were apparent in treated dams, beginning on PND 4; the range of clinical signs broadened and increased in severity during the remainder of the treatment period. By PND 21, two of the dams had been sacrificed moribund (PNDs 18 and 20), and there were numerous signs (clinical, behavioral, and functional observational battery) of neurotoxicity in the surviving dams. No histopathologic evidence of degeneration in sciatic nerve preparations from treated dams was found.

Increased mortality and reduced body weights were observed in offspring of AA-treated dams during the lactation period and were likely the result of maternal toxicity. Likewise, clinical signs and gross examination of offspring during the lactation period were consistent with inanition (i.e., little or no milk in the stomach). Body weight gain of postweaning males paralleled that of controls, although the mean body weight in the AA-treated group remained lower than that of controls throughout the postweaning observation period. Grip strength was significantly lower in the AA-treatment group of male weanlings when tested on PND 30 but was not significantly different from controls when tested on subsequent PNDs 60 and 90.

The study identifies 25 mg/kg-day for 21 days during lactation as a LOAEL producing progressive signs of neurobehavioral disorders, including hindlimb foot splay in Sprague-Dawley rat dams without histologic evidence of sciatic nerve damage. Nursing offspring of exposed dams showed reduced weight gain, increased mortality, and little or no evidence of milk in their stomachs. After weaning, surviving pups showed signs of recovery, including normal weight gain and increasing grip strength over time. Characteristic signs of AA neurotoxicity, such as hindlimb splaying, were not observed in the offspring.

Other developmental toxicity studies

Genotoxic effects observed in the germ cells of mice following i.p. injection of 125 mg/kg acrylamide included a weakly positive result for sperm head DNA dealkylation and a positive result for sperm head protamine alkylation (Sega et al., 1989). Significant increases in sperm head abnormalities were observed in epididymal samples taken from male ddY mice that had received AA in the drinking water at a concentration of 1.2 mM for 4 weeks (Sakamoto and Hashimoto, 1986).

Edwards (1976) treated Porton strain rats with AA (purity not specified) in the diet. In the first experiment, eight females were given 200 ppm in powdered feed from the day a plug

was found until parturition. Offspring were apparently reared by their dams and were followed until 6 weeks of age with weekly weights taken and observations made for abnormal gait. The dams were described as showing "slight abnormalities of gait" at the times the litters were born. There were no external abnormalities. The birth weights were similar to a control group (it is not clear if this control group was the same as the control group used in the second experiment, described below), and litters were described as gaining weight normally until weaning, without abnormalities of gait. No detailed information was presented.

In a second experiment by Edwards (1976), six pregnant females were given 400 ppm AA in the diet from the day of mating until 20 days thereafter when they underwent cesarean section. Six control dams were fed powdered diet without AA. Uteri were examined for resorptions (presumably uteri: the text states that placentas were examined for resorptions). One third of fetuses underwent Wilson sectioning, and the remaining fetuses were processed in alizarin red for skeletal evaluation. Maternal feed intake was reduced in the AA group (12.0 ± 0.8 grams/rat/day, mean ± SEM) compared to the control group (23.0 ± 1.8 g/rat/day). The weights of the rats were not given (assuming a 300 gram pregnant rat, 12 grams/rat/day feed containing 400 ppm AA represents a daily dose of 16 mg/kg-day). Fetal weights were reduced by AA treatment (acrylamide 2.4 ± 0.05 grams, control 3.2 ± 0.05 grams, mean ± SEM). (The *p*-value reported by the authors using the Student t-test was >0.2; however, the t-test performed by CERHR gave $p < 0.0001$.) Four fetuses were found dead in one uterine horn in the AA-treated group, and three resorptions were present in one litter in the control group. There were no fetuses with abnormalities and "there was no increase in the occurrence (approx. 10%) of a naturally occurring defect in the rib structure." No data were shown.

In a third experiment, Edwards (1976) administered 100 mg/kg AA in water i.v. to each of four pregnant rats on GD 9 (plug date unspecified). The rationale for this timing was the statement that GD 9 is when the nervous system is most susceptible to teratogenic effects. Pups were apparently delivered and reared by their dams and on the third day of life, pups were examined for external appearance and righting reflex. Offspring were followed for 6 weeks during which the day of eye opening was noted and animals were evaluated for gait and were weighed weekly. Two rats from each litter (sex unspecified) were perfused with formaldehyde/acetic acid/methanol, and brains, spinal cord, and peripheral nerves were evaluated by light microscopy (sectioning and staining unspecified). Two rats/litter (sex unspecified) were killed with a barbiturate for dissection for gross abnormalities. Brain weight was recorded. Four pregnant control rats were injected with saline and presumably handled in the same manner. There were no differences among groups in birth weight, pup weight 24 hours or 3 days after birth, righting reflex, or day of eye opening (data were not shown). There were no abnormalities of nervous system tissues by gross examination or by light microscopy.

In summary of all three studies, due to the limited number of doses, very limited number of pregnant rats/group, limited number of outcomes measured, and missing data necessary for full evaluation of this report, the conclusions presented in the report are questionable.

Bio/dynamics Inc. (1979) administered AA in the feed to female Sprague-Dawley CD rats at 0, 25, or 50 ppm for 2 weeks prior to mating, and from GDs 0 to 19. Acrylamide intake was estimated at 1.75–1.90 and 3.45–3.82 mg/kg-day in the 25 and 50 ppm dose groups, respectively. Litters were standardized to three male and three female pups on PND 4 and pups were examined for postnatal growth and mortality until weaning (PND 21). A slight but significant reduction in body weight gain was observed in the 50 ppm dams during the premating period. No difference among treatment groups was observed for mating and pregnancy indices, gestation length, neonatal viability, live litter size at birth, pup survival throughout the lactation period, and pup weights. Albert Einstein College of Medicine (1980) conducted a histopathologic evaluation of brain and spinal cord and sciatic, tibial, and plantar nerves and reported that AA-associated changes were confined to scattered nerve fiber degeneration in the sciatic and optic nerves. The incidence and severity of these histologic effects were not provided.

In a study conducted at the National Institute for Environmental Health and Sciences, Walden et al. (1981) evaluated the activity of five intestinal enzymes in the offspring of AA-treated Sprague-Dawley rats. Dams were treated from GD 6 to 17 (insemination = GD 0) with AA (purity not given) 20 mg/kg-day or water by gavage for a total cumulative dose of 200 mg/kg. There were 17 dams in each treatment group. On the day of birth (PND 0), pups in each treatment group were pooled and divided among dams to produce four groups: control dams with control pups (C-C); treated dams with treated pups (T-T); control dams with treated pups (C-T); and treated dams with control pups (T-C). Four pups were removed from each litter without regard to sex for intestinal enzyme analysis on PND 14, 21, and 60. The first 10–15 cm of intestinal mucosa was scraped and homogenized (the report implies that the scrapings of the four animals were pooled). Kinetic spectrophotometric assays were performed for alkaline phosphatase, citrate synthase, and lactate dehydrogenase. Endpoint spectrophotometric assays were performed for acid phosphatase and β-glucuronidase. Dams were killed on PND 24, after weaning, and intestinal enzymes were measured by the same methods. The results of differences (either increases or decreases) in enzyme activities for pups in the different groups were indicative of prenatal effects (C-T compared with C-C), lactational effects (T-C compared with C-C), or enhancement of prenatal effects (T-T compared with C-T) and are presented in Table 4-24. Statistical analysis was performed by Mann-Whitney U-test ($2p < 0.05$). The results indicate that prenatal exposure to AA in Sprague-Dawley dams at the doses stated above, and lactational exposure to pups, significantly changed intestinal enzyme levels in pups during early development. It is unknown whether these changes result in subsequent adverse structural or

functional effects. There were no differences in maternal body weight or in litter averages for pup number, weight, or sex ratio. Dam intestinal enzyme levels did not differ from this exposure level of AA.

Table 4-24. Differences in marker enzymes in the small intestine of pups cross-fostered to acrylamide-treated or control dams during postnatal lactation

Intestinal enzyme	Effect[a]	Postnatal day		
		14	21	60
Alkaline phosphatase	Prenatal[b]	↑	↑	↓
	Lactational[c]	–	↑	↓
	Enhancement of prenatal effect[d]	↑	↑	↑
Citrate synthase	Prenatal	–	–	–
	Lactational	–	–	–
	Enhancement of prenatal effect	–	–	–
Lactate dehydrogenase	Prenatal	–	–	–
	Lactational	–	↑	–
	Enhancement of prenatal effect	–	–	–
Acid phosphatase	Prenatal	↑	–	↓
	Lactational	↑	–	–
	Enhancement of prenatal effect	–	↓	↑
β-glucuronidase	Prenatal	–	↑	–
	Lactational	–	↑	–
	Enhancement of prenatal effect	↓	↑	–

[a]↑ = Increase; ↓ = decrease; – = not significantly different. All reported effects are significant at the $2p < 0.05$ level using the Mann-Whitney U-test.
[b]C-T values compared with C-C values.
[c]T-C values compared with C-C values.
[d]T-T values compared with C-T values.

Source: Walden et al. (1981).

A study by Rutledge et al. (1992) is unique in that female mice were dosed with AA selectively during the perifertilization period at 125 mg/kg i.p. 1, 6, 9, or 25 hours after mating. These times represented fertilization, the early pronuclear stage, pronuclear DNA synthesis, and the two-cell stage, respectively. On GD 17, the uteri were inspected for resorptions, embryonic death, and live fetuses. Live fetuses were inspected for external abnormalities. The number of live fetuses was decreased and the number of resorptions was increased at all treatment times. Among live fetuses, abnormalities were increased with treatment 6, 9, and 25 hours after mating. In spite of the lack of important details in the paper and a discrepancy between text and table in reporting the results, this study showed that an acute administration of AA at a high dose during the perifertilization period can produce very early death or structural malformations.

Walum and Flint (1993) evaluated the effect of AA (purity not given) on rat midbrain cells (obtained from embryos collected on day 13 postmating) in culture. This brain area is one rich in both dopamine and gamma-aminobutyric acid (GABA) receptors developmentally. In

this assay, sometimes called micromass culture, neural epithelial cells in suspension aggregate into foci of interconnected cells. A reduction in the number of such foci without a reduction in cell number or viability is taken as evidence of disruption of developmental processes. In this study, 10 μg/mL AA was determined to reduce the number of foci by 25% without decreasing cell number, assessed by neutral red staining and protein content. Uptake of dopamine and GABA were also decreased by AA exposure (the text indicates that GABA uptake was "virtually" unaffected; the data table shows a statistically significant 8% reduction in GABA uptake). The authors concluded that AA may reduce the "differentiation and development of dopaminergic projections" in the developing rat brain. This study provides an in vitro assessment of a potential mechanism of AA toxicity and a suggestion of how this mechanism might be established. This approach is a good beginning for whole-animal researchers to follow-up concerning these events within an in vivo model.

4.4 HERITABLE GERM CELL STUDIES

Qualitative characterization

Two recent reviews of studies in mice for heritable germ cell effects from exposure to acrylamide (Favor and Shelby, 2005; NTP/CERHR, 2004) have both concluded that AA induces transmissible genetic damage in male germ cells of mice in the form of reciprocal translocations and gene mutations. The studies consisted of five heritable translocation studies (Adler et al., 2004, 1994, 1990; Generoso et al., 1996; Shelby et al., 1987) and two specific mouse locus assays (Ehling and Neuhäuser-Klaus, 1992; Russell et al., 1991). No experiments have studied the potential for AA to induce heritable mutations in the female germ line. The heritable germ cell effect in male mice is consistent with the extensive evidence supporting dominant lethal effects in male murine test animals.

Favor and Shelby (2005) summarized their conclusions as follows: (1a) AA is mutagenic in spermatozoa and spermatid stages of the male germ line; (2) in these spermatogenic stages AA is mainly or exclusively a clastogen; (3) per unit dose, i.p. exposure is more effective than dermal exposure; and (4) per unit dose, GA is more effective than AA. They further note that, since stem cell spermatogonia persist and may accumulate mutations throughout the reproductive life of males, assessment of induced mutations in this germ cell stage is critical for the assessment of genetic risk associated with exposure to a mutagen. Further research is needed to resolve the conflicting results between two specific-locus mutation experiments with respect to the stem cell spermatogonial effects.

The NTP/CERHR panel also noted that AA-induced transmissible genetic damage can lead to genetic disorders and infertility in subsequent generations, but these risks were not included in the expert panel's quantitative evaluation of LOAELs for risk to the general population because of the lack of testing at dose levels below where reproductive and

developmental toxic effects were observed. The panel did hypothesize that, considering the magnitude of the response detected for heritable translocations at the lowest dose level tested (40 mg/kg-day × 5 days or 50 mg/kg as a single dose), it is likely that such effects would occur at doses lower than these.

The seven heritable germ cell studies in mice are briefly discussed below, and the results, as tabulated by Favor and Shelby (2005), are included in Tables 4-25, 4-26, and 4-27. These studies are also listed in Appendix B, Table B-1 that summarizes the mutagenicity assay results.

Heritable translocation studies

Shelby et al. (1987) administered AA i.p. at 40–50 mg/kg-day for 5 consecutive days to male C3H/El mice. Matings on days 7–10 following the last injection yielded a high frequency of translocation carriers in the F1 male population, demonstrating that AA is an effective inducer of translocations in postmeiotic germ cells. The proportions of male progeny that were sterile or semi-sterile after paternal treatment with 50 and 40 mg/kg-day for 5 days were 49/125 and 39/162, respectively, compared with 17/8095 in the historical control. All ten of the semi-sterile males sampled from the 5 × 50 treatment for cytogenetic analysis of spermatocytes had translocations.

Table 4-25. Frequency of translocation carriers in offspring derived from males exposed to acrylamide or glycidamide

Dose[a] (mg/kg)	Mating interval[b]	F_1 progeny tested		Translocation carriers[c]		Reference
		Males	Females	Males	Females	
Historical control	—	11,292[d]		7 (0.06)		Generoso et al. (1996)
	—	9,890[e,f]		5 (0.05)[f]		Adler et al. (2002)
50 AA i.p.	7–16	362[f]		2 (0.55)[f]		Adler et al. (1994)
100 AA i.p.	7–16	367[f]		10 (2.72)[f]		Adler et al. (1994)
100 GA i.p.	3.5–7.5	669		135 (20.17)		Generoso et al. (1996)
5 × 40 AA i.p.	7–10	162		39 (24.07)		Shelby et al. (1987)
5 × 50 AA i.p.	7–10	125		49 (39.20)		Shelby et al. (1987)
5 × 50 AA i.p.	7–11	57	48	17 (29.82)	6 (12.5)	Adler (1990)
5 × 50 AA i.p.	36–42	556	449	2 (0.36)	0 (0)	Adler (1990)
5 × 50 AA dermal	1.5–8.5	258	217	28 (10.85)	13 (5.99)	Adler et al. (2004)

[a]5 × 40 and 5 ×50 represent 40 or 50 mg acrylamide/kg on 5 consecutive days.
[b]Days posttreatment.
[c]See text for methods to ascertain translocation carriers. Frequency (%) of translocation carriers given in parentheses.
[d]Laboratory historical control used for statistical comparisons of the translocation frequencies reported by Shelby et al. (1987) and Generoso et al. (1996).
[e]Laboratory historical control used for statistical comparisons of the translocation frequencies reported by Adler (1990) and Adler et al. (1994, 2004).
[f]Both male and female F_1 animals were tested but not reported separately.

Source: Favor and Shelby (2005).

Table 4-26. Results for specific locus mutations recovered in offspring of male mice exposed i.p to 50 mg/kg acrylamide on 5 consecutive days.

Mating interval (days posttreatment)	Number of offspring	Number of mutations[a]
1–7	113	0 (0)
8–14	1506	2 (0.13)
15–21	5077	1 (0.02)
22–28	5191	0 (0)
29–35	5312	0 (0)
36–42	5353	1 (0.02)
43–49	6419	1 (0.02)
>49	17,112	0 (0)
Historical control	801,406	43 (0.01)

[a]Frequencies (%) of specific locus mutations given in parentheses.
Sources: Data from Russell et al. (1990); table from Favor and Shelby (2005).

Table 4-27. Results for specific locus mutations recovered in offspring of male mice exposed to acrylamide as a single 100 or 125 mg/kg i.p. dose

Dose (mg/kg)	Mating interval (days posttreatment)	Number of offspring	Number of mutations[a]
Historical control	—	248,413	22 (0.01)
100	1–4	1362	0 (0)
	5–8	2226	1 (0.04)
	9–12	2421	2 (0.08)
	13–16	2453	0 (0)
	17–20	2574	0 (0)
	21–42	2925	0 (0)
	>42	23,489	6 (0.03)
125	1–4	771	0 (0)
	5–8	1924	2 (0.10)
	9–12	1948	1 (0.05)
	13–16	2419	0 (0)
	17–20	2598	0 (0)

[a]Frequencies (%) of specific locus mutations given in parentheses.

Note: Only the 100 mg/kg-treated males were used to establish a permanent monogamist mating starting on day 21 to assay for effects on spermatogonia (i.e., for effects ≥43 days posttreatment).

Sources: Data from Ehling and Neuhäuser-Klaus (1992); table from Favor and Shelby (2005).

Adler et al. (1990) administered acrylamide i.p. at 50 mg/kg-day for 5 consecutive days to male C3H/E1 mice, which were then mated to untreated female 102/E1 mice on days 7–11 and again on days 36–42 posttreatment. There were 23 translocation heterozygotes among 105 progeny from the offspring of the 7–11 day mating interval. Among the offspring of the treated males, there were 17 male translocation carriers among 57 male offspring and 6 female translocation carriers among 48 female offspring (male vs. female, $p < 0.05$). In the second mating interval (36–42 days after treatment), 1005 offspring were produced, of which 2 males were translocation carriers. This rate did not differ from the historical control in the author's laboratory when considered on a total-offspring basis but was significantly greater than the historical control ($p = 0.03$) if considered on a male-offspring basis. All semi-sterile and sterile mice from treated parental males were analyzed cytogenetically, with 22/25 semi-sterile mice and 3/4 sterile mice confirmed as translocation carriers. This study provides further evidence for AA-induced chromosomal damage in postmeiotic rodent germ cells.

Adler et al. (1994) administered acrylamide i.p. as a single 50 or 100 mg/kg dose to male C3H/E1 mice, which were then mated on days 7–16 posttreatment to untreated female 102/E1 mice. Translocation carriers among the F1 progeny were selected by a sequential procedure of fertility testing and cytogenetic analysis, including G-band karyotyping, to determine the chromosomes involved in the respective translocations. The frequency of confirmed translocation carriers was 2/362 in the 50 mg/kg treatment group and 10/367 in the 100 mg/kg treatment group. Both frequencies were significantly greater than the historical control, 5/9890. Clustering was not apparent, as indicated by the fact that all translocations were unique.

Adler et al. (2004) conducted heritable translocation tests with dermal exposure of male mice to AA. Male C3H/El mice were treated with five dermal exposures of 50 mg/kg AA and mated 1.5–8.5 days after the end of exposure to untreated female 102/El mice. Pregnant females were allowed to come to term and all offspring were raised to maturity. Translocation carriers among the F1 progeny were selected by a sequential fertility testing and cytogenetic analysis including G-band karyotyping and M-FISH. A total of 475 offspring were screened and 41 translocation carriers were identified. The observed translocation frequency after dermal exposure was 8.6% as compared to 21.9% after similar i.p. exposure (Adler, 1990). The calculated ratio of end effects in this study of i.p. vs. dermal exposure is 0.39.

Favor and Shelby (2005) summarized the cytogenetic analysis from the Adler et al. (1990, 1994, 2004) studies to emphasize the appearance of complicated chromosomal rearrangements induced by AA. Among the 77 semi-sterile and sterile animals analyzed, 66 were carriers of reciprocal translocations between two chromosomes, 2 carried translocations among three chromosomes, 6 were carriers of two independent reciprocal translocations each between two chromosomes, 2 were carriers of a reciprocal translation between two chromosomes

plus an inversion on a third chromosome, and 1 animal carried a translocation among three chromosomes plus a reciprocal translocation between two chromosomes.

Generoso et al. (1996) administered a single i.p. dose of glycidamide at 100 mg/kg to male (C3H/RL × 101/RL)F1 mice. Among the 669 male progeny of GA-treated sires, 135 (20.18%) were confirmed as heterozygous translocation carriers, compared with 6% from the historical controls. The GA treatment generated a much higher frequency of translocations in male progeny than the comparable 100 mg/kg i.p. dose from AA reported in Adler et al. (1994) (20.17% vs. 2.72%). Although the mating interval was different (3.5–6.5 days posttreatment for GA and 7–10 days posttreatment for AA) and thus the spermatogonial stages were different and the studies were conducted in two different laboratories, the results demonstrate that GA is a potent inducer of chromosomal damage in postmeiotic rodent germ cells.

Specific locus studies

Russell et al. (1991) evaluated specific locus mutations, as well as fertility (measured as litter size/fertile female) and dominant lethals resulting from AA exposure to male mice from an i.p. 50 mg/kg-day dose for 5 consecutive days. Males were mated at specific intervals after mating to T-stock females homozygous for a (non-agouti), b (brown), cch (chinchilla), p (pink-eyed dilution), d (dilute), se (short ear), and s (piebald). Acrylamide was effective in the first 2 weeks posttreatment, corresponding to germ cells exposed in the spermatozoa or spermatid stages. The results confirmed previous dominant lethal studies and germ cell stages in which the treatment induced dominant lethals jointly yielded the highest frequency of specific locus mutations. Specific locus mutations occurred in 5/28,971 offspring with exposures 1–7 weeks after treatment, which was significantly higher than the historical control rate of 43/801,406 ($p = 0.026$ in a one-tailed Fisher Exact test). The two mutants arising from matings 1 and 2 weeks after treatment represented a significantly higher mutation rate than the three mutants arising from matings in weeks 3–7; the rate in this latter period was not significantly higher than the control rate. No mutations were recovered in 17,112 offspring derived from treated stem cell spermatogonia (fertilizations occurring >49 days posttreatment). The major conclusions are that AA is mutagenically active in the late spermatid–spermatozoa stages, the recovered mutations are associated with chromosomal aberration-type events (deletions and/or translocations), and AA is not mutagenically active in stem cell spermatogonia. Russell et al. (1991) reported that two specific locus mutations recovered in offspring derived from fertilizations (in which the male gametes were exposed to AA at the spermatozoa and spermatid stages) were homozygous lethal, of which one was associated with a cytogenetically visible deletion, and concluded that the specific locus mutations were due to large, multilocus deletions.

Ehling and Neuhäuser-Klaus (1992) exposed male mice to a single i.p. dose of AA at 100 or 125 mg/kg. Immediately after treatment, males were housed with untreated, test-stock

females homozygous for a (non-agouti), b (brown), cch (chinchilla), p (pink-eyed dilution), d (dilute), se (short ear), and s (piebald). For the 100 mg/kg-treated males, a permanent monogamist mating was established, starting on day 21. The offspring of the permanent mating were classified according to their day of conception into those derived from treated spermatocytes and differentiating spermatogonia (conception 21–42 days posttreatment), and those from treated spermatogonia (≥43 days posttreatment). Ehling and Neuhäuser-Klaus (1992) grouped their specific locus results for conceptions occurring in the intervals days 5–8 and 9–12 posttreatment, respectively, and reported an increased frequency of mutations due to exposure of parental males to these levels of AA. They reported that, of the six specific-locus mutations recovered following AA exposure of spermatids or spermatozoa, four had reduced viability, one was sterile, and one was homozygous lethal. As in the Russell et al. (1991) study, the authors concluded that the specific-locus mutations recovered in offspring derived from parental exposure to AA were associated with multi-locus deletions. Unlike Russell et al. (1991), who reported no increase in the frequency of specific-locus mutations in offspring derived from germ cells exposed as stem-cell spermatogonia, Ehling and Neuhäuser-Klaus (1992) observed a significant increase in the frequency of specific-locus mutations following exposure of spermatogonia to AA. Favor and Shelby (2005) reevaluated the mating intervals to more directly compare the results and noted that in the results of Russell et al. (1991) for spermatogonial exposure (days >42 posttreatment), the frequency of specific-locus mutations, 1/23,531, was not significantly higher than the frequency in the historical control. By contrast, Ehling and Neuhäuser-Klaus (1992) demonstrated a significantly higher specific-locus mutation frequency in treated spermatogonia (6/23,489) than in their historical control. The difference in the specific-locus mutation frequency for spermatogonia exposed to AA between Russell et al. (1991) (higher total accumulated dose, 50 mg AA/kg on 5 consecutive days) and Ehling and Neuhäuser-Klaus (1992) (lower dose, 100 mg AA/kg) approached significance ($p = 0.070$, Fisher's Exact test, two-tailed). Further, the intervals between treatment and conception for all specific-locus mutations recovered in the spermatogonia exposure group were noted by Ehling and Neuhäuser-Klaus (1992). One mutation resulted from a conception 43 days posttreatment and represented an exposure at the differentiating spermatogonial stage. Russell et al. (1991) also recovered one specific-locus mutation following exposure at this stage. The remaining five mutations recovered for treatment of spermatogonia by Ehling and Neuhäuser-Klaus (1992) all had conceptions much later (70, 181, 201, 234, and 436 days posttreatment) and represented exposures of stem-cell spermatogonia.

The two specific locus mutation studies provide evidence for specific locus mutations in rodent spermatid and spermatozoal stage germ cells. Resolution of the conflicting results between these two studies with respect to induced mutations in stem cell spermatogonia will require further research. This research is needed because mutations in stem cell spermatogonia

represent a more persistent and serious lesion that will be transmitted to all descendent spermatids, in contrast with the later stage mutations that can be inherited only from fertilization by that directly affected spermatid. There are also implications for determining risk from exposure and the dose duration for a reference value since, as Favor and Shelby (2005) note, if the mutagenic activity of AA is confined to postspermatogonial stages, the risk of effects would be relative to the dose accumulated during the sensitive postspermatogonial stages and this would be only a fraction of the lifetime accumulated exposure. If, however, stem cell spermatogonia are sensitive to mutation induction by AA, the risk would be relative to lifetime accumulated dose up to the time of fertilization.

4.5. OTHER DURATION OR ENDPOINT-SPECIFIC STUDIES

4.5.1. Neurotoxicity Studies

The oral toxicity animal studies described in detail in Sections 4.2 and 4.3 include those most relevant to describing dose-response relationships for chronic exposure. Numerous additional reports have been published in which AA-induced neurotoxicity has been assessed in animal species following single or repeated oral exposure to AA. For example, both Fullerton and Barnes (1966) and Tilson and Cabe (1979) observed clinical signs of neurotoxicity in rats following single oral dosing with AA in the range of 100 to 200 mg/kg; repeated administration at lower dose levels also resulted in neurotoxic signs. Aldous et al. (1983) reported overt signs of neurotoxicity as early as day 4 in rats administered AA by gavage at a dose level of 50 mg/kg-day.

Dixit et al. (1981) noted neurotoxicity in rats following 14 days of oral treatment at a dose level of 25 mg/kg-day. Severe loss of hindlimb function was reported as early as day 21 in rats administered AA in the diet for up to 90 days at a concentration that resulted in an estimated dose of 30 mg/kg-day (McCollister et al., 1964). Fullerton and Barnes (1966) noted slight leg weakness in rats after 40 weeks of dietary exposure at a concentration that resulted in a dose ranging from approximately 6 to 9 mg/kg-day (according to the authors); the effect did not appear to become more severe during the remaining 8 weeks of exposure.

Alterations in gait (home-cage and open-field assessment of neuromuscular function and equilibrium) were reported in adult male and female Long-Evans rats administered i.p. injections of AA at doses as low as 1 mg/kg-day for as little as 30 to 60 days (Moser et al., 1992). Acrylamide was administered 5 days/week for 13 weeks and included dose levels of 1, 4, and 12 mg/kg-day. Neurobehavioral observations were performed prior to dosing, at treatment days 29–31 and 58–62, and immediately following treatment termination. Significantly increased foot splay was observed at 4 mg/kg-day (females) and 12 mg/kg-day (males and females) at 60-day examination. All other signs of neurotoxicity (impaired mobility and righting reflex, decreased

grip strength, and axonal degeneration in peripheral nerves and spinal cord) were seen only at the high dose (12 mg/kg-day).

Other investigators have reported AA-induced neurotoxicity in mice (Gilbert and Maurissen, 1982; Hashimoto et al., 1981), cats (Post and McLeod, 1977; McCollister et al., 1964), dogs (Hersch et al., 1989; Satchell and McLeod, 1981), and monkeys (Eskin et al., 1985; Maurissen et al., 1983; McCollister et al., 1964).

4.5.2. Other Cancer Studies

The potential of acrylamide to initiate skin tumors has been examined in female SENCAR mice (40/group, 6 to 8 weeks of age) exposed via oral (gavage), i.p. injection, and dermal application (Bull et al., 1984a). Acrylamide was dissolved in distilled water for oral and injection routes and in ethanol for dermal applications. Acrylamide was administered at dose levels of 0, 12.5, 25, or 50 mg/kg-day, 6 times during a 2-week period for each route (total AA doses of 0, 75, 150, or 300 mg/kg). Two weeks later, dermal doses of a promoter, 1.0 µg 12-O-tetradecanoylphorbol-13-acetate (TPA) (in 0.2 mL acetone) were applied to the shaved back 3 times/week for 20 weeks. Two types of control groups (20–40 mice/group) were included for each route of administration: (1) vehicle initiation with TPA promotion; and (2) 50 mg/kg-day AA plus vehicle promotion. All animals were killed at 52 weeks, and all gross lesions in the skin were histologically examined. The incidences of histologically confirmed squamous cell carcinomas or squamous cell papillomas for the 0, 12.5, 25, or 50 mg/kg-day AA groups with TPA, followed by the incidence for the 50 mg/kg-day group without TPA are shown in Table 4-29.

Table 4-29. Acrylamide initiation of squamous cell carcinomas or papillomas in female SENCAR mice

	Skin carcinomas[a]					Skin papillomas[a]				
	Dose (mg/kg-day)									
	With TPA				No TPA	With TPA				No TPA
	0	12.5	25	50	50	0	12.5	25	50	50
Oral	0/34	2/35	7/33[b]	6/38[b]	0/17	0/34	3/35	8/33[b]	11/38[b]	0/17
Intraperitoneal	0/35	2/38	4/36	4/35	0/17	0/35	2/38	3/36	6/35[b]	0/17
Dermal	0/36	1/38	2/35	3/34	0/20	5/36	3/38	3/35	2/34	0/20

[a]Denominator is the number of surviving mice at 52 weeks with acceptable nonautolyzed tissues.
[b]Significantly different ($p < 0.05$) from the vehicle initiation/TPA promotion group by Fisher's Exact test.

Source: Bull et al. (1984a).

Incidences were also reported for the number of skin tumor-bearing mice/total mice in each group (Bull et al., 1984a). In this analysis, tumors were described as skin masses with diameter >1 mm that were detected during a minimum of 3 consecutive weeks in the study.

Incidences for the 0, 12.5, 25, or 50 mg/kg-day/+TPA promotion groups, followed by the 50 mg/kg-day/vehicle promotion group, for the three routes of administration are displayed in Table 4-30.

Table 4-30. Acrylamide initiation of skin tumor masses > 1mm in female SENCAR mice

	Skin tumor masses with diameter >1 mm				
	Dose (mg/kg-day)				
	With TPA				No TPA
	0	12.5	25	50	50
Oral	2/40	12/40[a]	23/40[a]	30/40[a]	0/20
Intraperitoneal	0/40	10/40[a]	13/40[a]	21/40[a]	0/20
Dermal	7/40	4/40	11/40	18/40[a]	0/20

[a]Significantly different ($p < 0.05$) from the vehicle initiation/+TPA promotion group by Fisher's Exact test.

Source: Bull et al. (1984a).

Overall, the data indicate that AA at oral dose levels of 25 or 50 mg/kg-day initiated TPA-promoted skin tumors in SENCAR mice. However, the incidences of histologically confirmed skin tumors were not statistically significantly elevated in mice receiving initiating doses of AA by i.p. injection or dermal administration, with the exception of papillomas in mice exposed to 50 mg/kg-day by i.p. injection followed by TPA promotion.

In another skin tumor initiation-promotion study, female Swiss-ICR mice (40/group) were administered AA in oral doses of 0, 12.5, 25, or 50 mg/kg-day, 3 times a week for 2 weeks (Bull et al., 1984b). Two weeks later, 2.5 µg TPA in acetone was applied to the shaved backs, 3 times a week for 20 weeks. Another group of 40 mice received 6 doses of 50 mg/kg-day AA during 2 weeks, followed by dermal application in acetone without TPA for 20 weeks. Mice were examined for skin papillomas on a weekly basis, until sacrifice at 52 weeks after start of the initiation period. The skin and lungs were preserved for histologic examination of all gross lesions. The combined incidence of mice with histologically confirmed skin papillomas or carcinomas for the 0, 12.5, 25, or 50 mg/kg-day AA groups with TPA, followed by the incidence for the 50 mg/kg-day group without TPA were as follows (* indicates significantly different [$p < 0.05$] from the vehicle/+TPA promotion group by Fisher's Exact test; denominator is the number of mice surviving to 52 weeks with acceptable nonautolyzed tissue): 0/35, 2/34, 3/32, 10/32*, and 1/36. Respective incidences for skin carcinomas alone were: 0/35, 1/34, 3/32, 4/32*, and 1/36. The data indicate that orally administered AA (50 mg/kg-day, 6 times during a 2-week period) initiated histologically confirmed mouse skin tumors promoted by TPA.

Support for the skin tumor initiation activity of AA is provided by an analysis in which tumors were described as skin masses with diameter >1 mm that were detected during a minimum of 3 consecutive weeks in the study (Bull et al., 1984b). In this analysis, incidences of skin-tumor bearing animals were 0/40, 4/40, 4/40, and 13/40* for the 0, 12.5, 25, and 50 mg/kg-

day /+TPA groups, respectively, and 10/40* for the 50 mg/kg-day/vehicle promotion group. Incidences in the 50 mg/kg-day AA-exposed groups were statistically significantly elevated (* $p < 0.05$ by Fisher's Exact test) compared with the vehicle/+TPA control group.

Lung tumors were also found in the Swiss-ICR mice that survived to 52 weeks (Bull et al., 1984b). The combined incidences of mice with histologically confirmed alveolar bronchiolar adenomas or carcinomas for the 0, 12.5, 25, or 50 mg/kg-day/+TPA promotion groups, followed by the incidence for the 50 mg/kg-day/vehicle promotion were as follows: 4/36, 8/34, 6/36, 11/34*, and 14/36*. The respective incidences for carcinomas were: 1/36, 2/34, 1/36, 1/34, and 10/36*. The incidences for combined adenomas and carcinomas were statistically significantly (Fisher's Exact test, * $p < 0.05$) elevated in both groups treated with 50 mg/kg-day 6 times during 2 weeks, but only 1/11 lung tumors in the 50-mg/kg-day/+TPA group was a carcinoma, in contrast to 10 carcinomas/14 lung tumors in the 50-mg/kg-day/–TPA group.

Bull et al. (1984a) also performed mouse lung adenoma bioassays on groups of 8-week-old male and female A/J mice, a strain that is very susceptible to lung tumor formation. Acrylamide was administered to mice (16/sex/group) via i.p. injection at doses of 1, 3, 10, 30, or 60 mg/kg-day, 3 times a week for 8 weeks. Untreated and vehicle control (distilled water) groups were also employed. The mice injected with 60 mg/kg-day showed severe peripheral neuropathy and deaths within the first 3 weeks of treatment and were not examined for lung tumor development. Surviving mice in other groups were sacrificed at 8 months, lungs were fixed, and surface adenomas were counted after 24 hours. Acrylamide exposure caused increased incidences of mice with lung tumors at dose levels ≥3 mg/kg. Incidences were 12/30 and 3/31 for untreated and vehicle controls, compared with 14/33, 15/33*, 21/31*, and 28/30* for the 1, 3, 10, and 30 mg/kg-day groups, respectively (* indicates significantly different from combined control incidence by Fisher's Exact test). Some evidence was also presented for increasing average number of lung tumors/mouse ("tumor yield") with increasing AA exposure: 0.4 ± 0.5, untreated control; 0.1 ± 0.3, vehicle control; 0.6 ± 0.8, 1 mg/kg; 0.8 ± 1.0, 3 mg/kg; 1.2 ± 1.4, 10 mg/kg; and 2.2 ± 1.5, 30 mg/kg. In a later report, Bull et al. (1984b) reported that the tumor yield in this study "displayed a reasonably strong relationship with dose ($p < 0.03$)" but did not provide specific information on the statistical analysis performed.

Robinson et al. (1986) compared skin and lung tumor yields (number of tumors/mouse) in several strains of mice (SENCAR, BALB/c, A/J, and ICR) injected i.p. with single 50 mg/kg doses of AA followed by topical application of TPA three times weekly for 20 weeks. Groups of 60 mice of each strain received initiating injections with AA or water (vehicle); 40 mice in each group then received TPA at the following dose levels: 1.0 μg for SENCAR, 5.0 μg for BALB/c, and 2.5 μg for A/J and ICR. The mice were sacrificed at 36 weeks. Microscopic examinations were conducted on all gross lesions found in lungs and skin and only lung adenomas and skin papillomas were included in the tumor count and calculation of tumor yield. One experiment

included all four strains, and a second experiment only examined SENCAR mice. Lung tumor yields were statistically significantly increased by the AA treatment (0.42 tumors/mouse), compared with vehicle controls (0.04 tumors/mouse), in the SENCAR strain but not in the BALB/c, A/J, or ICR strains. However, in the other experiment with SENCAR mice, lung tumor yields were not statistically significantly elevated (0.38 vs. 0.22 tumors/mouse). Skin tumor yields were statistically significantly elevated in SENCAR mice in the two experiments (0.25 vs. 0.08 tumors/mouse and 0.38 vs. 0.05 tumors/mouse) but were not significantly elevated in the other three strains. Robinson et al. (1986) only reported mean skin and lung tumor yield data, so the value of the reported data are only of limited use for cancer hazard identification purposes.

4.6. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION

4.6.1. Neurotoxicity Studies

Several groups (Padilla et al., 1993; Harry, 1992; Sickles, 1991) have put forth the theory that AA slows fast axonal transport within axons and that this produces the subsequent effects on the nerve terminal and the axon. Sickles (1991) examined the rate and quantity of protein transport in rat sciatic nerve following a single injection of 50 mg/kg AA and found an immediate reduction of 48% in the quantity of protein transported down the rat sciatic nerve. Transport remained depressed for 16 hours, and recovery occurred from 16 to 24 hours, reaching control levels at 24 hour postinjection. Harry (1992) dosed adult male rats with 40 mg/kg i.p. for nine consecutive days and found a 75% decrease in the amount of accumulated material by 24 hours. Padilla et al. (1993) treated male Long-Evans rats with a dose of 50 mg/kg AA i.p. twice a day for 5 weeks and observed that fast axonal transport in the sciatic nerve of these animals was decreased by 6.5–9.4%.

Other researchers have developed a hypothesis that nerve terminals are the primary site of AA-induced neurotoxicity and that axonal degeneration is secondary to nerve terminal changes (Lehning et al., 2003a, 2002, 1998; Lopachin et al., 2002a). Groups of adult male Sprague-Dawley rats were exposed to either i.p. injection of 50 mg/kg-day of AA for up to 11 days or via drinking water at 28 mM AA (estimated oral dose of 21 mg/kg-day) for 38 to 49 days. Gait abnormalities were observed in both treatment groups but appeared earlier at the higher dose rate. Using the de Olmos silver stain method (de Olmos et al., 1994), the authors observed nerve terminal degeneration in both treatment groups and proposed that nerve terminal degeneration precedes axonal degeneration, which occurred primarily at the subchronic, albeit, lower dose rate. This same pattern of lesion was observed in the cerebellum, the brainstem, the spinal cord, and the forebrain (Lehning et al., 2003a,b).

There remains uncertainty as to which is the most sensitive lesion and what is the sequence of events in the mode of action leading to clinical signs of neurotoxicity. Sickles et al.

(2002a) suggest that, because the most distal sites (i.e., nerve terminals) are likely to be the most vulnerable to alterations in fast axonal transport, they should not be considered as a separate site. The primary target currently under investigation for reduction in fast axon transport is the motor protein, kinesin (Sickles et al., 2002b). The primary target currently under investigation for nerve terminal damage is synaptosomal proteins involved in the presynaptic transmission (LoPachin et al., 2004). Both kinds of proteins contain sulfhydryl groups as sites for AA binding, and in both modes of action there is additional research needed to show a direct relationship between the protein functional inhibition and the observed functional deficits (LoPachin and Canady, 2004; Sickles et al., 2002a).

4.6.2. Genotoxicity Studies

Appendix B (Table B-1) summarizes results of numerous published mutagenicity tests for acrylamide including the dominant lethal mutation assays discussed in a previous section. Results from in vivo dominant lethal mutation assays involving i.p. exposure of mice (Adler et al., 2000; Shelby et al., 1987), oral exposure of mice (Chapin et al., 1995; Sakamoto and Hashimoto, 1986) or rats (Tyl et al., 2000a,b; Sublet et al., 1989; Working et al., 1987a; Smith et al., 1986; Zenick et al., 1986), and dermal exposure of mice (Gutierrez-Espeleta et al., 1992) have been consistently positive. Since the oral exposure studies were described in detail in Section 4.3.1, results from dominant lethal mutation assays were generally not included in Appendix B.[4] Heritable germ cell studies in male mice were consistently positive for heritable translocations (Adler et al., 2004, 1994, 1990; Generoso et al., 1996; Shelby et al., 1987) and specific mouse locus (Ehling and Neuhäuser-Klaus, 1992; Russell et al., 1991). No experiments studied the potential for AA to induce heritable mutations in the female germ line. The heritable germ cell studies are listed in Appendix B and are discussed in Section 4.3.3.

Manjanatha et al. (2006) evaluated the somatic cell mutagenic potential of AA and GA in an in vivo genotoxicity study in male and female Big Blue (BB) mice. BB mice were administered 0, 100, or 500 mg/L of AA or equimolar doses of GA in drinking water for 3–4 weeks. The estimated daily exposures to AA for males and females were 19 and 25 mg/kg-day, respectively, for the low dose of 100 mg/L (4-week exposure) and 98 and 107 mg/kg-day for the high dose of 500 mg/L (3 weeks only due to clinical signs of neurotoxicity). The estimated daily exposure to GA for males and females were 25 and 35 mg/kg-day for the low dose of 120 mg/L (4 weeks) and 88 and 111 mg/kg-day for the high dose of 600 mg/L (4 weeks). Micronucleated reticulocytes (MN-RETs) were assessed in peripheral blood within 24 hours of the last treatment, and lymphocyte Hprt and liver cII mutagenesis assays were conducted 21 days following the last

[4] It is further acknowledged that male-mediated dominant lethal effects can be mediated by effects on altered male mating performance, sperm motility and/or morphology, as well as effects on genetic integrity of the sperm (Perreault, 2003).

treatment. The types of cII mutations induced by AA and GA in the liver were determined by sequence analysis. The frequency of MN-RETs was increased 1.7–3.3-fold in males treated with the high doses of AA and GA ($p \leq 0.05$; control frequency = 0.28%). Both doses of AA and GA produced increased lymphocyte Hprt mutant frequencies (MFs), with the high doses producing responses that were 16–25-fold higher than those of the respective control ($p \leq 0.01$; control MFs = $[1.5 \pm 0.3] \times 10^{-6}$ and $[2.2 \pm 0.5] \times 10^{-6}$ in females and males, respectively). Also, the high doses of AA and GA produced significant 2–2.5-fold increases in liver cII MFs ($p \leq 0.05$; control MFs = $[26.5 \pm 3.1] \times 10^{-6}$ and $[28.4 \pm 4.5] \times 10^{-6}$). Molecular analysis of the mutants indicated that AA and GA produced similar mutation spectra and that these spectra were significantly different from that of control mutants ($p \leq 0.001$). The predominant types of mutations in the liver cII gene from AA- and GA-treated mice were G:C→T:A transversions and –1/+1 frameshifts in a homopolymeric run of guanosines. The results indicate that both AA and GA are mutagenic in mice. The MFs and types of mutations induced by AA and GA in the liver are consistent with AA exerting its mutagenicity in BB mice via metabolism to GA.

Ghanayem et al. (2005b) demonstrated the absence of AA-induced genotoxicity in CYP2E1-null mice as evidence of a GA-mediated genotoxic effect in somatic cells. Female wild-type and CYP2E1-null mice were administered acrylamide (0, 25, 50 mg/kg) by i.p. injection once daily for 5 consecutive days. Twenty-four hours after the final treatment, blood and tissue samples were collected. Erythrocyte micronucleus frequencies were determined by flow cytometry, and DNA damage was assessed in leukocytes, liver, and lung using the alkaline (pH >13) single cell gel electrophoresis (Comet) assay. Results included significant dose-related increases in micronucleated erythrocytes and DNA damage in somatic cells induced in AA-treated wild-type mice but not CYP2E1-null mice. These results were consistent with the observations in a similar study in male germ cells, where dose-related increases in dominant lethal mutations were detected in uterine contents of female mice mated to AA-treated wild-type males but not CYP2E1-null males (Ghanayem et al., 2005a) (discussed in Section 4.2.1).

Numerous previous tests were performed to evaluate AA-induced chromosomal alterations in mammalian systems in vivo; most tests employed i.p. injection of AA at concentrations in the range of 25 to 200 mg/kg. Tests for chromosomal aberrations in bone marrow cells yielded both positive (Adler et al., 1988; Čihák and Vontorková, 1988) and negative (Krishna and Theiss, 1995; Shiraishi, 1978) results. Similar assays of mouse spleen lymphocytes, splenocytes, and spermatogonia were all negative for chromosomal aberrations (Kligerman et al., 1991; Adler, 1990; Backer et al., 1989; Adler et al., 1988). Significant increases in chromosomal aberrations were observed in spermatocytes of mice that had been administered an i.p. dose of 100 mg/kg (Adler, 1990), but the frequency of aneuploid sperm detected by fluorescence in situ hybridization (FISH) was not increased by single i.p. injections of 60 or 120 mg/kg AA in male mice (Schmid et al., 1999). Consistent with AA induction of

chromosomal aberrations in sperm, the frequency of zygotes with chromosomal aberrations was significantly elevated in zygotes from females mated to males exposed to 50 mg/kg AA by i.p. injection for 5 days before mating (Marchetti et al., 1997). Tests were positive for early cleavage stages of mouse zygotes (Pacchierotti et al., 1994) and embryos (Valdivia et al., 1989), positive for polyploidy or aneuploidy (Shiraishi, 1978), and negative for spindle disturbances (Adler et al., 1993) in mouse bone marrow cells.

Acrylamide-induced increases in micronuclei were seen in bone marrow cells, reticulocytes, spleen lymphocytes, and splenocytes of mice and spermatids of rats and mice (Paulsson et al., 2002; Lähdetie et al., 1994; Russo et al., 1994; Xiao and Tates, 1994; Collins et al., 1992; Kligerman et al., 1991; Čihák and Vontorková, 1990, 1988; Backer et al., 1989; Adler et al., 1988; Knaap et al., 1988) but not in rat bone marrow cells (Paulsson et al., 2002; Krishna and Theiss, 1995). Synaptonemal complex irregularities (asynapsis in meiotic prophase) were slightly increased in germ cells of male mice following i.p. injection of AA, without a significant increase in aberrations (Backer et al., 1989). Tests for heritable translocations and reciprocal translocations in male mice yielded positive results (Adler et al., 1994; Shelby et al., 1987).

Acrylamide was found to induce chromosomal alterations (chromosomal aberrations, cell division aberration, chromosome enumeration, polyploidy, spindle disturbances) in a number of in vitro mammalian cell test systems at concentrations as low as 0.01 to 1 mg/mL (Adler et al., 1993; Tsuda et al., 1993; Warr et al., 1990; Knaap et al., 1988; Moore et al., 1987). A test for micronuclei in spermatids collected from Sprague-Dawley rats yielded negative results at concentrations up to 0.05 mg/mL (Lähdetie et al., 1994).

Earlier evidence for AA-induced DNA damage and repair included positive results in a spore rec assay (Tsuda et al., 1993), DNA breakage in mice following i.p. injection of AA doses ≥25 mg/kg (Sega and Generoso, 1990), in vitro unscheduled DNA synthesis (UDS) in human mammary epithelial cells (Butterworth et al., 1992), and in vivo UDS in male mouse germ cells (Sega et al., 1990). Testing for UDS in male rats in vivo/in vitro yielded positive results in spermatocytes and negative results in hepatocytes (Butterworth et al., 1992).

Acrylamide tested positive for sister chromatid exchange in mammalian cells both in vitro (Tsuda et al., 1993; Knaap et al., 1988) and in vivo (Russo et al., 1994; Kligerman et al., 1991; Backer et al., 1989). Both positive (Park et al., 2002; Tsuda et al., 1993; Banerjee and Segal, 1986) and negative (Abernethy and Boreiko, 1987) results were obtained in cell transformation assays.

Results of reverse mutation assays in bacterial test systems did not indicate a mutagenic response at AA concentrations ranging from 10 to 10,000 μg/plate with or without metabolic activation (Müller et al., 1993; Tsuda et al., 1993; Jung et al., 1992; Knaap et al., 1988; Zeiger et al., 1987; Hashimoto and Tanii, 1985; Lijinsky and Andrews, 1980). A fluctuation test in *Klebsiella pneumoniae* was also negative for mutagenicity (Knaap et al., 1988).

Genotoxicity was not observed in a test for sex-linked recessive lethality in *Drosophila melanogaster* following abdominal injection of a 50 mM solution of AA (Knaap et al., 1988), but positive results were obtained when *D. melanogaster* larvae were fed concentrations ≥1 mM (Tripathy et al., 1991). Somatic mutation and recombination assays were positive for genotoxicity in *D. melanogaster* exposed by larval feeding at concentrations ≥1 mM (Batiste-Alentorn et al., 1991; Tripathy et al., 1991; Knaap et al., 1988).

Positive results were obtained for gene mutation in mouse lymphoma cells in vitro at concentrations as low as 0.3 mg/mL (Barfknecht et al., 1988; Knaap et al., 1988; Moore et al., 1987). This response was seen both with and without metabolic activation. Negative results were obtained for gene mutation in Chinese hamster V79H3 cells at the highest concentration tested (7 mM) without activation (Tsuda et al., 1993).

Additional studies on the genotoxic potential of GA include positive results to *Salmonella typhimurium* strains TA100 and TA1535 (Hashimoto and Tanii, 1985) and mouse lymphoma cells (Barfknecht et al., 1988) but not *K. pneumoniae* (Voogd et al., 1981). Glycidamide induced unscheduled DNA synthesis in mouse spermatids in vivo (Sega et al., 1990), in human epithelial cells in vitro (Butterworth et al., 1992), in one of two tests for unscheduled DNA synthesis in rat hepatocytes in vitro (Butterworth et al., 1992; Barfknecht et al., 1988), and in (C3H/RL × C57BL)F1 male mice given single i.p. injections of 150 mg/kg GA (Generoso et al., 1996). Glycidamide (125 mg/kg by i.p. injection) induced dominant lethal mutations in male JH mice mated with nonexposed female SB mice (Generoso et al., 1996). Glycidamide treatment (100 mg/kg, i.p. injection) of male (C3H × 101/RL)F1 mice (mated with nonexposed (SEC × C57BL)F1 female mice) induced heritable translocations in male offspring at a frequency about twofold greater than spontaneous frequencies in historical controls (Generoso et al., 1996). Synthetic GA induced a similar frequency for micronuclei in erythrocytes per unit of in vivo dose in the mouse as obtained in a study in the same laboratory where animals were treated with AA, and GA was endogenously generated as a metabolite (Paulsson et al., 2003). This equality in potency of GA, whether its in vivo dose is established by injection of synthetic GA or through metabolism of AA, supports the view that GA is the predominant genotoxic factor in AA exposure.

Formation of DNA adducts

Glycidamide forms DNA adducts in mice and rats (see Figure 3-2) (Doerge et al., 2005a; Gamboa et al., 2003; Segerbäck et al., 1995). DNA adduct formation was seen in liver, lung, kidney, brain, and testis of male mice and rats following i.p. injection of 46–53 mg/kg acrylamide (Segerbäck et al., 1995; Sega et al., 1990).

Doerge et al. (2005a) measured DNA adducts following a single i.p. administration of AA and GA to adult B6C3F1 mice and F344 rats at 50 mg/kg AA or an equimolar dose of GA (61 mg/kg), and reported GA-derived DNA adducts of adenine and guanine formed in all relevant tissues in both males and females where tumors had been reported, including liver, brain, thyroid, leukocytes, mammary gland, and testis in rats and liver, lung, kidney, leukocytes, and testis in mice. Dosing rats and mice with an equimolar amount of GA typically produced higher levels of DNA adducts than observed with AA. Kinetics of DNA adduct formation and accumulation were measured following oral administration of a single dose of AA (50 mg/kg) or from repeat dosing (1 mg/kg-day), respectively. The formation of these DNA adducts is consistent with previously reported mutagenicity of AA and GA in vitro, which involved reaction of GA with adenine and guanine bases. These results provide strong support for a mutagenic mechanism of AA carcinogenicity in rodents.

Acrylamide has been observed to form DNA adducts in vitro, but the formation rate is very slow (Solomon et al., 1985).

Besaratinia and Pfeifer (2004) treated normal human bronchial epithelial cells and Big Blue mouse embryonic fibroblasts (that carry a lambda phage cII transgene) in vitro with AA, its primary epoxide metabolite GA, or water (control) and then subjected the cells to terminal transferase-dependent polymerase chain reaction to map the formation of DNA adducts within the human gene encoding the tumor suppressor p53 gene (TP53) and the cII transgene. The frequency and spectrum of GA-induced mutations in cII were examined by using a lambda phage-based mutation detection system and DNA sequence analysis, respectively. All statistical tests were two-sided. Acrylamide and glycidamide formed DNA adducts at similar specific locations within TP53 and cII, and DNA adduct formation was more pronounced after GA treatment than after AA treatment at all doses tested. Acrylamide-DNA adduct formation was saturable, whereas the formation of most GA-DNA adducts was dose-dependent. Glycidamide treatment dose-dependently increased the frequency of cII mutations relative to control treatment ($P<.001$). Glycidamide was more mutagenic than AA at any given dose, and the spectrum of GA-induced cII mutations was statistically significantly different from the spectrum of spontaneously occurring mutations in the control-treated cells ($P=.038$). Compared with spontaneous mutations in control cells, cells treated with GA or AA had more A-->G transitions and G-->C transversions and GA-treated cells had more G-->T transversions ($P<.001$). These results support the hypothesis that the mutagenicity of AA in human and mouse cells is based on the capacity of its epoxide metabolite GA to form DNA adducts.

4.7. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS

4.7.1. Oral

Neurological impairment has been established as a human health hazard from AA exposure, predominantly based on studies of effects from occupational inhalation and dermal exposure (see Section 4.5.2) (Tilson, 1981; Spencer and Schaumberg, 1974). There are few reports of health effects in humans associated with oral exposure to AA. However, corroborative case reports of neurological impairment from oral exposure include one of persistent peripheral neuropathy in a subject who intentionally ingested 18 grams AA crystals (Donovan and Pearson, 1987). In another report, signs of central and peripheral neurological deficits were observed in family members exposed to AA in well water at a concentration of 400 ppm; both oral and dermal exposure to AA were likely (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.

Numerous studies in animals provide evidence of neurotoxic effects in males and females and reproductive effects in males as the most sensitive noncancer effects associated with oral exposure to AA (summarized in Table 4-31). The studies in Table 4-31 provided the information needed to characterize the dose-response relationships for noncancer effects.

Examination of NOAELs and LOAELs for the various effects noted in Table 4-31 indicates that the lowest effect levels are for degenerative peripheral nerve changes in rats exposed to 1 mg/kg-day AA in drinking water for 90 days (Burek et al., 1980) or 2 mg/kg-day (Johnson et al., 1986) or 2 or 3 mg/kg-day (Friedman et al., 1995) for 2 years. Comprehensive histologic examinations of all major organs and tissues in these rat studies revealed no other exposure-related nonneoplastic lesions at dose levels below 5 mg/kg-day (Friedman et al., 1995; Johnson et al., 1986; Burek et al., 1980) (see Table 4-31). Although studies selected for inclusion in Table 4-31 only examined rats and mice, Table 4-32 lists reports of AA neurological impairment in other species (cats, dogs, monkeys, and additional mouse studies) exposed via intraparenteral administration or orally at higher dose levels.

Table 4-31. Noncancer effects in animals repeatedly exposed to acrylamide by the oral route

Reference/species	Exposure conditions (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effect
Burek et al., 1980 F344 rat, M&F	0, 0.05, 0.2, 1, 5, or 20 90 days in DW	0.2 1 5 5 5	1 5 20 20 20	Degenerative nerve changes (EM) Degenerative nerve changes (LM) Hindlimb foot splay Decreased body weight (8–20%) Atrophy of testes & skeletal muscle
Johnson et al., 1986 F344 rat, M&F	0, 0.01, 0.1, 0.5, or 2.0 2 years in DW	0.5 2 0.5 0.5 2	2 ND 2 2 ND	Degenerative nerve changes (LM) Hindlimb foot splay Decreased body weight (<5%, M only) Early mortality after 24 weeks Other nonneoplastic lesions
Friedman et al., 1995 F344 rat, M&F	0, 0.1, 0.5, or 2.0 (M) 0, 1.0, or 3.0 (F) 2 years in DW	0.5(M) 1.0(F) 2.0(M) 3.0(F) 0.5(M) 1.0(F) 0.5 2.0(M) 3.0(F)	2.0(M) 3.0(F) ND ND 2.0(M) 3.0(F) 2.0 ND ND	Degenerative nerve changes (LM) Hindlimb foot splay Decreased body weight (8–9%) Early mortality after 60 weeks Other nonneoplastic lesions
Tyl et al., 2000a F344 rat, M&F	0, 0.5, 2.0, or 5.0 2 generations in DW	2.0 ND ND 5.0(F) 0.5(M)	5.0 5.0(M) 0.5(M) ND (F) 2.0(M)	MM implantation losses (F0&F1) Degenerative nerve changes (LM) Hindlimb foot splay (F0 M only) Body weight effects Decreased body weight (4–6%)
Chapin et al., 1995 CD-1 mouse, M&F	0, 0.8, 3.1, or 7.5 2 generations in DW	3.1 7.5 3.1 7.5 3.1(F)	7.5 ND 7.5 ND 7.5(F)	MM implantation losses (F0&F1) Degenerative nerve changes (F1,LM) Mild grip strength deficits (F1&F2) Hindlimb foot splay Decreased body weight (8%, F1 only)
Zenick et al., 1986 Long-Evans rat, M	0, 4.6, 7.9, or 11.9 10 weeks in DW; mated w/ nonexposed F	ND 4.6	7.9 7.9	MM implantation losses Hindlimb foot splay
Zenick et al., 1986 Long-Evans rat, F	0, 5.1, 8.8, or 14.6 9 weeks in DW; mated w/ nonexposed M	5.1 5.1 14.6	8.8 8.8 ND	Decreased maternal body weight (6%) Decreased pup body weight (30–35%) Other reproductive performance endpoints (fertility, implantation loss)
Smith et al., 1986 Long-Evans rat, M	0, 1.5, 2.8, or 5.8 80 days in DW; mated w/ nonexposed F	1.5 5.8 5.8	2.8 ND ND	MM postimplantation losses Peripheral nerve changes (LM) Hindlimb foot splay
Sakamoto and Hashimoto, 1986 ddY mouse, M	0, 3.3, 9.0, 13.3, or 16.3 4 weeks in DW; mated w/ nonexposed F	9.0 13.3 13.3	13.3 16.3 16.3	MM decreased fetuses/dam Slight hindlimb weakness Decreased sperm counts, abnormal sperm morphology
Sakamoto and Hashimoto, 1986 ddY mouse, F	0, 18.7 4 weeks in DW; mated w/ nonexposed M	18.7 ND	ND 18.7	Female reproductive performance Slight hindlimb weakness

Table 4-31. Noncancer effects in animals repeatedly exposed to acrylamide by the oral route

Reference/species	Exposure conditions (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effect
Field et al., 1990	0, 2.5, 7.5, or 15	7.5	15	Decreased maternal weight gain
Sprague-Dawley	GD 6–20 by gavage	15	ND	Fetal malformations or variations
rat, F		15	ND	Hindlimb splay, maternal
Field et al., 1990	0, 3, 15, or 45	15	45	Decreased maternal weight gain
CD-1 mouse, F	GD 6–17 by gavage	45	ND	Fetal malformations or variations
		15	45	Hindlimb splay, maternal
Wise et al., 1995	0, 5, 10, 15, or 20	10	15	Decreased maternal weight gain
Sprague-Dawley	GD 6–10 by gavage	10	15	Hindlimb splay, maternal
rat, F		ND	5	Decreased body weight in offspring
		10	15	Increased overall horizontal activity, decreased auditory startle response in offspring
Friedman et al.,	0, 25 (maternal	ND	25	Hindlimb foot splay, maternal
1999a	doses) PND 0–21	25	ND	Degenerative nerve changes (LM), maternal
Wistar rat, F	by gavage	25	ND	Hindlimb foot splay in offspring

DW = drinking water LM = light microscopy ND = not determined
EM = electron microscopy LOAEL = lowest-observed-adverse-effect level
F = female M = male NOAEL = no-adverse-effect level
GD = gestation days MM = male-mediated PND = postnatal days

Table 4-32. Neurological effects following exposure to acrylamide in species other than the rat and mouse

Reference/Species	Exposure conditions (dose, route, duration)	Effect
McCollister et al., 1964 Cats (n = 2)	Single 100 mg/kg i.p. dose	After 24 hours, one was unconscious and was sacrificed, the other had severe neurotoxicity.
Post and McLeod, 1977 Cats (2–3 kg)	15 mg/kg in food for up to 16 weeks	Progressively increasing neurotoxicity; by 12–16 weeks, severe poisoning, reduction in conduction velocity, damage to large and small myelinated fibers in peripheral nervous system.
Hersch et al., 1989 Dogs (greyhounds, 22–30 kg)	5.7 mg/kg-day via ingested capsule for 6–7 weeks	Progressive, but reversible dysfunction of the pulmonary stretch receptors and their myelinated vagal afferents.
Satchell and McLeod 1981 Dogs (greyhound)	7 mg/kg-day in feed for 8 weeks	Sensorimotor peripheral neuropathy and megaesophagus suggesting an axonopathy of the vagus nerve.
Eskin et al., 1985 Monkeys (macaque)	10 mg/kg-day in juice, 5 days/week for 6–10 weeks	Axonal swellings with neurofilament accumulation in the distal optic tract and lateral geniculate nucleus.
Maurissen et al., 1983 Monkeys (pigtail)	10 mg/kg-day in juice, 5 days/week until appearance of mild toxicity (n = 4; average for 54 days; average total dose 400 mg/kg)	Loss of balance, impaired coordination, tremor (these symptoms reversed relatively soon after dosing); reduced vibration sensitivity and remained impaired for several months after dosing.
McCollister et al., 1964 Monkeys (5.1 kg)	total of 200 mg/kg of four consecutive 50 mg/kg i.v. doses	Death.
Gilbert and Maurissen, 1982 Mice (Balb/c)	25.8 mg/kg-day (250 ppm) acrylamide in drinking water for 12 days (total estimated dose 310 mg/kg)	Decreased retention time and increased hindlimb splay.
Hashimoto et al., 1981 Mice (ddY strain)	1/5 to 1/2 of the LD_{50} (107 mg/kg) administered by gavage twice weekly for 8–10 weeks	241 mg/kg was the total dose for half maximal inhibition of rotarod performance.

LoPachin et al. (2002b) reported measures of gait characteristics as a sensitive behavioral measure for the onset and progression of AA neurotoxicity, but the study protocols cited in Table 4-31 were not oriented towards neurobehavioral endpoints and did not evaluate gait abnormalities. Instead, hindlimb foot splay, a gross characteristic sign of AA-induced peripheral neuropathy, was measured in several of the studies cited in Table 4-31. Changes in foot splay have been observed in most studies at oral dose levels above the lowest less-than-lifetime and chronic doses associated with histologic signs of peripheral nerve damage (1–3 mg/kg-day), with the exception of one study that reported statistically significantly increased incidences of F0-generation F344 rats with hindlimb foot splay following exposure to a dose level as low as 0.5 mg/kg-day (Tyl et al., 2000a) (Table 4-31). This observation does not appear consistent with other observations, including the absence of hindlimb foot splay in F1 generation rats in the same study exposed to doses as high as 5 mg/kg-day (Tyl et al., 2000a) and F344 rats exposed to drinking water doses as high as 2–3 mg/kg-day for 2 years (Friedman et al., 1995; Johnson et al., 1986) or 5 mg/kg-day for 90 days (Burek et al., 1980). Neurobehavioral studies with protocols

and endpoints that are suitable for quantifying the dose-response are a research need, and efforts are ongoing to measure more sensitive neurobehavioral responses to AA.

Acrylamide induces adverse reproductive and developmental effects, but study data suggest these effects occur at higher doses than those resulting in neurotoxicity. Pre- and postimplantation losses and decreased numbers of live fetuses have been observed following repeated prebreeding oral exposure of rats and mice to AA at doses in the range of 3 to 8 mg/kg-day (Chapin et al., 1995; Sakamoto and Hashimoto, 1986; Smith et al., 1986; Zenick et al., 1986) (see Table 4-31). Dominant lethality testing (Tyl et al., 2000a,b; Chapin et al., 1995; Smith et al., 1986) and crossover trials (Chapin et al., 1995; Sakamoto and Hashimoto, 1986; Zenick et al., 1986) indicate male-mediated reproductive effects (Table 4-31). More gross effects on male reproductive organs have been demonstrated at higher dose levels, e.g., exposure of F344 rats to 20 mg/kg-day AA in drinking water for 90 days produced severe testicular atrophy (Burek et al., 1980). Male germ cell assays (e.g., sperm abnormalities, heritable translocations, specific locus mutations) provide evidence of AA-induced male reproductive toxicity following drinking water (Sakamoto and Hashimoto, 1986) or i.p. exposures (Adler et al., 2004, 2000, 1994, 1990; Generoso et al., 1996; Ehling and Neuhäuser-Klaus, 1992; Russell et al., 1991; Sega et al., 1989; Shelby et al., 1987). No experiments have studied the potential for AA to induce heritable mutations in the female germ line. Prebreeding exposure of female mice to doses of 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 (Table 4-31). In these female-exposure studies, the only reproductive endpoint affected was body weight decreases in offspring of female Long-Evans rats exposed to 8.8 and 14.6 mg/kg-day (Zenick et al., 1986).

Comparing the study LOAEL values listed in Table 4-31 suggests that the onset of adverse effects for male reproductive toxicity results from lower levels of AA exposure (2.8–13.3 mg/kg-day) than those needed to produce clinical signs of neurotoxicity (15–20 mg/kg-day) but higher than those that result in peripheral nerve damage following less-than-lifetime or chronic exposures (1–2 mg/kg-day).

Developmental effects associated with oral exposure to AA are restricted to body weight decreases and neurobehavioral changes (e.g., decreased auditory startle response) in offspring of female Sprague-Dawley rats exposed to 5 and 15 mg/kg-day, respectively, on GDs 6–10 (Wise et al., 1995) (Table 4-31). No exposure-related fetal malformations or variations (gross, visceral, or skeletal) were found in Sprague-Dawley rats exposed to doses up to 15 mg/kg-day on GDs 6–20 or in CD-1 mice exposed to doses up to 45 mg/kg-day on GDs 6–17 (Field et al., 1990) (Table 4-31). These doses produced decreased maternal weight gains. No signs of hindlimb foot splay or other gross signs of peripheral or central neuropathy were noted in suckling offspring of

female Wistar rats that were given gavage doses of 25 mg/kg-day during the postnatal lactation period (Friedman et al., 1999a).

Subchronic or chronic exposure to AA doses in the 2–8.8 mg/kg-day range resulted in small body weight deficits (4–9% decreased compared with controls) in F344 rats (Tyl et al., 2000a; Friedman et al., 1995; Johnson et al., 1986), CD-1 mice (Chapin et al., 1995), and Long-Evans rats (Zenick et al., 1986). More pronounced decreases in body weight were seen at higher doses, but these also produced overt neurotoxicity (e.g., Burek et al., 1980).

4.7.2. Inhalation

Numerous reports have associated human exposure to AA with neurological impairment (Igisu and Matsuoka, 2002; Gjerløff et al., 2001; Hagmar et al., 2001; Mulloy, 1996; Calleman et al., 1994; Bachmann et al., 1992; Myers and Macun, 1991; Dumitru, 1989; He et al., 1989; Donovan and Pearson, 1987; Kesson et al., 1977; Mapp et al., 1977; Davenport et al., 1976; Igisu et al., 1975; Takahashi et al., 1971; Fullerton, 1969; Auld and Bedwell, 1967; Garland and Patterson, 1967). Most reports involved occupational exposure with potential for both inhalation and dermal exposure. Although exposure concentrations of AA were measured in some instances, studies describing reliable relationships between exposure concentrations and neurological responses in humans are not available. However, cross-sectional health surveillance studies of AA-exposed workers describe correlative relationships between hemoglobin adduct levels of AA (an internal measure of cumulative dose) and changes in a neurotoxicity index based on self-reported symptoms and clinical measures of neurological impairment (Calleman et al., 1994) or increased incidences of self-reported symptoms alone (Hagmar et al., 2001). These studies, however, do not provide reliable information on dose-response relationships for chronic inhalation exposure to AA because (1) they involved mixed inhalation and dermal exposure (in both groups of workers dermal exposure was thought to have been substantial); (2) the duration of exposure was less than chronic; (3) both groups of workers were exposed to confounding chemicals (acrylonitrile in the first and NMA in the second study); and (4) the internal measure of dose (N-terminal valine adducts of hemoglobin) is not specific for AA alone (e.g., NMA can form the same adduct).

Data on AA-induced toxicity in animals exposed by inhalation are limited to a single report of progressive signs of neuropathy and death in rats and dogs following acute-duration repeated exposure to aerosols of AA dust at a concentration of 15.6 mg/m^3 (Hazleton Laboratories, 1953).

4.7.3. Mode-of-Action Information

Since experimental acrylamide neuropathy was first reported (Hazleton Laboratories, 1953), acrylamide has been extensively studied in efforts to understand its toxicological

properties and mode of action (MOA) for the functional deficits observed in animal studies, including alterations in gait, hindfoot splay, impaired mobility and righting reflex, and decreased grip strength (Moser et al., 1992; Dixit et al., 1981; Tilson and Cabe, 1979; Fullerton and Barnes, 1966; McCollister et al., 1964). Similar muscle weakness and functional impairments have been observed in humans exposed to acrylamide (Hagmar et al., 2001; Calleman et al., 1994; He et al., 1989).

Early animal research associated AA functional neurotoxicity with central and peripheral distal axonopathy and more specifically with histopathologic findings of neurofilamentous accumulations in distal paranodal regions of large peripheral nerve fibers that appeared to cause local axon swelling and subsequent degeneration of myelin (Spencer and Schaumberg, 1977, 1974). Axon degeneration was observed to progress proximally toward the cell body region, a process known as "dying back." Based on these findings, neurofilaments were thought to be a target for AA toxicity. Other potential pathways for AA-induced axonopathy included interference with nerve cell body metabolism and delivery of nutrients to the axon (Spencer et al., 1979; Cavanagh, 1964), interruption of axonal protein transport (Pleasure et al., 1969), disruption of axon cytoskeleton (Lapadula et al., 1989), diminished axolemma Na^+,K^+-ATPase activity (LoPachin and Lehning, 1994), and reduction of fast anterograde axonal transport capacity (Harris et al., 1994; Padilla et al., 1993; Harry, 1992; Sickles, 1991).

After four decades of research, the cellular and molecular site and the mode of action for AA-induced neurotoxicity remain unresolved, although compromise of fast axonal transport is the central theme for several of the hypotheses (Sickles et al., 2002b). With respect to neurofilaments and fast axon transport, a series of studies on normal and transgenic mice (lacking neurofilaments) demonstrated that the presence or absence of neurofilaments did not alter AA's reduction of fast axonal transport in central (optic) or peripheral (sciatic) nerves and that the reduction mode of action is independent of toxicant-induced modifications or accumulations of neurofilaments (Stone et al., 2001, 2000, 1999). Sickles et al. (2002b) further advanced the hypothesis for reduction in fast transport as the MOA in a review of the literature and the identification of the motor protein, kinesin, as a primary target. Kinesin has a reduced affinity in vitro for microtubules following preincubation with varying concentrations of AA (Sickles et al., 1996). Sickles et al. (2002b) proposed that kinesin and microtubules are covalently modified by AA leading to a reduction in their mutual affinity and, subsequently, the level of fast axonal transport.

Lehning et al. (1998) reported that relatively low dose oral subchronic (26–45 days) exposure to AA (e.g., 20 mg/kg-day from drinking water) induced axonal degeneration (tibial nerve) while shorter-term (11 day) i.p. doses at higher levels (50 mg/kg-day) did not, yet both dosing regimens resulted in moderate levels of behavioral neurotoxicity. No apparent differences were observed in a comparison of AA metabolism and toxicokinetics by the above

dosing regimens that provided an explanation for the occurrence of degeneration with the subchronic but not acute dosing regimen (Barber et al., 2001). These results support a hypothesis that the MOA for axon degeneration is conditional on dose rate and an epiphenomenon (i.e., a secondary phenomenon accompanying, and resulting from, the primary MOA for functional neurotoxicity). LoPachin et al. (2002a) proposed that this primary MOA involves the nerve terminal region and that the primary sites are synaptosomal proteins involved in presynaptic transmission (LoPachin et al., 2004). LoPachin et al. (2004) hypothesized that AA alters neurotransmitter release by interacting with sulfhydryl groups on synaptosomal proteins.

Although there is controversy over which events are primary and which are epiphenomena, both proposed MOAs agree that binding of AA (or metabolites) to sulfhydryl rich proteins is a key event. Both research teams also recognize the need for additional research to determine relative binding rates and to demonstrate a direct relationship between protein inhibition (kinesin or synaptosomal or other proteins) and observed functional deficits (LoPachin and Canady, 2004; Sickles et al., 2002a).

The neurotoxic potential of the AA metabolite, glycidamide, also needs further evaluation. Costa et al. (1992) administered either AA or GA to groups of male Sprague-Dawley rats by i.p. injection at doses of 25 or 50 mg/kg-day (AA) or 50 or 100 mg/kg-day (GA) for 8 days and assessed hindlimb foot splay and rotarod performance. In the AA-treated rats, performance was significantly reduced on both rotarod and foot splay tests, relative to controls. Glycidamide-treated rats exhibited decreased rotarod performance but no evidence of hindlimb foot splay. The lack of this effect suggests that AA-induced peripheral neuropathy may not involve GA. Abou-Donia et al. (1993), however, report that GA is capable of inducing peripheral neuropathy in male Sprague-Dawley rats (six/group) given i.p. injections of 50 mg/kg-day AA or GA for up to 13 days. Acrylamide-exposed rats developed hindlimb weakness and altered gait by day 5, with hindlimb paralysis occurring in all rats by day 13. Glycidamide-exposed rats developed severe ataxia by day 11, and hindlimb paralysis developed in all treated rats by day 13. The apparent inconsistency between the two reports concerning the ability of GA to induce peripheral neuropathy remains unresolved. Some of the inter- and intraspecies differences in dose-response may result from differences in AA toxicokinetics (as discussed previously in the section on species differences in AA and GA metabolism). For example, it was observed that mice had approximately a five-fold increase in internal exposure to GA compared with rats due to an increased rate of GA formation in the mouse (Twaddle et al., 2004; Barber et al., 2001; Miller et al., 1982).

The MOA for AA-induced reproductive toxicity is poorly understood. Positive results of germ cell mutagenicity assays and reproductive toxicity tests indicate that some aspects of reproductive toxicity may be mediated by mutagenic effects on male germ cells (see Sections

4.3.1, 4.3.3, and 4.4.3) (Costa et al., 1992). Mechanistic proposals have also been made for a common MOA for neurotoxic and male fertility effects (e.g., effects on mounting, sperm motility, and intromission) involving modifications of kinesin and sulfhydryl groups of other proteins by AA and/or GA and a separate mechanism for male dominant lethal mutations involving clastogenic effects from AA and/or GA interactions with protamine or spindle fiber proteins in spermatids and/or direct alkylation of DNA by GA (Perrault, 2003; Tyl and Friedman, 2003; Tyl et al., 2000b; Sega et al., 1989; Adler et al., 2000).

Sega et al. (1989) proposed AA alkylation of protamine in late-stage spermatids as a mechanism for AA-induced dominant lethal effects based on a parallel time course for protamine alkylation and dominant lethal effects in spermatids of mice treated with AA. This observation was repeated by Adler et al (2000), who further proposed that the GA metabolite is the ultimate clastogen in mouse spermatids based on the results of enzyme inhibition studies. Zenick et al. (1994) summarized the MOA as follows:

> Protamines are highly basic (arginine and lysine rich) proteins that also contain numerous cysteine residues. During epididymal transit and spermatozoal maturation, the cysteine sulfhydryls are oxidized to form both inter- and intramolecular disulfide bonds. These confer even greater stability on sperm nuclei such that they become resistant to disruption by any means, including anionic detergent treatment, unless a disulfide-reducing agent is applied. This remarkably stable structure packages sperm DNA such that it remains transcriptionally inert and protected from damage during transit through both the epididymis and the female tract. Only after the sperm have entered the oocyte are the disulfide bonds in its chromatin reduced, thus initiating the rapid decondensation of the sperm nucleus with replacement of protamines by somatic histones, and subsequent reactivation of the male genome. Chemicals that disrupt sperm chromatin packaging by altering the synthesis of disposition of testis-specific transitional proteins (which first replace somatic histones prior to themselves being replaced with protamine) or protamines, or by binding to free sulfhydryls and thus preventing protamine cross-linking, may contribute to genetic damage, perhaps by an indirect mechanism or by making the chromatin more vulnerable other DNA-binding chemicals.

The hypothesis that AA-induced germ cell and somatic mutations in male mice require CYP2E1-mediated epoxidation of AA to GA received strong support from studies by Ghanayem et al. (2005a,b) where dose-responses for germ-cell and somatic mutagenicity were compared between male CYP2E1-null and wild-type mice treated with AA. In both studies, effects were not observed in the CYP2E1-null mice, while treated wild-type male mice responded with dose-related increases in resorption moles (i.e., chromosomally aberrant embryos), decreases in the numbers of pregnant females and the proportion of living fetuses, and somatic cell mutations. These results support further evaluation of CYP2E1 polymorphisms in human populations as a possible determinant of variability in, and susceptibility to, AA genotoxicity in the human population.

Support for the occurrence of DNA alkylation in the MOA leading to dominant lethals includes the detection of DNA adducts of GA in various tissues from mice and rats following single i.p. injections of 50 mg/kg AA (Segerbäck et al., 1995). The mechanistic proposals presented by Tyl and Friedman (2003) appear to be consistent with other proposals that the primary direct biological reactivity of AA involves binding to proteins (in vitro direct binding of AA to DNA is very slow), AA is converted to GA in rats and humans, and GA can react both with proteins and with DNA (Dearfield et al., 1995).

4.8. EVALUATION OF CARCINOGENICITY

4.8.1. Summary of Overall Weight of Evidence

In accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), AA is characterized as "likely to be carcinogenic to humans." This characterization is based on the following findings: (1) chronic oral exposure of F344 rats to AA in drinking water induced statistically significant increased incidences of thyroid follicular cell tumors (adenomas and carcinomas combined in both sexes), scrotal sac mesotheliomas (males), and mammary gland fibroadenomas (females) in two bioassays; (2) oral, i.p., or dermal exposure to AA initiated skin tumors that were promoted by TPA in SENCAR and Swiss-ICR mice; and (3) i.p. injections of AA induced lung adenomas in strain A/J mice.

The available human studies on potential AA carcinogenicity are for either dietary exposures—four case-control studies (Mucci et al., 2005, 2004, 2003; Pelucchi et al., 2006) and one prospective study (Mucci et al., 2006) or occupational exposures from inhalation and/or dermal exposure—three cohort mortality studies (Marsh et al., 1999; Collins et al., 1989; Sobel et al., 1986). These studies are judged as providing limited data. No statistically significant increased risks for cancer-related deaths were found in the cohort mortality studies of AA workers with the exception that, in an exploratory dose-response analysis of the most comprehensive study, an increased risk for pancreatic cancer was reported in a subgroup with the highest cumulative AA exposure. In the four recent case-control studies and one prospective study, no statistically significant associations were found between frequent consumption of foods with high or moderate levels of AA and cancer incidence for large bowel, bladder, kidney, renal cell breast, colorectal, oral cavity, pharynx, esophagus, larynx, ovary, or prostate cancer. These studies evaluated Swedish or Italian populations, not U.S. populations or U.S. diets (i.e., no studies on U.S. populations have been reported to date). Some of the sites observed in the animal studies (thyroid, testicular, CNS) have not been evaluated, and there are limitations in some of the study methods and cohort sizes.

There is one case-control study that evaluated whether diet during preschool age (3–5-year-olds) affected a woman's risk of breast cancer later in life and reported a slightly increased OR associated with consumption of French fries, but there is considerable uncertainty in the

accuracy of the results from a recall questionnaire administered to mothers for diets in their preschool children from an estimated 40–60 years earlier and no information on the AA content of the foods in the diet (Michels et al., 2006).

There are no animal data on the carcinogenicity of chronic inhalation exposure to AA or human data exclusively for an inhalation exposure. EPA's *Guidelines for Carcinogen Risk Assessment* (2005) indicate that for tumors occurring at a site other than the initial point of contact, the weight of evidence for carcinogenic potential may apply to all routes of exposure that have not been adequately tested at sufficient doses.

The majority of the data support a mutagenic MOA for AA carcinogenicity. Acrylamide has been reported to induce gene mutations and chromosomal aberrations in somatic and germ cells of rodents in vivo and cultured cells in vitro, to transform cells of mouse cell lines, and to form adducts with protamines in germ cells. The mutagenic potential of GA is well-characterized in studies of the induction of gene mutations in bacteria, unscheduled DNA synthesis in a variety of test systems, and formation of DNA adducts. An alternative MOA of disruption of hormone levels or activity has been proposed for some of the tumors observed in animal studies, but the data supporting such an MOA are limited or lacking.

4.8.2. Synthesis of Human, Animal, and Other Supporting Evidence

Cohort mortality studies of acrylamide workers at several locations in the United States and the Netherlands (Marsh et al., 1999; Collins et al., 1989) and a location in Michigan (Sobel et al., 1986) have not found statistically significant increased risks for cancer-related deaths compared with national cancer mortality rates in whole-cohort analyses. Four case-control studies (Mucci et al., 2005, 2004, 2003; Pelucchi et al., 2006) and one prospective study (Mucci et al., 2006) have found no statistically significant associations between increased levels of AA in the diet and increased risk for a variety of cancer types, including large bowel, bladder, kidney, renal cell, breast, colorectal, oral cavity, pharyngeal, esophageal, laryngeal, ovarian, or prostate cancers. These studies evaluated Swedish or Italian populations, not U.S. populations or U.S. diets (i.e., no studies on U.S. populations have been reported to date). Some of the tumor sites observed in animal studies (thyroid, testis, CNS) have also not been evaluated, and there are limitations in some of the study methods and cohort sizes. One case-control study reported an increased OR for female breast cancer in later life (OR = 1.27; 95% CI = 1.12–1.44) from increased consumption of French fries in preschool diet, but the results are of questionable use in this assessment due to uncertainties in the accuracy of the diet recall (from an estimated 40–60 years ago) and lack of information on AA content in the foods (Michels et al., 2006).

In an exploratory exposure-response analysis in which U.S. workers in one of the cohorts were grouped into exposure categories, an increased risk for pancreatic cancer was calculated for the group with the highest cumulative AA exposure category (≥0.30 mg/m^3-years: SMR 2.26,

95% CI 1.03–4.29, based on nine pancreatic cancer deaths) (Marsh et al., 1999). The risk for pancreatic cancer in the four cumulative exposure categories did not increase monotonically from the lowest to highest category. A monotonic increase in SMR with another measure of exposure, duration of employment, was observed, but the SMRs for pancreatic cancer were not statistically significantly elevated in any of the four duration categories.

Since the relationship between increased exposure and increased risk for pancreatic cancer is tenuous, and has only been observed in one epidemiologic study, the available human evidence supporting AA carcinogenicity is considered to be limited to inadequate. The epidemiology study results, and the limitations of the studies to detect increased cancer mortality risks, however, are limited to discount potential AA carcinogenicity in humans, as suggested by the positive animal study carcinogenicity results. Limitations in the epidemiology studies include small cohort size and limited follow-up period (Sobel et al., 1986); large proportion of short-term workers in the cohort, low exposures, incomplete smoking habit information, and incomplete follow-up period (Marsh et al., 1999); and relatively low dietary exposures, a relatively short time frame for exposure information (5 years of recalled dietary habits), poor characterization of AA levels in the food items, variability in levels among different brands, and few food items in the diet known to have high levels of AA. Although a variety of cancer sites in humans were evaluated in the case-control and prospective epidemiology studies that reported no increased risk from dietary exposures (large bowel, kidney, renal cell, bladder, breast, ovary, prostate, oral/pharyngeal), some of the sites observed in the animal studies have not yet been evaluated (thyroid, testicular, CNS). No studies on U.S. populations have been reported to-date. The only study that showed a positive association had questionable data on diet composition and AA content in the diet (Michels et al., 2006).

Cancer studies in test animals include two 2-year drinking water administration studies in F344 rats (Friedman et al., 1995; Johnson et al., 1986), skin tumor initiation assays involving oral, i.p., or dermal initiating applications of AA and dermal promotion by TPA in SENCAR and Swiss-ICR mice (Bull et al., 1984a,b), and a lung adenoma i.p. administration assay in strain A/J mice (Bull et al., 1984a). The results from the two chronic oral exposure studies in rats are presented in Table 4-33.

Table 4-33. Incidence of tumors with statistically significant increases in both 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[e]	–
Tunica vaginalis mesothelioma	3/60	–	0/60	7/60	11/60[e]	–	10/60[e]	–
Johnson et al., 1986; females								
Follicular cell adenoma/carcinoma	1/58	–	0/59	1/59	1/58	–	5/60[e]	–
Mammary adenocarcinoma	2/60	–	1/60	1/60	2/58	–	6/61	–
Mammary benign	10/60	–	11/60	9/60	19/58	–	23/61[e]	–
Mammary benign + malignant[a]	12/60		12/60	10/60	21/58		29/61	
Friedman et al., 1995; males[b]								
Follicular cell adenoma/carcinoma	3/100	2/102[d]	–	12/203	5/101	–	17/75[e]	–
Tunica vaginalis mesothelioma[c]	4/102	4/102	–	9/204	8/102	–	13/75[e]	–
Friedman et al., 1995; females[b]								
Follicular cell adenoma/carcinoma	1/50	1/50	–	–	–	10/100	–	23/100[e]
Mammary benign + malignant	7/46	4/50	–	–	–	21/94[e]	–	30/95[e]

[a]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.
[b]Two control groups were included in the study design to assess variability in background tumor responses.
[c]Incidences reported herein are those originally reported by Friedman et al. (1995) and not those reported in the reevaluation study by Iatropoulos et al. (1998).
[d]The data reported in Table 4 in Friedman et al. (1995) lists 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.
[e]Statistically significant.

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

Tumor types that were consistently observed to increase in both chronic rat drinking water bioassays included statistically significant increases in thyroid follicular cell adenomas or carcinomas in male and female rats, tunica vaginalis testis (i.e., scrotal sac) mesotheliomas in male rats, and mammary gland tumors (adenomas, fibroadenomas or fibromas) in female rats at dose levels of 0.5 to 3 mg/kg-day but not at dose levels of 0.1 or 0.01 mg/kg-day (Friedman et al., 1995; Johnson et al., 1986). Data from both studies are sufficient to describe relationships between administered dose levels and cancer responses. The Friedman et al. (1995) bioassay included 204 male rats in the 0.1 mg/kg-day group to increase statistical power sufficient to detect a 5% incidence of scrotal sac mesotheliomas over an expected background incidence of this tumor in F344 rats of about 1%.

Findings of statistically significant increased incidences of adrenal pheochromocytomas in male rats, oral cavity tumors in female rats, CNS 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) and, with the exception of the CNS tumors, are not considered to add weight to the evidence for acrylamide carcinogenicity in animals. With

respect to the CNS tumors, Friedman et al. (1995) reported no significant increase in glial tumors of brain and spinal cord, however, not all of the animal brains or spinal cords in the treatment groups were examined (Rice, 2005), and seven cases of a morphologically distinctive category of primary brain tumor described as "malignant reticulosis" were reported but excluded from the authors' analysis (see Tables 4-13 and 4-14). CNS tumors are therefore considered to be one of the tumor types replicated in the Friedman et al. (1995) study, even though the incomplete brain and spinal cord tumor data set from this study precludes a quantitative analysis of CNS tumor incidence in the characterization of the dose-response analysis.

Results from the mouse skin tumor initiation assays add considerable weight to the evidence for acrylamide carcinogenicity in animals. Oral administration of AA, 6 times over a 2-week period, followed by dermal application of the tumor promoter, TPA, for 20 weeks, induced statistically significant increased incidences of histologically confirmed skin tumors (squamous cell papillomas and carcinomas) at 52 weeks in two mouse strains, SENCAR and Swiss-ICR (Bull et al., 1984a,b). Similar initiation treatments of the SENCAR strain involving i.p. injections or dermal applications of AA (followed by TPA promotion) induced statistically significant increased incidences of palpable skin masses during the course of the 52-week observation period but were not as effective as oral administration (Bull et al., 1984a). These findings provide evidence that AA can initiate tumor development in mice, a process that is thought to involve a mutagenic mode of action. These findings are consistent with the positive findings for AA and GA genotoxicity in numerous tests.

Other evidence of the carcinogenicity of acrylamide in mice is provided by the observations that statistically significant increased incidences of lung tumors were found in A/J mice 8 months after i.p. injection of AA 3 times a week for 8 weeks (Bull et al., 1984a) and in Swiss-ICR mice 52 weeks after starting a 2-week oral administration AA initiation protocol followed by dermal TPA application for 20 weeks (Bull et al., 1984b).

As discussed in Section 4.4.3 and tabulated in Appendix B, acrylamide mutagenicity has been extensively studied. Although AA did not induce mutations in bacterial assays (with or without mammalian metabolic activation systems), results from certain other mutagenicity tests have been predominantly positive and provide supporting evidence for the human carcinogenic potential of AA. The positive results include demonstrations of chromosomal aberrations in in vitro exposed mammalian cells (Tsuda et al., 1993; Warr et al., 1990; Moore et al., 1987); in vitro cell transformation of Syrian hamster embryo cells (Park et al., 2002); chromosomal aberrations or micronuclei in bone marrow of mice given i.p. injections of 50–100 mg/kg (Čihák and Vontorková, 1990, 1988; Adler et al., 1988); formation of DNA adducts of GA following i.p. injection of 50 mg/kg of AA in mice and rats (Segerbäck et al., 1995); and dominant lethal mutations in mice given one to five i.p. injections of 40–125 mg/kg AA (Shelby et al., 1987), in

rats exposed to 2.8 mg/kg-day in drinking water for 80 days (Smith et al., 1986), and in mice exposed to five consecutive dermal doses of 50–125 mg/kg AA (Gutierrez-Espeleta et al., 1992).

In addition, the epoxide metabolite of AA, glycidamide, has been shown to be mutagenic to *S. typhimurium* strains TA100 and TA1535 (Hashimoto and Tanii, 1985) and mouse lymphoma cells (Barfknecht et al., 1988) but not to *K. pneumoniae* (Voogd et al., 1981). Glycidamide induced unscheduled DNA synthesis in mouse spermatids in vivo (Sega et al., 1990), human epithelial cells in vitro (Butterworth et al., 1992), in one of two tests for unscheduled DNA synthesis in rat hepatocytes in vitro (Butterworth et al., 1992; Barfknecht et al., 1988), and in (C3H/RL × C57BL)F1 male mice given single i.p. injections of 150 mg/kg GA (Generoso et al., 1996). Glycidamide (125 mg/kg by i.p. injection) induced dominant lethal mutations in male JH mice mated with nonexposed female SB mice, without producing discernible effects on mating performance (Generoso et al., 1996). Glycidamide treatment (100 mg/kg by i.p. injection) of male (C3H × 101/RL)F1 mice mated with nonexposed females induced heritable translocations in male offspring (Generoso et al., 1996).

4.8.3. Mode of Action for Carcinogenicity

The mode of action (MOA) discussion considers all of the tumor types observed in the animal assays and the events that might lead to increased incidence in those tumors. The tumor types of interest include the following: (1) the consistently observed increase in thyroid follicular cell adenomas or carcinomas in male and female rats, tunica vaginalis testis (i.e., scrotal sac) mesotheliomas in male rats, and mammary gland tumors (adenomas, fibroadenomas or fibromas) in female rats following chronic oral exposure (Friedman et al., 1995; Johnson et al., 1986); (2) the CNS tumors reported in the Johnson et al. (1986) study, supported by the brain tumor data in Friedman et al. (1995), although an incomplete analysis of all of the animals in the latter study precluded the inclusion of brain tumors in the quantitative dose-response analysis; (3) the initiated skin tumors following oral, i.p., or dermal exposure to AA in SENCAR and Swiss-ICR mice (Bull et al., 1984a,b); and (4) the lung adenomas following i.p. doses of AA in A/J mice (Bull et al., 1984a).

At present, the mechanistic sequence of events by which AA induces these tumor types is not completely defined. The majority of the data, however, support a mutagenic MOA for AA carcinogenicity. An alternative MOA has been proposed for some of the tumors observed in the animal bioassays (i.e., disruption of hormone levels or activity), but data supporting this MOA are limited or lacking.

4.8.3.1. *Hypothesized Mode of Action—Mutagenicity*

A number of study results support a mutagenic MOA for acrylamide-induced carcinogenicity (including Besaratinia and Pfeifer, 2007; Besaratinia and Pfeifer, 2005; Schmid

et al., 1999; Dearfield et al., 1995; Segerbäck et al., 1995; Moore et al., 1987). Acrylamide has been reported to induce genotoxicity (gene mutations and some types of chromosomal aberrations [i.e., translocations]) in somatic and germ cells of rodents in vivo and cultured cells in vitro, to transform cells of mouse cell lines, and to form DNA adducts in somatic cells. The mutagenic potential of GA is well-characterized in studies of the induction of gene mutations in mammalian cells, and in the formation of DNA adducts. The available data indicate that the major genotoxic effects of AA are clastogenic, which may involve covalent modifications of proteins by AA and GA, and that the mutagenic events that lead to tumors from exposure to AA are produced by GA via direct alkylation of DNA.

Specifically, evidence in support of a mutagenic MOA for carcinogenicity includes the following:

- Acrylamide is metabolized by CYP2E1 to the DNA-reactive epoxide, GA;

- AA and GA are genotoxic in the Big Blue mouse following oral exposures, significantly increasing lymphocyte Hprt and liver cII mutation frequencies (MFs). Molecular analysis of the mutants indicated that AA and GA produced similar mutation spectra that were significantly different from controls consistent with AA exerting its genotoxicity in BB mice via metabolism to GA. The predominant types of mutations in the liver cII gene from AA and GA-treated mice were G:C ->T:A transversions and -1/+1 frameshifts in a homopolymeric run of Gs.
- DNA adducts of GA have been detected in mice and rats exposed to AA and GA in all relevant tissues in both males and females where tumors have been reported, including liver, brain, thyroid, leukocytes, mammary gland, and testis in rats; and liver, lung, kidney, leukocytes, and testis in mice.
- Glycidamide is mutagenic in short-term bacterial assays.
- Glycidamide is mutagenic in male and female mouse somatic cells following oral exposure and in male mouse germ cells (heritable translocations) following intraparenteral exposure.
- Acrylamide induces heritable translocations in male mouse germ cells following intraparenteral or dermal administration, and specific locus mutations in male germ cells following intraparenteral administration.
- Positive mouse lymphoma assay results (with the caveat that it is not definitively known whether these somatic cell mutations resulted from AA-induced chromosomal alterations [chromatid and chromosome breaks and rearrangements] or GA-DNA adducts).
- Dominant lethal mutations have been demonstrated in rodents following subchronic oral exposure at AA dose levels in the 2.8 to 13.3 mg/kg-day range, which is near the range of chronic dose levels associated with carcinogenic effects in rats (0.5 to 3 mg/kg-day).

Description and identification of key events

The proposed sequence of events for a mutagenic MOA for AA is as follows:

(1) AA is metabolized to the relatively long-lived epoxide, GA, in rats and humans, and GA reacts both with proteins and with DNA;

(2) GA binding to DNA results in mutations that persist in viable somatic cells; and

(3) GA's mutagenic activity leads to carcinogenicity and the formation of tumors observed in the animal bioassays.

It is not known whether alterations in protein function due to the formation of both parent compound- and reactive metabolite-protein adducts have an effect on cell replication or proliferation or both. The primary mutagenic activity of AA, however, is proposed to result from the direct binding of the GA metabolite to DNA. In vitro studies indicate that direct binding of AA to DNA is slow.

Strength, consistency, and specificity of the association between exposure to acrylamide and mutagenic activity that could lead to the formation of tumors

There is ample evidence in the literature for the ability of acrylamide and glycidamide (administered via different routes of exposure) to induce a variety of genotoxic effects in mammalian cells (Besaratinia and Pfeifer, 2007; Rice, 2005; Doerge et al., 2005a; Ghanayem et al., 2005a; Gamboa et al., 2003; Generoso et al., 1996; Dearfield et al., 1995; Segerbäck et al., 1995; Adler et al., 1994; Ehling and Neuhäuser-Klaus, 1992; Russell et al., 1991; Knaap et al., 1988; Moore et al., 1987).

Some genotoxic endpoints and cell assays may be considered to be less relevant to carcinogenic potential than others. For example, genotoxicity results in germ cells are less relevant than toxicity in somatic cells where tumors are formed. Further, some effects on germ cells that appear to be transmitted via genetic alterations may be due to alternative causes. Dominant lethals in males, for example, may be due not only to genotoxic events in the sperm but alternatively to nongenetic interactions with proteins critical to the formation and function of the sperm. Other genotoxic phenomena, such as chromosome breaks, are not heritable. Also, alterations in chromosome numbers (aneuploidy) are usually due to protein effects and do not involve a mutagenic MOA. Epidemiology studies that evaluated the association between increased cytogenetic damage and enhanced cancer risk report no significant association between the sister chromatid exchange or micronuclei frequencies and subsequent cancer incidence or mortality (Hagmar et al., 1998; Bonassi et al., 2004). Other measures, such as unscheduled DNA synthesis may be attributable to either DNA damage or general cytotoxicity and, therefore, may not be directly attributable to mutagenicity.

The strongest direct evidence to supporting a mutagenic MOA for acrylamide's carcinogenic effects consists of positive findings of stable mutations in viable somatic cells.

Such evidence, and support that GA is the predominant mutagenic agent following exposure to AA, includes the following:

1) significant increases in somatic cell mutations following in vivo oral exposures of the Big Blue mouse to either AA and GA, and similar mutagenicity spectra between AA and GA (Manjanatha et al., 2006);

2) formation of GA-DNA adducts at similar specific locations within the cII gene in Big Blue mouse embryonic fibroblasts (that carry a lambda phage cII transgene)and the tumor suppressor p53 gene (TP53) in normal human bronchial epithelial cells following exposure to AA or GA in vitro (Besaratinia and Pfeifer, 2004);

3); detection of DNA adducts of GA in various mouse and rat tissues following single i.p. administration of AA and GA (Doerge et al., 2005a; Segerbäck et al., 1995);

4) demonstration that AA-induced germ and somatic cell mutations in male mice require CYP2E1-mediated epoxidation of AA (Ghanayem et al., 2005a,b);

5) positive results for GA in Salmonella typhimurium strains TA100 and TA1535 (Hashimoto and Tanii, 1985);

6) detection of heritable translocations in mice following single i.p. injections of GA doses of 100–150 mg/kg (Generoso et al., 1996); and

7) positive results for gene mutation in mouse lymphoma cells in vitro at concentrations as low as 0.3 mg/mL (Barfknecht et al., 1988; Knaap et al., 1988; Moore et al., 1987).

The results of Manjanatha et al. (2006) studies on significantly increased in vivo mutation frequencies in the Big Blue (BB) mouse following oral exposure to AA and GA are consistent with AA's ability to induce heritable mutations in mammalian cells. Average daily AA exposure from drinking water at the low dose of 100 mg/L (4-week exposure) was 19 mg/kg-day for male and 25 mg/kg-day for female BB mice; the high dose of 500 mg/L (3 weeks only due to clinical signs of neurotoxicity) yielded average daily exposures of 98 mg/kg-day for males and 107 mg/kg-day for females. GA exposures were 25 and 35 mg/kg-day for males and females, respectively, administered the low dose of 120 mg/L (4 weeks), and 88 and 111 mg/kg-day administered the high dose of 600 mg/L (4 weeks). Both doses of AA and GA produced significantly increased lymphocyte Hprt mutant frequencies, with the high doses producing responses that were 16–25-fold higher than those of the respective control. The high doses of AA and GA also produced significant 2–2.5-fold increases in liver cII MFs. Molecular analysis of the mutants indicated that AA and GA produced similar mutation spectra that were significantly different from controls consistent with AA exerting its genotoxicity in the BB mice via metabolism to GA. The predominant types of mutations in the liver cII gene from AA and GA-treated mice were G:C ->T:A transversions and -1/+1 frameshifts in a homopolymeric run of Gs.

Acrylamide and glycidamide react with nucleophilic sites in macromolecules (including hemoglobin and DNA) in Michael-type additions (Segerbäck et al., 1995; Bergmark et al., 1993, 1991; Solomon et al., 1985). Solomon et al. (1985) conducted in vitro studies for the reaction of acrylamide with calf thymus DNA and with various deoxynucleosides including 2'-deoxyadenosine (dAdo), 2' deoxycytidine (dCyd), 2'-deoxyguanosine (dGua), and 2'-deoxythymidine (dThd), and demonstrated the formation of 2-formamidoethyl and 2-carboxyethyl adducts via Michael addition. Acrylamide reacted extremely weakly with both the nucleosides and calf thymus DNA, even under in vitro conditions, producing only small quantities of adducts only after incubations of 40 days even at high acrylamide concentrations.

Segerbäck et al. (1995) reported much higher rates of DNA-adduct formation from acrylamide-generated glycidamide than from acrylamide itself. In analyzing either calf thymus DNA incubated with S-9 fraction in vitro or liver DNA from mice treated in vivo with radiolabeled AA, approximately 90% of the radioactivity released during hydrolysis co-chromatographed with a standard synthesized from the reaction of glycidamide and deoxyguanosine, N-7-(2-carbamoyl-2-hydroxyethyl)guanine. The amount of this adduct formed in vivo was measured in a number of organs from both rats and mice administered 46-53 mg AA/kg i.p., and was found to be in the range of 5-62 pmol/mg DNA. The amount of guanine adduct that would have been formed solely from AA at this dose was estimated to be much less, in the low fmol range, which would be negligible compared with the observed levels.

Besaratinia and Pfeifer (2004) treated normal human bronchial epithelial cells and Big Blue mouse embryonic fibroblasts (that carry a lambda phage cII transgene) in vitro with AA, its primary epoxide metabolite GA, or water (control) and then subjected the cells to terminal transferase-dependent polymerase chain reaction to map the formation of DNA adducts within the human gene encoding the tumor suppressor p53 gene (TP53) and the mouse embryonic fibroblast cII transgene. Acrylamide and glycidamide formed DNA adducts at similar specific locations within TP53 and cII, and DNA adduct formation was more pronounced after GA treatment than after AA treatment at all doses tested. Acrylamide-DNA adduct formation was saturable, whereas the formation of most GA-DNA adducts was dose-dependent for all doses tested. Glycidamide formed more adducts than AA at any given dose, and the spectrum of GA-induced cII mutations was statistically significantly different from the spectrum of spontaneously occurring mutations in the control-treated cells (P=.038). Compared with spontaneous mutations in control cells, cells treated with GA or AA had more A-->G transitions and G-->C transversions and GA-treated cells had more G-->T transversions (P<.001). These results support the hypothesis that the mutagenicity of AA in human and mouse cells is based on the capacity of its epoxide metabolite GA to form DNA adducts.

Doerge et al. (2005a) confirmed that GA-derived DNA adducts of adenine and guanine were formed in all tissues examined from either AA or GA dosing, including target tissues

identified in rodent carcinogenicity bioassays and nontarget tissues including liver and leukocytes in rats and liver, lung, kidney, leukocytes and testis in mice, indicating wide-spread occurrence. They measured DNA adducts following a single i.p. administration of either AA or GA to adult B6C3F1 mice and F344 rats at 50 mg/kg AA or an equimolar dose of GA (61 mg/kg). Kinetics of DNA adduct formation and accumulation were also measured following oral administration of a single dose of AA (50 mg/kg) or from repeat dosing (1 mg/kg-day for up to 50 days). The formation of the DNA adducts was consistent with previously reported mutagenicity of AA and GA in vitro involving reactions of GA with adenine and guanine bases. Repeated dosing of rats and mice with AA administered in the drinking water resulted in production of steady state serum levels of GA, and in accumulation of N7-GA-guanine adducts in liver. Steady state levels of N7-GA-Gua were attained in approximately 14 days with a formation half-life of about 4 days in male and female mice, and in female rats. Male rats reached a maximum level at 14 days, but subsequently had an apparent slow decline in adduct level. The findings indicate that DNA damage from exposure to AA can accumulate to a level that is dependent on the frequency of consumption, the amount consumed, and depurination rate.

Ghanayem et al. (2005a) compared germ-cell mutagenicity in male CYP2E1-null and wild-type mice treated with AA, and provided the first unequivocal demonstration that AA-induced germ cell mutations in male mice required CYP2E1-mediated epoxidation of AA to GA. CYP2E1-null and wild-type male mice were treated by i.p. injection with 0, 12.5, 25, or 50 mg AA/kg bw in 5 mL saline/kg-day for 5 consecutive days. At defined times after exposure, males were mated to untreated B6C3F1 females. Females were killed in late gestation, and uterine contents were examined. Dose-related increases in resorptions (chromosomally aberrant embryos), and decreases both in the numbers of pregnant females and the proportion of living fetuses were seen in females mated to AA-treated wild-type mice. No changes in any fertility parameters were seen in females mated to AA-treated CYP2E1-null mice. Of importance to the argument that GA is the putative mutagen in AA's mutagenic MOA, a further study by Ghanayem et al. (2005b) demonstrated the absence of AA-induced genotoxicity in somatic cells in CYP2E1-null mice compared with wild-type mice treated with AA.

Generoso et al. (1996) had previously evaluated AA's ability to induce dominant lethal mutations and heritable translocations in male mice spermatids, and demonstrated that GA produced responses that were consistent with the proposal that in vivo conversion to GA is responsible for the observed mutagenicity (i.e., heritable translocations) of AA in male mice. Positive results for gene mutation were also observed in mouse lymphoma cells in vitro with concentrations of AA as low as 0.3 mg/mL (Barfknecht et al., 1988; Knaap et al., 1988; Moore et al., 1987). Moore et al. (1987) evaluated activity of AA without exogenous activation in L5178Y/TK+/- -3.7.2C mouse lymphoma cells at the thymidine kinase locus, and noted AA induced almost exclusively small-colony mutants, indicating clastogenic activity, including

chromatid and chromosome breaks and rearrangements. Thus, the positive results in these assays, although relevant for heritable mutations can not be definitively attributable to GA related DNA mutations or AA related chromosomal alterations.

Acrylamide and 15 of its analogues have been tested for mutagenicity in five TA strains of Salmonella typhimurium (Hashimoto and Tanii, 1985). Acrylamide and most of its analogues were not mutagenic, neither in the standard Ames assay either with or without Aroclor 1254-induced S9 liver fraction, nor in the plate incubation or liquid preincubation procedures. However, three of the epoxides including glycidamide (the other two were N,N-diglycidyl acrylamide and glycidyl methacrylamide) were mutagenic in one or two strains both with and without the S9 fraction.

Overall, the available in vivo mutagenicity data indicate that acrylamide, via conversion to its active epoxide metabolite, glycidamide, can form DNA adducts, point mutations, and frameshift mutations that persist in viable mammalian (including human) somatic cells.

Mutations occur in target tissues where tumors have been observed

Doerge et al. (2005a) provide the strongest evidence that acrylamide-induced mutagenicity (via glycidamide) can be associated with the target tissues where tumors are observed in the animal bioassays. They report that GA-derived DNA adducts of adenine and guanine were formed in all target tissues identified in rodent carcinogenicity bioassays as well as a number of non-target tissues including liver, brain, thyroid, leukocytes, mammary gland, and testis in rats; and liver, lung, kidney, leukocytes and testis in mice.

There is little information to causally associate the events between GA–DNA adduct formation, the occurrence of a stable mutation, and the development of a tumor. It is also not known why some tissues are more prone to tumor formation than others with similar levels of GA-DNA adducts. Other tissue-specific events may be occurring. Klaunig and Kamendulis (2005) reported the effects of AA reactivity with DNA and altered cell growth in the target tissues identified in the chronic oral bioassays. DNA synthesis was examined in F344 rats treated with AA at 0, 2, or 15 mg/kg-day for 7, 14, or 28 days. Acrylamide increased DNA synthesis in the target tissues (thyroid, testicular mesothelium, adrenal medulla) at all doses and time points examined. In contrast, in a nontarget tissue (liver), no increase in DNA synthesis was seen. Examination of DNA damage using single cell gel electrophoresis (the Comet assay) showed an increase in DNA damage in the target tissues but not in nontarget tissue (liver). In addition, a cellular transformation model, the Syrian hamster embryo (SHE) cell morphological transformation model, was used to examine potential mechanisms for the observed carcinogenicity of AA. SHE cell studies showed that GSH modulation by AA was important in the cell transformation process. Treatment with a sulfhydryl donor compound (N-acetyl cysteine) reduced AA transformation, while depletion of GSH (buthionine sulfoximine) resulted

in an enhancement of transformation. Acrylamide was thus shown to increase both DNA synthesis and DNA damage in mammalian tissues and cells, suggesting that DNA reactivity and cell proliferation, in concert, may contribute to the observed AA-induced carcinogenicity in the rat target tissues.

Dose-response concordance and temporal relationship

Empirical support for dose-response concordance and a temporal sequence of events is generally limited. Specifically, the only data of positive mutagenicity in the dose range of the chronic rat bioassays (0.5 to 3 mg/kg-day) to demonstrate dose-response concordance is the increase in N7-GA-guanine adducts to steady state levels in female rat livers after approximately 14 days of repeat dosing of approximately 1 mg AA/kg bw/day in the drinking water. In male rat livers, the adduct levels at this same regimen were consistently lower than in females, and the increase to a maximum at approximately 14 days was followed by an apparent slow decline in adduct levels (Doerge et al., 2005a).

There are also some data from mouse skin tumor initiation bioassays and several in vivo genotoxicity assays (including dominant lethal mutation assays) that provide evidence of mutagenicity from AA exposure in the range of 3 to 50 mg/kg-day.

Acrylamide's ability to initiate mouse skin tumors has been demonstrated at oral dose levels as low as 12.5 mg/kg-day (Bull et al., 1984a,b). Oral administration of AA (three times a week for 2 weeks, followed by dermal application of the cancer promoter, TPA) caused statistically significant increased incidences of skin-tumor-bearing SENCAR mice at 12.5, 25, or 50 mg/kg-day dose levels and statistically significant increased incidences of histologically confirmed skin adenomas or carcinomas at 25 or 50 mg/kg-day (Bull et al., 1984a). In this study, oral administration was more effective at initiating skin tumors than i.p. injection or dermal application at equivalent dose levels. In Swiss-ICR mice, a similar initiation-promotion protocol caused statistically significantly increased incidences of the same endpoints at oral doses of 50 mg/kg-day but not at 12.5 or 25 mg/kg-day (Bull et al., 1984b). The power to detect statistically significant changes in these studies, however, is limited by the number of animals in each exposure group (n = 40). For example, in the Swiss-ICR study, statistical significance could not be demonstrated for the difference between the control incidence (0/40) and the incidences of skin-tumor bearing animals in the 12.5 mg/kg-day (4/40) and 25 mg/kg-day groups (4/40). Thus, the available data give some indication that AA tumor initiation activity increases with increasing dose level, but these data are inadequate to determine whether oral dose levels of 0.5–3 mg/kg-day would also initiate mouse skin tumors.

Dominant lethal mutations following repeated exposure to AA in drinking water (e.g., implantation losses or decreased fetuses/dam) have been observed in male F344 rats exposed for

at least 12 weeks to 5 mg/kg-day, but not to 2 mg/kg-day (Tyl et al., 2000a); male Swiss CD-1 mice exposed for at least 15 weeks to 7.5 mg/kg-day, but not to 3.1 mg/kg-day (Chapin et al., 1995); male Long-Evans rats exposed for 72 days to 2.8 mg/kg-day, but not to 1.5 mg/kg-day (Smith et al., 1986); and male ddY mice exposed for 4 weeks to 13.3 mg/kg-day, but not to 9.0 mg/kg-day (Sakamoto and Hashimoto, 1986). There is currently insufficient information, however, to determine if the events leading to the dominant lethals are relevant or not to a mutagenic MOA.

Studies designed to examine in vivo clastogenic effects in mammals from subchronic or chronic exposures at lower doses are limited to the reports of no chromosomal aberrations in spermatogonia or spermatocytes in male Long-Evans rats exposed for 72 days to drinking water doses between 1.5 and 5.8 mg/kg-day (Smith et al., 1986) and the dominant lethal effects described above with subchronic exposure to doses in the range of 2.8 to 7.5 mg/kg-day in several studies (Tyl et al., 2000a; Chapin et al., 1995; Smith et al., 1986). These results, however, indicate only that genotoxic effects on male germ cells can occur following subchronic duration oral exposure to dose levels in the vicinity of the chronic dose levels that induced carcinogenic effects in rats, and again it is uncertain whether or not the events are these results are relevant to a mutagenic MOA for AA.

Allen et al (2005) attempted dose-response modeling of AA in vivo genotoxicity data to extrapolate the response for chromosomal aberrations or sister chromatid exchange from the relatively high administered doses in these assays (50–150 mg/kg) to the 2 mg/kg-day dose used in the chronic oral bioassays that significantly increased thyroid tumors in F344 rats. The intent of this approach was to move the analysis of genotoxicity assay results from qualitative conclusions of "negative or positive" results (as listed in the table in Appendix B) to more useful quantitative characterizations of the dose response that support or refute dose-response concordance between mutagenic events and increased tumorigenicity. In their analysis of the AA data (based on a variety of dose-response modeling approaches and a benchmark response level of 10% for occurrence of chromosomal damage), the authors report that a 2 mg/kg-day dose would result in levels indistinguishable from background (i.e., zero exposure), suggesting little concordance between these studies and the observed tumorigenicity in rats. The analysis, however, has a number of serious (if not fatal) flaws and assumptions, including some addressed by the authors (e.g., comparing short-term, high-dose effects with long-term, low-dose effects, comparing results in mice with results in rats, assuming low-dose response relationships based on extrapolations from very high doses, and limited sample sizes), as well as others not well addressed, including the assumption that chromosomal damage is the primary mutagenic event (rather than DNA adducts or other DNA damage), not evaluating mutagenic events in target tissues (i.e., not considering the toxicokinetics of AA) or at different life stages (not considering the toxicodynamics of AA), and that very small increments above background are not important

(i.e., disregarding the one hit, one tumor hypothesis), or, alternately, that it is acceptable to apply a benchmark response of 10% to mutagenic events assumed to lead to tumor formation when the generally accepted "minimal" risk level for carcinogenicity is 0.0001% (i.e., one in a million, not one in ten). Nonetheless, attempts to quantitate mutagenic dose response is clearly in the right direction, and warrants further support and research.

In summary, the Doerge et al. (2005a,b) data demonstrates formation of GA-DNA adducts in tissues throughout the body as a result of the rapid and wide distribution of AA and GA from any route of exposure (i.e., a high volume of distribution). Additional indicators of potential mutagenicity discussed above that occur within hours or days of treatment support these events as precursor events to the formation of tumors, although the administered doses were much higher than those given to the test animals in the chronic bioassays.

Biological plausibility and coherence

DNA adducts and mutations in genes have been implicated in the carcinogenic effects of a variety of chemicals and drugs (polycyclic aromatic hydrocarbons, vinyl chloride, benzene, tamoxifen). Thus, it is biologically plausible that the formation of DNA adducts is a causal event in the carcinogenicity of AA. However, the fact that adducts are detected in nontarget organs underscores the importance of not assuming that adducts by themselves are sufficient to produce tumors. Only certain DNA adducts lead to perturbed gene structure and function. Although biologically plausible, quantitating the cancer risk from DNA adducts requires an ability to identify the critical adducts based on the nature of the chemical and the variety and quantity of adducts formed, DNA repair rates, the proliferation rate of the target cells needed to "fix" the adducts into mutations, and the mutagenic potency of those fixed adducts in critical genes. Highly sensitive methods are also needed to correlate DNA adducts in target organs with carcinogenic response to firmly establish both dose-response concordance and temporal sequence.

Human relevance

The basic biology of DNA adduct formation and subsequent perturbation of gene structure and function is believed to be similar between test animals and humans. Thus, a mutagenic MOA is considered a biologically relevant MOA in humans. Qualitatively, there is considerable evidence in test animal and mammalian cells to support the relevance of a mutagenic MOA for AA in humans. Quantitative data are only available in one in vitro assay measuring mutagenicity directly in human cells (human bronchial epithelial cells) (Besaratinia and Pfeifer, 2004).

Conclusion

There is evidence from a variety of studies of glycidamide's mutagenicity in mammalian (including human) somatic cells that supports a mutagenic MOA for AA that would be operational in both test animals and humans. The mutagenicity of AA is indicated through its ability to induce gene mutations and chromosomal aberrations in somatic and germ cells of rodents in vivo and cultured cells in vitro and cell transformation in mouse cell lines, and its ability to form adducts with protamines in germ cells. The mutagenicity of GA is characterized by its induction of gene mutations in bacteria, unscheduled DNA synthesis in a variety of test systems, and ability to form DNA adducts. The available data indicate that the major mutagenic effects of AA are clastogenic, which may involve covalent modifications of proteins by AA and GA, and direct alkylation of DNA by GA (Doerge et al., 2005a; Besaratinia and Pfeifer, 2004; Schmid et al., 1999; Dearfield et al., 1995; Segerbäck et al., 1995; Moore et al., 1987,). Support for the genetic damage in somatic and germ cells of mice treated with AA being dependent upon metabolism of the parent compound to GA by CYP2E1 comes from studies in CYP2E1-null male mice (Ghanayem et al., 2005a,b), and the similar mutation spectra that AA and GA produced in the Big Blue male and female mice (Manjanatha et al., 2006).

There is some support for the temporal sequence in that mutagenic events (e.g., GA-DNA adducts) have been observed in target tissues, and these occur soon after exposure to AA, although most of these studies are at doses of AA higher than those of the bioassays. Additional data are needed to further demonstrate the temporal sequence of events between the formation of DNA adducts, the development of mutations, and the formation of tumors; and to establish dose-response concordance to firmly establish that a GA-DNA adduct is an obligate precursor event in tumor formation. Additional data are also needed to resolve why only hormonally responsive tissues were observed to have increased tumors in the Friedman et al. (1995) chronic rat bioassay, whereas GA-DNA adducts have been observed in a much wider array of tissues.

4.8.3.2. *Alternative Mode of Action—Disruption of Hormone Levels or Signaling*

An alternative MOA via disruption of hormone levels or hormone signaling has also been suggested for the acrylamide-induced tumors in hormonally sensitive tissues (mammary gland and thyroid) or in a tissue adjacent to hormonally sensitive tissue (tunica vaginalis, the scrotal sac mesothelium) (Shipp et al., 2006; Environ, 2002; KS Crump Group, Inc., 1999a,b). Although this is a possible MOA, at present there are only limited or absent supporting data.

The hypothesized sequence of events for the induction of tunica vaginalis and mammary gland tumors is as follows: dopamine agonist activities promote age-related hormonal changes that, in turn, stimulate sustained cell proliferation in the tunica vaginalis and mammary gland, leading to progression to mesothelioma and fibroadenoma, respectively. For the thyroid tumors the events are alteration of a signal transduction pathway, leading to persistent stimulation of cell

proliferation in thyroid follicular cells and eventual progression to follicular cell adenomas (Shipp et al., 2006; Environ, 2002; KS Crump Group, Inc., 1999a,b).

In support of the hypothesis for dopamine agonist activity (at the D2 dopamine receptor), AA has been shown to decrease circulating levels of prolactin in male F344 rats. The relevance of the carcinogenicity of chemicals that induce Leydig cell tumors in rats via dopamine agonist activity is an issue of scientific debate, because human Leydig cells (as well as Leydig cells in other animal species, except male rats) do not decrease their luteinizing hormone (LH) receptors in response to decreased prolactin. Because of the evidence for dopamine agonist activity of AA in male rats and evidence to suggest that the malignancy of the tunica vaginalis mesotheliomas in F344 rats was linked to the extent of Leydig cell neoplasia, it has been proposed that the mesotheliomas may not be relevant to humans. Additional supporting evidence would include demonstration of a lack of mesotheliomas in other animal species chronically exposed to AA; however, these data are not currently available.

In contrast to male rats, there is little empirical evidence to support this alternative MOA in female rats. Marked changes in circulating levels of prolactin have not been observed in female F344 rats exposed to AA for up to 28 days. There is also no direct evidence that AA displays D1 dopamine agonist activity in female rats, which could enhance ovarian progesterone secretion and subsequently stimulate cell proliferation in the stromal/fibroblast cells of the rat mammary gland.

With respect to thyroid tumors, short-term (2–7 days) exposure of female F344 rats to AA caused follicular cell morphometric changes (decreased colloid area and increased cell height) without significantly changing circulating levels of thyroid hormones or thyroid stimulating hormone (TSH). Other studies indicated that AA doses as high as 25 mg/kg-day for up to 28 days did not induce consistent, biologically significant changes in thyroid hormones or TSH levels. Thus, current data do not support a MOA by which AA alters thyroid hormone homeostasis. Direct evidence that AA may cause follicular cell proliferation by an alternative MOA involving stimulation of a cAMP cascade (without changes in TSH levels) is not currently available. TSH-induced mitogenic activities are mediated largely by cAMP, which in turn may activate protein kinase (PKA)-dependent and independent processes.

Tunica Vaginalis Mesotheliomas

Description and identification of key events

The events in the proposed hormonal pathway MOA for AA-induced formation of tunica vaginalis mesotheliomas is as follows: (1) AA increases dopamine levels or functions as a dopamine receptor agonist; (2) a dopamine agonist-induced decrease in prolactin levels then down-regulates LH receptors on rat Leydig cell membranes, leading to decreases in testosterone production; (3) there is a subsequent compensatory increase in serum LH to maintain testosterone at normal levels (Clegg et al., 1997; Cook et al., 1999; Prentice and Meikle, 1995);

and (4) the increase in LH stimulates sustained cell proliferation in the tunica vaginalis with eventual progression to mesotheliomas.

Experimental support for the hormonal pathway MOA in male rats

Strength, consistency, and specificity of association

Serum prolactin levels have been observed to decrease in AA-exposed male rats, but not females (Friedman et al., 1999b; Khan et al., 1999; Ali et al., 1983; Uphouse et al., 1982). These studies were instigated because it is well known that dopamine plays a predominant role in hypothalamic suppression of pituitary secretion of prolactin (Yamada et al., 1995; Neuman, 1991), and AA has been demonstrated to increase striatal dopamine receptors in rats (Agrawal, 1981a,b; Bondy et al., 1981; Uphouse and Russell, 1981). The results suggest that AA, in inhibiting prolactin secretion by the pituitary, may act as a dopamine agonist, at least in male rats.

In an unpublished study, male and female F344 rats (approximately 8 weeks of age at beginning of exposure) were exposed to AA in drinking water providing doses of 0, 4.1, 12, 19, or 25 mg/kg-day for up to 28 days (Friedman et al., 1999b). Serum prolactin levels in males were decreased after 14 days of treatment: percentage decreases (compared with controls) were 17, 36, 81, and 87% for the 4.1 through 25 mg/kg-day groups, respectively. The values at the two highest exposure levels were statistically significantly different from control values. Percentage decreases in the mean values for the 4.1 through 25 mg/kg-day males at 28 days were 0, 5, 44, and 33%, but none of the mean values were statistically significantly different from control values at 28 days.

Circulating levels of prolactin in female F344 rats showed no consistent dose-related changes, compared with controls, after 14 or 28 days of AA exposure (Friedman et al., 1999b, unpublished) or, in another published study with 28-day-old females, after gavage administration of 2 or 15 mg/kg-day AA for 2 or 7 days (Khan et al., 1999).

In earlier studies, serum prolactin levels were shown to be decreased in male F344 inbred rats 24 hours after oral administration of 100 mg/kg AA (Uphouse et al., 1982). The decrease in prolactin levels was statistically significant in rats that were not handled for 3 minutes a day for 7 days before AA administration but was not significant in rats that received this handling pretreatment protocol. Serum prolactin levels were also decreased in male F344 rats (8 to 10 weeks of age at the start of the study) following 20 daily i.p. injections of 10 or 20 mg/kg AA (Ali et al., 1983).

The available animal studies do not support a consistent AA effect on dopamine levels or receptors in various brain regions.

Acrylamide has been shown to produce changes in the dopaminergic system in some short-term oral exposures to AA (5, 10, or 20 mg/kg-day, 10 times during 14 days, or single

doses of 50, 100, or 200 mg/kg) with increases in dopamine receptors (assayed as increased binding of [^{3}H]-spiroperidol) in the striatal brain region of young (6-week-old) Sprague-Dawley or F344 male rats (Agrawal, 1981a,b; Bondy et al., 1981; Uphouse and Russell, 1981). In contrast, 24 hours post dosing, rats orally exposed to 10 mg/kg AA for 10 consecutive days had a decreased response to apomorphine (a dopamine receptor agonist) compared with nonexposed controls (Bondy et al., 1981). Bondy et al. (1981) noted that similar, apparently paradoxical, results were also reported for another neurotoxicant, haloperidol. It was proposed that AA might induce damage to the dopaminergic pathways such that apomorphine would not elicit a response even in the presence of an excess number of dopamine receptors.

Oral exposure of pregnant F344 rats to 20 mg/kg-day on GDs 7–16 was also reported to induce decreased dopamine receptors in offspring assayed 2 weeks after birth but not at 3 weeks (Agrawal and Squibb, 1981). Repeated oral exposure to AA (10 times during 14 days) also caused an increase in other neurotransmitter receptors: acetylcholine striatal receptors (at 5, 10, or 20 mg/kg-day), GABA cerebellar receptors (at 20 mg/kg-day), glycine medullar receptors (at 20 mg/kg-day), and serotonin frontal cortical receptors (at 20 mg/kg-day) (Bondy et al., 1981). The biological and mechanistic significance of these findings of effects of AA on levels of neurotransmitter receptors remains uncertain.

Exposure to AA also has been reported to cause changes in levels of dopamine in some regions of the rat brain, but changes have been inconsistently observed across studies (Ali, 1983; Ali et al., 1983; Rafales et al., 1983; Agrawal et al., 1981a). Mean striatal dopamine concentrations were higher than control values by about 22–31% in 6-week-old male Sprague-Dawley rats, 24 hours after administration of single i.p. injections of 50, 100, or 150 mg/kg, but the difference was not statistically significant (Agrawal et al., 1981a). Male 10-week-old F344 rats given single i.p. injections of 50 or 100 mg/kg AA showed no significant change in levels of dopamine in the frontal cortex or striatum; in contrast, following 10 consecutive injections of 10 mg/kg-day, levels of dopamine and a metabolite, dihydroxyphenylacetic acid, were significantly decreased in the frontal cortex but not changed in the striatum or hypothalamus (Ali et al., 1983).

In another study, 8- to 10-week-old male F344 rats were administered 20 consecutive i.p. injections of 10 or 20 mg/kg AA, resulting in significantly increased dopamine levels in the caudate nucleus compared with controls; however, levels of dopamine in the frontal cortex or the hypothalamus were not significantly affected (Ali, 1983). In male Long-Evans rats exposed to 100 mM AA in drinking water for 6 weeks, there were no changes in concentrations of dopamine and its metabolites, dihydroxyphenylacetic acid and homovanillic acid in the nucleus accumbens, septal area, corpus striatum, or thalamus compared with controls (Rafales et al., 1983).

Acrylamide-exposed rats showed increased psychomotor stimulation from amphetamine, compared with controls, that was associated with short-term elevations of 5-hydroxyindoleacetic

acid in several brain regions and a lesser elevation of dopamine in the nucleus accumbens but not in the septal area, corpus striatum, or thalamus (Rafales et al., 1983).

Dose-response concordance

Only a few studies are available to support a dose-response relationship of acrylamide on circulating prolactin levels via an effect on the dopaminergic system in male rats and influence on circulating levels of hormones. Serum testosterone levels in male F344 rats were statistically significantly decreased following 28 days of exposure to AA in drinking water at dose levels of 19 and 25 mg/kg-day but not at lower dose levels (Friedman et al., 1999b). For groups exposed to 0, 1.4, 4.1, 12, 19, or 25 mg/kg-day, respective mean testosterone values (± SD, in units of ng/mL) were 1.1 ± 0.7, 2.1 ± 1.1, 2.2 ± 1.4, 0.5 ± 0.3, 0.3 ± 0.4, or 0.1 ± 0.1. Decreased serum levels of testosterone have also been observed in male F344 rats exposed to 20 daily i.p. injections of 10 or 20 mg/kg AA (Ali et al., 1983).

Temporal relationship

If acrylamide-induced decreases in circulating levels of prolactin actually lead to physical or hormonal changes in Leydig cell tumors, such changes may subsequently stimulate the development of spontaneously initiated or AA-initiated mesothelial cells in the scrotal sac (i.e., tunica vaginalis) into mesotheliomas. These types of actions have been proposed by Tanigawa et al. (1987) to explain the higher spontaneous incidences of genital serosal mesotheliomas in male F344 rats compared with other rat strains, such as Sprague-Dawley, that do not show high spontaneous incidences of Leydig cell tumors. Older male F344 rats, surviving between about 80 and 120 weeks, are well documented to display spontaneous Leydig cell tumors at high (80–100%) incidences, and spontaneous mesotheliomas, predominantly in the genital serosa, at low (3–4%) incidences (Tanigawa et al., 1987; Solleveld et al., 1984; Goodman et al., 1979). The male F344 rats in the AA bioassays were not an exception to this occurrence. The appearance of Leydig cell tumors in aging F344 rats shows a temporal relationship with age-related changes in the synthesis or secretion of gonadal and adrenohypophyseal hormones (Amador et al., 1985; Turek and Desjardins, 1979). In addition, persistently elevated levels of prolactin (produced by transplantation of anterior pituitaries from adult females or by treatment with diethylstilbestrol) have been shown to inhibit the development of spontaneous Leydig cell tumors in aging male F344 rats (Bartke et al., 1985).

Biological plausibility and coherence

The mechanism by which AA may increase dopamine receptors or other neurotransmitter receptors is unknown. One hypothesis that has been proposed involves AA down-regulation of the microtubular system and disintegration of neurofilaments followed by blockage of intracellular transport of receptors and their subsequent accumulation (Ho et al., 2002). This hypothesis was based on observations that exposure of cultured brain neurons from chicken embryos to 10 mM AA induced increased levels of $GABA_A$ receptors, decreased levels of tubulin proteins, and decreased numbers of microtubules and neurofilaments in the neuron cell body. Similar experiments examining AA effects on dopamine receptors and associated changes in tubulin protein levels and numbers of neurofilaments in cultured brain neurons are not available.

Human relevance

A reevaluation of the most recent of the two AA drinking water cancer bioassays for tumors in reproductive tissues (Iatropoulos et al., 1998) in male rats originally assessed as having tunica vaginalis mesotheliomas (Friedman et al., 1995) provides some support for the proposal that acrylamide-induced mesotheliomas in F344 rats may not be relevant to humans (Shipp et al., 2006). In the reevaluation, all rats diagnosed with malignant mesothelioma were assessed as having 75% or 100% of the testes occupied by Leydig cell neoplasia, whereas rats with mesothelial hyperplasia or benign mesothelioma were assessed as having 50% or less of the testes occupied by Leydig cell neoplasia (Iatropoulos et al., 1998).[5] These observations suggest that the extent of Leydig cell neoplasia and the development of malignant mesotheliomas in these rats may have been linked.

Most of the possible mechanisms proposed for the chemical induction of Leydig cell hyperplasia and adenomas involve elevation of serum LH and/or a change in Leydig cell responsiveness to LH as the key event (Cook et al., 1999; Clegg et al., 1997). Several other mechanisms involving elevations of LH or other disruptions of the hypothalamic-pituitary-testis axis could possibly result in an adverse human response (Cook et al., 1999; Clegg et al., 1997).

Conclusion

In summary, there is some evidence to suggest that acrylamide can promote or enhance age-related decreases in serum prolactin and testosterone in older male F344 rats (Friedman et al., 1999b; Khan et al., 1999; Ali et al., 1983; Uphouse et al., 1982) and that this enhancement may lead to the development of tunica vaginalis mesotheliomas due to larger adjacent Leydig

[5] In another study of the tunica vaginalis testis mesotheliomas reported in Friedman et al. (1995), it was concluded, based on light and electron microscopy, that tumors in the acrylamide-exposed rats did not differ

cell tumors (Iatropoulos et al., 1998). Because the response to decreased circulating levels of prolactin in this sequence of events may be specific to male F344 rats (and not occur in humans or other animal species), AA-induced tunica vaginalis mesotheliomas in older F344 rats may not be relevant to humans. Additional support for this proposal, such as the lack of mesotheliomas in other rat strains or other animal species exposed chronically to AA, however, is not available. In conclusion, a hormone-mediated MOA for the observed mesotheliomas is possible but data are lacking to link key events with tumor formation.

Mammary Gland Fibroadenomas

Description and identification of key events

The events in the proposed hormonal pathway MOA for acrylamide induction of mammary gland fibroadenomas in female F344 rats are as follows: an age-related decrease in dopamine, leading to increased secretion of prolactin by the pituitary, followed by increased and sustained release of progesterone from the ovary, leading to a sustained cell proliferative response in stromal/fibroblast cells of the mammary gland and eventual progression to fibroadenomas (Shipp et al., 2006).

Experimental support for the hormonal pathway MOA in female rats

Strength, consistency, specificity of association

The hypothesis proposes that AA acts as a dopamine agonist on D1 dopamine receptors in the ovary to further enhance secretion of progesterone in aging rats. Direct in vitro or in vivo evidence showing that AA interacts with D1 dopamine receptors and subsequently enhances progesterone secretion in female rats is not currently available.

Dose-response concordance

Circulating levels of prolactin in female F344 rats showed no consistent, dose-related changes, compared with controls, after 14 or 28 days of AA exposure (Friedman et al., 1999b, unpublished) or, in another published study with 28-day-old females, after gavage administration of 2 or 15 mg/kg-day AA for 2 or 7 days (Khan et al., 1999).

Temporal relationship

No in vitro or in vivo evidence were available to support a temporal relationship between AA interaction with D1 dopamine receptors, subsequent enhanced progesterone secretion in female rats, and development of mammary tumors.

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morphologically from tumors in the control rats (Damjanov and Friedman, 1998). This study, however, did not specifically compare morphological features of Leydig cell tumors between acrylamide-exposed and control rats.

Biological plausibility and coherence

Although the proposed hormonal pathway MOA for AA-induced mammary fibroadenomas in female F344 rats is possible, there are no empirical data directly linking AA to an enhancement of any particular process in the proposed cascade of events (e.g., AA acting as an agonist for D1 dopamine receptors, leading to enhanced progesterone secretion from rat, but not human, ovary cells.

Human relevance

It has been proposed (Shipp et al., 2006) that the increased incidences of mammary gland fibroadenomas in the AA bioassays are not relevant to humans because fibroadenomas in women are associated with either an increase in estrogen or a decrease in progesterone or both (Smith, 1991) and not an increase in progesterone as in aging female rats; because increased prolactin does not lead to increased progesterone secretion in humans or other primates (Neumann, 1991); and because the dopamine agonist, SKF-38393, acting at D1 dopamine receptors in rat ovary cells, stimulates progesterone secretion (Mori et al., 1994) but does not appear to stimulate progesterone secretion in human ovary cells (Mayerhofer et al., 1999).

Conclusion

Although empirical support is inadequate or lacking for this proposed MOA, it is a possible MOA, assuming that AA-induced fibroadenomas in female F344 rats are produced by AA enhancement of the normal age-related mode of development of spontaneous fibroadenomas. However, the possible human relevance of AA-induced mammary gland fibroadenomas cannot be ruled out with confidence at this time, because there is no empirical evidence directly linking AA to an enhancement of any particular process in the proposed cascade of events (e.g., AA acting as an agonist for D1 dopamine receptors, leading to enhanced progesterone secretion from rat, but not human, ovary cells).

Thyroid Tumors

Description and identification of key events

The events in the proposed hormonal pathway MOA for AA-induced formation of thyroid tumors in male and female F344 rats are alteration of a signal transduction pathway, leading to persistent stimulation of cell proliferation in thyroid follicular cells and eventual progression to follicular cell adenomas (Environ, 2002; KS Crump Group, Inc., 1999a,b).

Experimental support for the hormonal pathway MOA in male and female rats

Strength, consistency, specificity of association

Both of the available chronic exposure studies reported statistically significant increased incidences of thyroid follicular cell adenomas, or combined adenomas and carcinomas, at the highest dose levels of 2–3 mg/kg-day (Friedman et al., 1995; Johnson et al., 1986). Chemicals that alter thyroid hormone homeostasis by interfering with synthesis or secretion of triiodothyronine (T3) or thyroxin (T4) or by increasing T3 or T4 metabolism can lead to compensatory release of TSH from the pituitary, which, if sustained, may induce thyroid follicular cell hyperplasia that may progress to neoplasia (U.S. EPA, 1998c).

There is no clear evidence to support the hypothesis that AA induces sustained follicular cell proliferation by altering thyroid hormone homeostasis. Exposure of female F344 rats to 2 or 15 mg/kg-day for 2 or 7 days induced follicular cell morphometric changes (decreased colloid area and increased cell height) without significantly changing circulating levels of T4 or TSH (Khan et al., 1999). In female F344 rats exposed to 2 or 15 mg/kg-day AA for 2 or 7 days, no statistically significant changes, compared with controls, were found in plasma levels of T4, TSH, or prolactin, in pituitary levels of TSH or prolactin, or in body, pituitary, or adrenal weights, whereas thyroid gland morphometry showed statistically significant decreased colloid area (56–57% decrease compared with control) and increased follicular cell height (18–22% increase compared with control) (Khan et al., 1999).

In an unpublished study, blood levels of T3, T4, or TSH were evaluated in male or female F344 rats exposed to AA in drinking water for 14 or 28 days at dose levels ranging from about 1 to 25 mg/kg-day (Table 4-34) (Friedman et al., 1999b). A significant decrease in T3 and T4 in high dose males is reported at 28 days, but T4 in high dose males increased at 14 days, and overall there is inadequate support for a consistent, significant change in blood levels of T3, T4, or TSH.

Table 4-34. Circulating thyroid hormone levels in F344 rats following exposure to acrylamide in drinking water for 14 or 28 days

Dose (mg/kg-day)		T3 (ng/dL)		T4 (ng/dL)		TSH (ng/mL)	
Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
14 days							
0	0	85.2 ± 14.4	78.8 ± 8.4	3.5 ± 0.5	2.8 ± 0.6	2.7 ± 1.1	2.1 ± 0.6
1.4	1.3	75.2 ± 16.0	77.5 ± 6.6	3.3 ± 0.3	2.8 ± 0.3	3.7 ± 1.7	2.2 ± 0.4
4.1	4.3	80.3 ± 7.7	91.0 ± 13	3.8 ± 0.3	3.4 ± 0.5[a]	3.1 ± 1.3	1.8 ± 0.3
12	9.0	81.6 ± 10.2	81.6 ± 8.7	3.6 ± 0.3	3.2 ± 0.5	2.9 ± 1.4	1.8 ± 0.4
19	19	92 ± 20.2	101.9 ± 10.3[a]	4.0 ± 0.5	3.2 ± 0.3	3.7 ± 1.0	2.1 ± 0.9
25	24	91.9 ± 13.2	89 ± 15	4.1 ± 0.4[a]	3.0 ± 0.8	2.8 ± 0.8	2.8 ± 0.2[a]
28 days							
0	0	90.8 ± 13.3	78.9 ± 13.5	3.9 ± 0.6	2.5 ± 0.7	2.0 ± 0.7	1.5 ± 0.4
1.4	1.3	90.6 ± 13.8	75.5 ± 13.0	4.0 ± 0.5	2.4 ± 0.6	2.3 ± 1.2	1.8 ± 0.6
4.1	4.3	82.0 ± 13.1	79.6 ± 8.2	3.9 ± 0.5	2.5 ± 0.4	2.1 ± 0.9	1.6 ± 0.2
12	9.0	80.3 ± 11.5	84.9 ± 4.4	3.7 ± 0.4	2.7 ± 0.3	2.1 ± 0.4	1.7 ± 0.4
19	19	71.2 ± 10.3[a]	81.6 ± 7.9	3.3 ± 0.5	2.7 ± 0.3	1.9 ± 0.4	1.9 ± 0.9
25	24	61.4 ± 32.4[a]	65.2 ± 23.6	2.6 ± 1.0[a]	2.4 ± 0.6	2.8 ± 1.2	1.6 ± 0.4

[a]Statistically significantly different ($p < 0.01$) from control by an unspecified statistical test with unspecified number. Available report does not specify if values are means ± SEM or SD.

Source: Friedman et al. (1999b).

In another unpublished study, no changes in plasma TSH levels were found in male Sprague-Dawley rats exposed to 2 or 15 mg/kg-day AA for up to 28 days by an unspecified route of administration, and evidence for a sustained statistically significant increase in DNA synthesis in the thyroid of exposed rats, compared with control rats, was not found (Klaunig, 2000, as cited in Environ, 2002). DNA synthesis in the thyroid was assayed as "BrdU incorporation and proliferating cell nuclear antigen (PCNA) expression", but further methodological details were not specified in the available report of this study. The results (as cited in Environ, 2002) are shown in Table 4-35. The quality of these data, however, is poor due to lack of information on methodological details and the fact that the data were neither published nor peer reviewed.

Klaunig and Kamendulis (2005) later reported that exposure of F344 rats to AA (0, 2, or 15 mg/kg-day) for 7, 14, or 28 days increased DNA synthesis in the target tissues (thyroid, testicular mesothelium, adrenal medulla) at all doses and time points examined but not in nontarget tissue (liver). They also reported increase in DNA damage in the target tissues but not in nontarget tissue (liver), which supports a mutagenic MOA.

Table 4-35. Plasma TSH, BrdU incorporation in thyroid, and PCNA expression in thyroid in male Sprague-Dawley rats exposed to acrylamide by an unspecified route for up to 28 days

Dose (mg/kg-day)	Day	TSH (ng/mL)	BrdU (units not reported)	PCNA (units not reported)
0	7	2.92 (0.90)	0.47 (0.11)	0.20 (0.07)
2		3.28 (1.12)	4.09 (1.04)[a]	2.64 (1.39)[a]
15		4.09 (2.16)	1.92 (0.55)	2.29 (0.91)[a]
0	14	5.02 (2.44)	2.31 (0.18)	0.11 (0.05)
2		4.41(1.89)	2.79 (1.69)	0.06 (0.04)
15		4.72(2.10)	5.60 (1.73)	2.24 (0.59)[a]
0	28	5.29 (2.44)	2.31 (0.18)	0.04 (0.02)
2		3.96 (1.64)	3.13 (1.53)	1.21 (0.89)
15		4.90 (2.55)	5.60 (1.73)	3.13 (1.77)

[a]Reported as statistically significant ($p < 0.05$), by ANOVA followed by Fisher's Least Significant Difference (LSD); values in parentheses were not specified. Methodological details concerning thyroid BrdU incorporation and PCNA expression were not provided in Environ (2002).

Source: Klaunig (2000) as cited in Environ (2002).

Dose-response concordance

No data are available to support dose-response concordance for the proposed effect on circulating thyroid hormone levels.

Temporal relationship

No data are available to support the temporal relationship between AA exposure, hormonal disruption, and formation of thyroid tumors to support this proposed MOA.

Biological plausibility and coherence

This hormonal pathway MOA is biologically plausible, and the occurrence of altered thyroid hormone homeostasis leading to thyroid follicular cell hyperplasia with potential progression to neoplasia is well established (U.S. EPA, 1998c).

Human relevance

If AA disruption of thyroid hormone homeostasis is supported by future studies, this proposed MOA for thyroid tumorigenicity could call into question the human relevance of the tumors.

Conclusion

Although this proposed MOA is possible for thyroid tumorigenicity in male and female rats (and possibly humans), there is little empirical support for AA alteration of thyroid hormone homeostasis.

4.8.3.3. *Conclusion About the Mode of Action*

The available data indicate that the most plausible MOA for the carcinogenicity of AA is a mutagenic MOA based upon the numerous and consistent study results on the mutagenicity of AA (or its GA metabolite) in both germ and somatic mammalian cells that support the events, dose-concordance, and temporal relationship of a mutagenic MOA. There is relatively little support for a hormonal pathway MOA for the tumor types observed in the animal studies, although this is a possible MOA and warrants further evaluation. It is also possible that there is a mixed MOA, i.e., an increased mutagenic burden in hormonally-sensitive tissues with or without disruption of the hormonal pathways.

4.9. SUSCEPTIBLE POPULATIONS

4.9.1. Possible Childhood Susceptibility

Neurotoxicity

No human data are available regarding age-related differences in susceptibility to acrylamide-induced neurotoxicity. Animal studies provide conflicting results. Some reports indicate that young animals may be less susceptible than older ones (Kaplan et al., 1973; Fullerton and Barnes, 1966), whereas other reports present evidence that young animals may be more sensitive (Ko et al., 1999; Suzuki and Pfaff, 1973).

Fullerton and Barnes (1966) administered 100 mg/kg AA orally to groups of 5-, 8-, 26-, and 52-week-old albino rats at weekly intervals and noted severe signs of peripheral neuropathy in the oldest group after three treatments. The 26-week-old rats were severely affected after four treatments, while rats whose treatment started at 5 weeks of age only showed "mild" clinical signs of peripheral neuropathy after 4 weeks of treatment.

Kaplan et al. (1973) injected 50 mg/kg-day AA i.p. to rats ranging in age from 5 to 14 weeks. Impaired rotarod performance appeared earlier in the older rats, but the younger rats recovered more slowly following the cessation of treatment.

Suzuki and Pfaff (1973) administered 50 mg/kg of AA to 1-day-old and adult rats three times a week for up to 18 injections. Signs of hindlimb weakness appeared several days earlier in the young pups, and degenerative histopathologic changes in peripheral nerves were more prominent in the pups than the adults.

Recently, Ko et al. (1999) demonstrated that mouse weanlings may be more susceptible to the adverse neurological effects of AA than young adult mice. Groups of male ICR mice were exposed to AA in the drinking water at concentrations of 0 or 400 ppm and observed for clinical signs, rotarod performance, peripheral nerve growth and function, and histopathologic evidence of peripheral neuropathy. Calculated AA doses were 91.8 ± 20.6 mg/kg-day for the 3-week-old mice and 90.8 ± 10.9 mg/kg-day for the 8-week-old mice. The younger (3-week-old) mice

exhibited earlier onset (7.1 ± 1.1 days vs. 15.6 ± 4.0 days in 8-week-old mice) and more rapid progression of AA-induced neuropathy.

Carcinogenicity

With respect to carcinogenicity, EPA has concluded by a weight-of-evidence evaluation that AA is carcinogenic by a mutagenic MOA. According to the *Supplemental Guidance for Assessing Susceptibility from Early Life Exposure to Carcinogens* (U.S. EPA, 2005b), those exposed to carcinogens with a mutagenic MOA are assumed to have increased early life susceptibility. Data for AA, however, are not sufficient to develop separate risk estimates for childhood exposure, thus the oral slope factor and inhalation unit risk (see Section 5.3.5) do not reflect presumed early life susceptibility for this chemical, and age-dependent adjustment factors (ADAFs) should be applied to this slope factor when assessing cancer risks for less than 16-year-old subpopulations or for lifetime exposures that begin in less than 2-year-olds. Example evaluations of cancer risks based on age at exposure are given in Section 6 of the *Supplemental Guidance*.

Aside from the assumption that early life stages are more susceptible to mutagens, there are limited data on early-life susceptibility to AA-induced carcinogenicity. Gamboa et al. (2003) measured DNA adduct formation in selected tissues of adult and whole body DNA of 3-day-old neonatal mice treated with AA and GA. In adult mice, DNA adduct formation was observed in liver, lung, and kidney with levels of N7-GA-Gua around 2000 adducts/10^8 nucleotides and N3-GA-Ade around 20 adducts/10^8 nucleotides. Adduct levels were modestly higher in adult mice dosed with GA as opposed to AA; however, treatment of neonatal mice with GA produced five- to seven-fold higher whole body DNA adduct levels than with AA. The authors suggest that this is due to lower oxidative enzyme activity in newborn mice. DNA adduct formation from AA treatment in adult mice showed a supralinear dose-response relationship, consistent with saturation of oxidative metabolism at higher doses.

Increased incidences of tumors in hormonally responsive tissues (thyroid gland, mammary gland, and tunica vaginalis mesothelium) have been noted in rats chronically exposed to AA in the diet (Friedman et al., 1995; Johnson et al., 1986). Since AA induced disruption of hormonal pathways or homeostasis is a possible MOA, additional studies are needed to evaluate this MOA and whether there is an increased susceptibility to AA induced hormonal disruption during early developmental stages.

As discussed in Section 3.3, CYP2E1 catalyzes the initial oxidation of AA to the epoxide derivative, GA, and there are age-related increases in CYP2E1 expression in humans as reported by Johnsrud et al. (2003). CYP2E1 was detected as early as the second trimester (0.35 pmol/mg microsomal protein), increasing approximately fivefold from neonatal levels (median = 8.8 pmol/mg microsomal protein) to post-90-day levels (41.4 pmol/mg microsomal protein). Levels

in older infants (>90 days old), children, and young adults up to 18 years old were relatively similar. A four-fold or greater intersubject variation was observed among samples from each age group, with the greatest variation, 80-fold, seen among neonatal samples. These results suggest that infants less than 90 days old would have decreased clearance of CYP2E1 substrates compared with older infants, children, and adults. However, the delivery rate of the substrate relative to the value of the Michaelis-Menten constant (Km) for CYP2E1 is an important determinant of the total amount metabolized (or parent compound cleared) (Lipscomb, 2004; Lipscomb et al., 2003), such that the higher the substrate concentration is relative to Km, the more profound the influence of enzyme level and differences in the enzyme's maximum velocity (V_{max}) on total clearance for a saturable enzyme like CYP2E1. There is no reason to suspect that the Km value of CYP2E1 in <90-day-old infants would be any different than the Km for CYP2E1 in older infants, so that a difference in susceptibility in neonates would mostly depend on levels of CYP2E1 and delivery rates of AA. There is therefore a research need to develop quantitative estimates of differences in clearance due to different levels of CYP2E1 for less than 90-day-old infants at high or low levels of AA exposure.

4.9.2. Possible Gender Differences

No data are available regarding gender-related differences in sensitivity to acrylamide in humans.

Acrylamide-induced adverse reproductive effects (male-mediated implantation losses and reduced number of fetuses, testicular atrophy) have been demonstrated in male rodents at dose levels that do not affect female reproductive performance (see Sections 4.3.1 and 4.5.1; see also Table 4-31). Part of the gender difference may be due to the AA or GA alkylation of sperm protamines late during spermiogenesis and resultant genetic damage (Perrault, 2003; Adler et al., 2000; Generoso et al., 1996; Sega et al., 1989; Sublet et al., 1989). Other modes may involve neurotoxic actions impairing copulatory behavior (Zenick et al., 1986) and sperm motility (Tyl et al., 2000b; Sublet et al., 1989), both of which are key determinants of male reproductive performance (see Section 4.3 for a more detailed discussion).

Acrylamide-induced neurological effects have been observed in both male and female rats at similar dose levels. Light microscopic examination of peripheral nervous tissue revealed evidence of distal axonal neuropathy in both sexes at doses of 2–3 mg/kg-day for up to 2 years (Friedman et al., 1995; Johnson et al., 1986, 1985; Burek et al., 1980). Male and female rats also exhibited similar clinical signs of neurotoxicity following repeated exposure to doses of 20 or 50 mg/kg-day (Burek et al., 1980; Fullerton and Barnes, 1966).

Chronic exposure of F344 rats to AA in drinking water induced increased incidences of thyroid follicular cell tumors (adenomas and carcinomas combined) in males and females, scrotal sac mesotheliomas in males, and mammary gland fibroadenomas in females (Friedman et al.,

1995; Johnson et al., 1986). These results show that both male and female rats are susceptible to AA-induced carcinogenic effects.

4.9.3. Other

No data are available regarding the effects of acrylamide on other potentially susceptible populations.

Genetic polymorphisms in the AA metabolizing P-450 enzyme CYP2E1 have been identified in humans (Hanioka et al., 2003) and studied for the impact of a susceptible population to alcohol toxicity (Verlaan et al., 2004) and to acrylonitrile, a chemical with similar metabolism to AA (Thier et al., 2002). The polymorphisms result in differences in the V_{max} of the enzyme (Hanioka et al., 2003) that could result in greater or lesser production of the GA metabolite and make some people more or less sensitive to adverse effects. The epidemiology evidence is not strong. There is some suggestive (i.e., not statistically significant) evidence that polymorphisms in CYP2E1 might confer a differential risk to alcohol-induced chronic pancreatitis (Verlaan et al., 2004) and that a slower CYP2E1-mediated metabolism of acrylonitrile might result in higher acrylonitrile-hemoglobin adducts (and lower N-(cyanoethyl)valine adducts from the metabolite) (Thier et al., 2002). As discussed for childhood susceptibility, however, the delivery rate of the substrate relative to the values of Km and V_{max} for CYP2E1 is an important determinant of the total amount metabolized (or parent compound cleared) (Lipscomb, 2004; Lipscomb et al., 2003). There is currently no quantitative estimate of differences in parent AA or GA tissue or blood levels that might result from CYP2E1 polymorphisms at high or low levels of AA exposure. It is also noted that, since both the parent AA and the metabolite GA have adverse effects, different catalytic activities of CYP2E1 may result in different spectra of adverse effects.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE

5.1.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

As discussed in Section 4.5.1, there are only a few reports of noncancer health effects in humans associated with oral exposure to AA, but occupational experiences involving inhalation and dermal exposures firmly establish neurological impairment as a potential human health hazard from acute and chronic exposure to AA. In contrast, the oral toxicity database for laboratory animals is robust and contains (as shown in Table 4-31): two 2-year carcinogenicity/toxicology drinking water studies in F344 rats; two two-generation reproductive toxicity studies, one in F344 rats and one in CD-1 mice; several single-generation reproductive toxicity studies involving prolonged prebreeding drinking water exposure of Long-Evans rats and ddY mice; and several developmental toxicity studies involving gestational exposure of Sprague-Dawley and Wistar rats and CD-1 mice.

Acrylamide induces transmissible genetic damage in male germ cells of mice in the form of reciprocal translocations and gene mutations. Such effects can lead to genetic disorders and infertility in subsequent generations. However, heritable adverse effects were not observed for the endpoints measured in the two-generation studies at the lower doses tested in these studies (Tyl et al., 2000a; Chapin et al., 1995). Further evaluation of the linearity or nonlinearity of the dose-response curve for these adverse heritable risks is a critical database need (see discussion on database uncertainty in Section 5.1.3).

The most sensitive effects noted in animals are degenerative peripheral nerve changes and male-mediated implantation losses (i.e., male-mediated dominant lethal mutations). The lowest observed exposure levels associated with peripheral nerve changes are (with NOAELs noted in parentheses): 1 mg/kg-day (0.2 mg/kg-day NOAEL) in male F344 rats exposed for 90 days (Burek et al., 1980) and 2 mg/kg-day (0.5 mg/kg-day NOAELs) in male F344 rats exposed for 2 years in two separate bioassays (Friedman et al., 1995; Johnson et al., 1986). The lowest exposure levels associated with male-mediated implantation losses are somewhat higher than those associated with degenerative nerve changes: 2.8 mg/kg-day (1.5 mg/kg-day NOAEL) in Long-Evans rats exposed for 80 days (Smith et al., 1986); 5 mg/kg-day (2.0 mg/kg-day NOAEL) in F0 and F1 F344 rats (Tyl et al., 2000a); 7.5 mg/kg-day (3.1 mg/kg-day NOAEL) in F0 and F1 CD-1 mice (Chapin et al., 1995); and 13.3 mg/kg-day (9.0 mg/kg-day NOAEL) in ddY mice exposed for 4 weeks (Sakamoto and Hashimoto, 1986). Comprehensive histologic examinations of all major organs and tissues in the chronic and subchronic rat bioassays found no exposure-related nonneoplastic lesions at other sites at dose levels below 5 mg/kg-day (Table 4-31). Hindlimb splaying, a gross characteristic sign of peripheral neuropathy, has been observed in most studies at oral exposure levels (about 9–25 mg/kg-day) well above the lowest doses (1–2

mg/kg-day) associated with microscopically detected degenerative peripheral nerve changes (Table 4-31). As discussed in Section 4.5.1, an exception is one report that exposure to 0.5 mg/kg-day AA induced hindlimb splaying in F0 male F344 rats (Tyl et al., 2000a), but this report is not consistent with other findings, including the absence of hindlimb splaying in F1 rats exposed to doses as high as 5 mg/kg-day in the same study and in rats exposed to doses as high as 2–3 mg/kg-day for 2 years and 5 mg/kg-day for 90 days (Table 4-31). Thus, microscopically detected degenerative peripheral nerve changes appear to be the most sensitive effect from oral exposure and are selected as the critical effect for deriving the RfD.

The two chronic 2-year drinking water studies (Friedman et al., 1995; Johnson et al., 1986) are selected as co-principal studies for deriving the RfD, and the final quantitative RfD value is based on the dose-response data from only the Johnson study. These studies are better candidates to derive the chronic RfD than the subchronic study (Burek et al., 1980), primarily due to more appropriate durations of exposure (lifetime vs. 90 days) and greater numbers of animals/exposure group (a range of 20 to 88/sex/group in the chronic studies vs. 10/sex/group in the subchronic study). All three studies included multiple dose groups, thereby providing information on characteristics of the dose-response relationship.

The subchronic, 90-day study (Burek et al., 1980) used a more sensitive electron microscopic technique to detect degenerative nerve changes vs. the light microscopy used in the 2-year bioassays. The chronic drinking water study by Johnson et al. (1986) examined nerves sampled at 18 and 24 months by electron microscopy but reported that the background of ultrastructural changes in aging rats was too high to discern differences between control and exposed groups. The Burek et al. (1980) study evaluated sciatic nerves from only three rats/exposure group (about 150 fields/rat)[6], and the changes noted 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 (see Table 4-8). 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). The 1 mg/kg-day LOAEL and 0.2 mg/kg-day NOAEL from this subchronic study were, therefore, based on a semiquantitative assessment of the electron microscopy data, i.e., the incidences of electron microscopic fields with any ultrastructural changes were higher in the 1, 5, and 20 mg/kg-day groups than in the 0, 0.05, and 0.2 mg/kg-day groups, and light microscopy of sciatic nerves revealed no signs of degeneration in the 0, 0.05,

[6] The incidences of fields with any alterations were: 68/450, 39/450, 44/350, 108/453, 149/443, and 239/435 for the 0, 0.05, 0.2, 1, 5, and 20 mg/kg-day groups. Approximately 150 fields were examined for each rat;

0.2, or 1 mg/kg-day groups, equivocal to very slight degeneration in 15/20 5 mg/kg-day rats, and moderate to severe degeneration in 20/20 20 mg/kg-day rats. The 1 mg/kg-day LOAEL, however, was for only very slight changes that were reversible by day 25 posttreatment, and the NOAEL from this study was limited to the selection of dose levels (i.e., there was no 0.5 mg/kg-day group as in the 2-year studies). Since benchmark analysis was not possible on this data set, the subchronic results are viewed as supporting the findings from the chronic studies. The two chronic studies presented better quantified NOAELs of 0.5 mg/kg-day and LOAELs of 2 mg/kg-day for persistent microscopically-detected AA-induced degenerative nerve changes from lifetime exposures.

5.1.2. Methods of Analysis—Including Models (PBTK, BMD, etc.)

All available models in the EPA Benchmark Dose Software (BMDS version 1.3.1) were fit to the incidence data for microscopically-detected degenerative nerve changes in male and female F344 rats from the two 2-year drinking water studies (Friedman et al., 1995; Johnson et al., 1986). The modeled data are shown in Table 5-1. The benchmark response (BMR) predicted to affect 5% of the population, BMR_5, was selected for the point of departure (POD). A BMR lower than a 10% extra risk was selected for the following reasons: (1) the 95% lower bound of the benchmark dose (BMD), $BMDL_5$, remained near the range of observation; (2) the 5% extra risk level is supportable given the relatively large number of animals used in the critical studies; and (3) the use of $BMDL_5$ is consistent with the technical guidance for BMD analysis which states that "while it is important to always report ED_{10}s [effective doses at 10% extra risk] and LED_{10}s [95% lower bounds of the ED_{10}s] for comparison purposes, the actual 'benchmark dose' used as a POD may correspond to response levels below (or sometimes above) 10%, although for convenience standard levels of 1%, 5%, or 10% have typically been used" (U.S. EPA, 1995).

The PBTK model used in this derivation is described in Section 3.5, and a table of the physiological and chemical parameters used in the model simulations is in Appendix E.

however, further statistical analysis was not possible because the numbers of fields with changes observed were not reported for each of the three rats singly from each group.

Table 5-1. Incidence data for degenerative changes detected by light microscopy in nerves of male and female F344 rats exposed to acrylamide in drinking water for 2 years

	Dose (mg/kg-day)							
Reference	**0**	**0**	**0.01**	**0.1**	**0.5**	**1.0**	**2.0**	**3.0**
Johnson et al., 1986 (incidence of rats with changes in tibial nerves: see Table 4.9)								
Males (moderate to severe)[a]	9/60	–	6/60	12/60	13/60	–	16/60[b]	–
Females (slight to moderate)[a]	3/60	–	7/60	5/60	7/60	–	16/61[c]	–
Friedman et al., 1995[d] *(incidence of rats with minimal to mild changes in sciatic nerves: see Table 4.12)*								
Males	30/83	29/88	–	21/65	13/38	–	26/49[c]	–
Females	7/37	12/43	–	–	–	2/20	–	38/86[c]

[a]Reported severity classes were very slight, slight, moderate, and severe. Males showed a high background of very slight and slight lesions; females showed a high background of very slight lesions.
[b]Statistically significant trend test (Mantel-Haenszel extension of the Cochran-Armitage test, $p < 0.05$) for pooled moderate and severe degeneration. Note: no statistical significance for the high dose group. Incidence for severe degeneration with dose level in parentheses (in mg/kg-day) was 1 (control), 1 (0.01), 0 (0.1), 0 (0.5), and 4 (2.0).
[c]Statistically significantly different from control incidences ($p < 0.05$).
[d]Two control groups were included in the study design to assess variability in background tumor responses; degeneration was reported to be characterized by vacuolated nerve fibers of "minimal-to-mild severity."

As shown in Appendix C, all models provided adequate fits to the data for changes in tibial nerves of male and female rats in the Johnson et al. (1986) study, as assessed by a chi-square goodness-of-fit test. The log-logistic model was the best fitting model for the male rat data as assessed by Akaike's Information Criterion (AIC). The probit model was the best fitting model for the female rat data as assessed by Akaike's Information Criterion (AIC). The log-logistic model was thus selected to estimate a benchmark dose (BMD) from the Johnson et al. (1986) data. The probit model was selected to estimate the BMD for the female rat data. Table 5-2 (same as Table C-4 in Appendix C) lists the predicted doses associated with 10%, 5% and 1% extra risk for nerve degeneration in female and male rats in the Johnson et al. (1986) study. The BMD_5 is the predicted dose associated with a 5% extra risk for degenerative lesions in either tibial or sciatic nerves, the $BMDL_5$ is the lower 95% confidence limit for the 5% extra risk. For male rats, the BMD_5 is 0.58 mg/kg-day, and the $BMDL_5$ is 0.27 mg/kg-day. For female rats, the BMD_5 is 0.67 mg/kg-day, and the $BMDL_5$ is 0.49 mg/kg-day.

Table 5-2. Predictions (mg/kg-day) from best-fitting models for doses associated with a 10, 5, and 1% extra risk for nerve degeneration in male and female rats exposed to acrylamide in drinking water

Model	BMD_{10} (ED_{10})	$BMDL_{10}$ (LED_{10})	BMD_5 (ED_5)	$BMDL_5$ (LED_5)	BMD_1 (ED_1)	$BMDL_1$ (LED_1)
Male						
Log-logistic	1.22	0.57	0.58	0.27	0.11	0.05
Female						
Probit	1.19	0.88	0.67	0.49	0.15	0.11

Source: Johnson et al. (1986).

Several models in the software provided adequate fits to the data for minimal to mild changes in sciatic nerves of male and female rats in the Friedman et al. (1995) study, as assessed by a chi-square goodness-of-fit test (Appendix C). The quantal-quadratic and gamma models provided the best fit of the male and female rat data, respectively, as assessed by AIC. Table 5-3 (same as Table C-7 in Appendix C) lists the predicted doses associated with 10%, 5% and 1% extra risk for nerve degeneration in female and male rats in the Friedman et al. (1995) study. The BMD_5 for minimal to mild changes in sciatic nerves for male rats is 0.77 mg/kg-day and the $BMDL_5$ is 0.57 mg/kg-day. For female rats, the BMD_5 is 2.25 mg/kg-day and the $BMDL_5$ is 0.46 mg/kg-day.

Table 5-3. Predictions (mg/kg-day) from best-fitting models for doses associated with 10, 5, and 1% extra risk for sciatic nerve changes in male and female rats exposed to acrylamide in drinking water

Model	BMD_{10} (ED_{10})	$BMDL_{10}$ (LED_{10})	BMD_5 (ED_5)	$BMDL_5$ (LED_5)	BMD_1 (ED_1)	$BMDL_1$ (LED_1)
Male						
Quantal quadratic	1.11	0.82	0.77	0.57	0.34	0.25
Female						
Gamma[a]	2.48	0.93	2.25	0.46	1.86	0.09

[a]Restrict power ≥1.

Source: Friedman et al. (1995).

5.1.3. RfD Derivation—Including Application of Uncertainty Factors

The male rats appeared to be slightly more sensitive than the female rats in the Johnson et al. (1986) and Friedman et al. (1995) studies, as reflected by slightly higher BMDLs for female rats (0.49 and 0.46 mg/kg-day, respectively) than for male rats (0.27 and 0.57 mg/kg-day, respectively). The lowest of the BMDLs from the Johnson et al. (1986) study (0.27 mg/kg-day for 5% extra risk for mild-to-moderate lesions) reflects the most sensitive response and was selected as the POD for deriving the RfD.

The recalibrated Kirman et al. (2003) PBTK model (discussed in Section 3.5) was used to estimate the internal dose in a rat that would result from an external exposure to the $BMDL_5$ of

0.27 mg/kg-day. Both PBTK model variations were used: (1) enzyme-catalyzed glutathione binding of AA and GA and (2) passive glutathione binding of AA and GA. AUC in blood for the parent compound, AA, was considered the most appropriate choice for the internal dose metric for neurotoxicity. Table 5-4 presents the model simulation results of the AUC for the male and female BMDs and BMDLs, the human equivalent daily intake that would produce that same AUC in humans from a drinking water exposure, and the drinking water concentration, assuming a 70 kg person who drinks 2 L/day. For the drinking water simulations in humans, the PBTK model used an ingestion pattern that divided the total daily intake among five daily water bolus ingestions, three of which were at meal times (8 am, 1 pm, and 6 pm), each amounting to 25% of the total daily intake, and two that were in-between meals (10:30 am and 3:30 pm), each amounting to 12.5% of the total daily intake.

Table 5-4. PBTK model simulation results for HEC based on the rat neurotoxicity BMD

	Female rats		Male Rats	
	BMD_5	**$BMDL_5$**	**BMD_5**	**$BMDL_5$**
BMD analysis results (mg/kg-day)	0.67	0.49	0.58	**0.27**
PBTK model 1—enzyme catalyzed glutathione binding AUC for AA in rat blood (μM-hour)	45.0	32.9	38.9	18.1
Human equivalent intake (mg/kg-day) for a comparable AA AUC in blood, based on a drinking water exposure	0.188	0.137	0.163	**0.076**
Drinking water concentration that would result in the HEC[a] daily intake (mg/L) (70 kg person, 2 L/day)	6.6	4.8	5.7	2.7
PBTK model 2—passive glutathione binding AUC for AA in rat blood (μM-hour)	46.1	33.7	39.9	18.5
Human equivalent intake (mg/kg-day) for a comparable AA AUC in blood, based on a drinking water exposure	0.193	0.141	0.167	**0.078**
Drinking water concentration that would result in the HEC daily intake (mg/L) (70 kg person, 2 L/day)	6.8	4.9	5.9	2.7

[a]HEC = human equivalent concentration.

Source: Johnson et al. (1986).

The human equivalent concentration (HEC)-adjusted POD from the PBTK model results based on the lowest of the BMDLs from the Johnson et al. (1986) study of 0.27 mg/kg-day for a 5% extra risk in males for mild-to-moderate lesions is 0.076 mg/kg-day for the model with enzyme catalyzed glutathione binding and 0.078 mg/kg-day for the model with passive glutathione binding. There is little difference between the two models results, and the slightly lower value of 0.076 mg/kg-day is chosen for the HEC-adjusted POD in light of the uncertainty as to which type of binding occurs in humans.

The $HEC_{PBTK\ model}$ adjusted POD was divided by a total uncertainty factor (UF) of 30: 3 for extrapolation for interspecies toxicodynamic differences ($UF_{A\text{-}TD}$: animal to human) and 10 for consideration of intraspecies variation (UF_H: human variability).

Total UF = 30
= 3 ($UF_{A\text{-}TD}$) × 1 ($UF_{A\text{-}TK}$) × 10 (UF_H) × 1 (UF_S) × 1 (UF_D)

An UF of 3 ($10^{1/2}$ = 3.16, rounded to 3) was selected to account for uncertainties in extrapolating from rats to humans for toxicodynamic differences ($UF_{A\text{-}TD}$). It is reasonable to assume that the neuropathic effects observed in rats are relevant to humans since peripheral neuropathy in humans has been widely associated with occupational (inhalation and dermal) exposure to AA, and cases of peripheral neuropathy associated with oral exposure have been reported. Available information is inadequate to quantify potential differences between rats and humans in the toxicodynamics of orally administered AA. The lack of a mechanistic basis or any quantitative information on toxicodynamic differences between rats and humans supports not reducing the $UF_{A\text{-}TD}$ from 3. The PBTK model simulations are used to account for intraspecies toxicokinetic differences, and thus the $UF_{A\text{-}TK}$ = 1 instead of the default value of 3.16 ($10^{1/2}$).

An UF of 10 was used to account for interindividual variability in toxicokinetics and toxicodynamics to protect potentially sensitive populations and lifestages (UF_H). Although male rats appear to be slightly more sensitive than female rats to AA-induced neurotoxicity and were the basis of the POD for the RfD, the extent of variation in sensitivity to AA within the human population is unknown. In the absence of this information, the default value of 10 was not reduced.

An UF for extrapolating from a subchronic exposure duration to a chronic exposure duration (UF_S) was not needed, because the point of departure was derived from a study with chronic exposure (i.e., the UF_S = 1).

An UF to account for database deficiency is not necessary (i.e., UF_D = 1). The oral toxicity database for laboratory animals repeatedly exposed to AA is robust and contains two 2-year carcinogenicity/toxicology drinking water studies in F344 rats and numerous shorter-term oral toxicity studies in animals; two two-generation reproductive toxicity studies, one in F344 rats and one in CD-1 mice; several single-generation reproductive toxicity studies involving prolonged prebreeding drinking water exposure of Long-Evans rats and ddY mice; and several developmental toxicity studies involving gestational exposure of Sprague-Dawley and Wistar rats and CD-1 mice. The database identifies nerve degeneration as the critical effect from chronic oral exposure. There are unresolved issues that warrant further research including the MOA of AA-induced neurotoxicity, the potential for behavioral or functional adverse effects not detected in the assays to date, and the uncertainty that heritable germ cell effects may occur at lower than previously reported doses. These issues, however, do not warrant applying a UF for database deficiencies.

Functional neurotoxic deficits have been observed in both animal and human studies, and at least two MOA precursor events have been proposed (i.e., central nerve terminal damage or reduction in fast axonal transport). Either of these precursor events might result in other serious behavioral or functional neurological deficits that were not detected in the bioassays. More research is needed to further evaluate more subtle irreversible adverse behavioral or functional effects in humans and laboratory animals. The magnitude of response at low doses, and the shape of the low dose-response curve for potentially serious heritable germ cell effects is also a research need. Some of these data needs are currently being addressed.

The RfD for acrylamide was calculated as follows:

$$\begin{aligned} \text{RfD} &= \text{HEC}_{\text{PBTK model}} \div \text{UF} \\ &= 0.076 \text{ mg/kg-day} \div 30 \\ &= 0.003 \text{ mg/kg-day}. \end{aligned}$$

5.1.4. Previous RfD Assessment

This RfD replaces the previous RfD for acrylamide of 0.0002 mg/kg-day entered into the IRIS database on September 26, 1988. The previous RfD was based on nerve damage (NOAEL of 0.2 mg/kg-day; LOAEL of 1 mg/kg-day) observed in a rat subchronic drinking water study (Burek et al., 1980). The RfD was derived by dividing the NOAEL by an UF of 1000: 10 for uncertainty in extrapolating from animals to humans, 10 for intrahuman variability, and 10 for uncertainty in extrapolating from a subchronic to a chronic exposure. The new RfD is based on a more recent chronic exposure studies (Johnson et al., 1986; Friedman et al., 1995), as well as current methodology for characterizing the dose-response curve and for determining the POD (i.e., the BMDL).

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

As discussed in Section 4.5.2, neurological impairment is a well-established human health hazard associated with acute and repeated occupational exposure involving inhalation of airborne AA and dermal contact with AA-containing materials. Studies describing reliable relationships, however, between exposure concentrations and neurological responses in humans or animals are not available. Two cross-sectional health surveillance studies of AA-exposed workers describe correlative relationships between hemoglobin adduct levels of AA (an internal measure of dose) and changes in a neurotoxicity index based on self-reported symptoms and clinical measures of neurological impairment (Calleman et al., 1994) or increased incidences in self-reported symptoms of neurological impairment and eye and respiratory irritation (Hagmar et

al., 2001). These studies, however, do not provide reliable information on dose-response relationships for chronic inhalation exposure to AA, because they involved mixed inhalation and dermal exposure (in both groups of workers, dermal exposure was thought to have been substantial), the duration of exposure was less than chronic, workers in both studies were exposed to confounding chemicals (acrylonitrile in the first study and NMA in the second), and the internal measure of dose (N-terminal valine adducts of hemoglobin) is not specific for AA alone (i.e., NMA can form the same adduct).

The PBTK model was used for a route-to-route extrapolation by simulating the internal AUC for acrylamide in the blood that results from an oral exposure at the $BMDL_5$ for male rats and then simulating the daily inhaled intake level that would be needed to produce a comparable AUC in humans (Table 5-5). The level of AA in the air for such an inhalation exposure is based on a 70 kg person who breathes 20 m^3/day. The benchmark response (BMR) predicted to affect 5% of the population, BMR_5, was selected for the point of departure (POD). A BMR lower than a 10% extra risk was selected for the following reasons: (1) the 95% lower bound of the benchmark dose (BMD), $BMDL_5$, remained near the range of observation; (2) the 5% extra risk level is supportable given the relatively large number of animals used in the critical studies; and (3) the use of $BMDL_5$ is consistent with the technical guidance for BMD analysis (U.S. EPA, 1995).

Justification for deriving an RfC from the oral RfD comes from: (1) considerable evidence from occupational experience involving dermal and inhalation exposure that AA-induced peripheral neuropathy (including development of the types of degenerative lesions observed in nerves of rats exposed via drinking water) is a well-established human health hazard; (2) evidence that tissue distribution in rats is similar following i.v., i.p., oral, dermal, and inhalation exposure to AA (Sumner et al., 2003; Kadry et al., 1999; Dow Chemical Co., 1984; Miller et al., 1982; Hashimoto and Aldridge, 1970); (3) evidence that the elimination kinetics of radioactivity from oral or i.v. administration of radiolabeled AA in rats was similar (Miller et al., 1982); and (4) lack of support for portal of entry effects. As a caveat, the RfC would not account for dermal absorption from AA vapor deposited on the skin, although it is not known how much vapor would be absorbed dermally since the human dermal exposure studies applied AA in a solution to the skin, then evaporated the liquid and covered the dried residue with gauze.

5.2.2. Methods of Analysis—Including Model (PBTK, BMD, etc.)

See Section 5.1 for derivation of the chronic oral RfD for acrylamide, Section 3.5 for a discussion of the PBTK model, and Appendix E for a table of the physiological and chemical parameters used in the model simulations.

5.2.3. RfC Derivation—Including Application of Uncertainty Factors

The $BMDL_5$ for degenerative nerve lesions in male rats exposed to acrylamide in drinking water for 2 years is taken as the POD for deriving the RfC. The $BMDL_5$ from males and females is listed in Table 5-5, along with model simulation results for the internal dose metric (AUC of AA in blood), the human equivalent inhalation daily intake required to produce that same AUC value, and the air concentration that would provide a 70 kg person who breathes 20 m^3 of air that amount of daily exposure. For the inhalation exposure, the PBTK model simulated a continuous 24-hour inhalation exposure (i.e., no variation in the amount of intake/unit time at any time throughout the day).

Table 5-5. PBTK model simulation results for HEC based on the rat neurotoxicity BMD

	Female rats		Male rats	
	BMD_5	**$BMDL_5$**	**BMD_5**	**$BMDL_5$**
BMD analysis results (mg/kg-day)	0.67	0.49	0.58	**0.27**
PBTK Model 1—enzyme catalyzed glutathione binding AUC for AA in rat blood (μM-hour)	45.0	32.9	38.9	18.1
Human equivalent intake (mg/kg-day) for a comparable AA AUC in blood, based on continuous air exposure	0.180	0.132	0.156	**0.073**
Air concentration that would result in the HEC daily intake (mg/m^3) (70 kg person, 20 m^3/day)	0.63	0.46	0.55	**0.25**
PBTK Model 2—passive glutathione binding AUC for AA in rat blood (μM-hour)	46.1	33.7	39.9	18.5
Human equivalent intake (mg/kg-day) for a comparable AA AUC in blood based, on continuous air exposure	0.188	0.138	0.163	**0.076**
Air concentration that would result in the HEC daily intake (mg/m^3) (70 kg person, 20 m^3/day)	0.66	0.48	0.57	0.27

Source: Johnson et al. (1986).

The HEC-adjusted POD from the PBTK model results is based on the lowest of the Johnson et al. (1986) study BMDLs of 0.27 mg/kg-day for a 5% extra risk in males for mild-to-moderate lesions. This HEC is 0.073 mg/kg-day for the model with enzyme catalyzed glutathione binding, and 0.076 mg/kg-day for the model with passive glutathione binding. There is little difference between the two models results, and the lowest value of 0.073 mg/kg-day is chosen for the HEC adjusted POD in light of the uncertainty as to which type of binding occurs in humans. In a 70 kg person inhaling 20 m^3/day air, the air concentration needed to result in the HEC adjusted POD would be 0.25 mg/m^3.

The $HEC_{PBTK\ model}$-adjusted POD was divided by a total UF of 30: 3 for extrapolation for interspecies toxicodynamic differences ($UF_{A\text{-}TD}$: animal to human) and 10 for consideration of intraspecies variation (UF_H: human variability).

Total UF = 30

$= 3\ (UF_{A\text{-}TD}) \times 1\ (UF_{A\text{-}TK}) \times 10\ (UF_H) \times 1\ (UF_S) \times 1\ (UF_D)$

An UF of 3 ($10^{1/2} = 3.16$, rounded to 3) was selected to account for uncertainties in extrapolating from rats to humans for toxicodynamic differences ($UF_{A\text{-}TD}$). It is reasonable to assume that the neuropathic effects observed in rats are relevant to humans since peripheral neuropathy in humans has been widely associated with occupational (inhalation and dermal) exposure to AA, and cases of peripheral neuropathy associated with oral exposure have been reported. Available information is inadequate to quantify potential differences between rats and humans in toxicodynamics of orally administered AA. The lack of a mechanistic basis or any quantitative information on toxicodynamic differences between rats and humans provides support for not reducing the $UF_{A\text{-}TD}$ from 3. The PBTK model simulations are used to account for intraspecies toxicokinetic differences, and thus the $UF_{A\text{-}TK} = 1$ instead of the default value of 3.16 ($10^{1/2}$).

An UF of 10 was used to account for interindividual variability in toxicokinetics and toxicodynamics to protect potentially sensitive populations and lifestages (UF_H). Although male rats appear to be slightly more sensitive than female rats to AA neurotoxicity and were the basis of the POD for the RfD, the extent of variation in sensitivity to AA within the human population is unknown. In the absence of this information, the default value of 10 was not reduced.

An UF for extrapolating from a subchronic exposure duration to a chronic exposure duration (UF_S) was not needed because the point of departure was derived from a chronic exposure study (i.e., the $UF_S = 1$).

An UF to account for database deficiency is not necessary for this derivation (i.e., $UF_D = 1$) because a PBTK model was used to conduct the route-to-route extrapolation from an oral POD and the oral POD was based on an adequate database. The oral toxicity database for laboratory animals repeatedly exposed to AA is robust and contains two 2-year carcinogenicity/toxicology drinking water studies in F344 rats and numerous shorter-term oral toxicity studies in animals; two two-generation reproductive toxicity studies, one in F344 rats and one in CD-1 mice; several single-generation reproductive toxicity studies involving prolonged prebreeding drinking water exposure of Long-Evans rats and ddY mice; and several developmental toxicity studies involving gestational exposure of Sprague-Dawley and Wistar rats and CD-1 mice. The database identifies nerve degeneration as the critical effect from chronic oral exposure. There are unresolved issues that warrant further research, including the MOA of AA neurotoxicity, the potential for behavioral or functional adverse effects not detected in the assays to date, and the uncertainty that heritable germ cell effects may occur at lower than previously reported doses. These issues, however, do not warrant applying a UF for database deficiencies.

The RfC for acrylamide is calculated as follows:

$$\begin{aligned} \text{RfC} &= \text{HEC}_{\text{PBTK model}} \div \text{UF} \\ &= 0.25 \text{ mg/m}^3 \div 30 \\ &= 0.008 \text{ mg/m}^3 \end{aligned}$$

5.2.4. Previous RfC Assessment

The previous IRIS assessment did not derive a RfC.

5.3. UNCERTAINTIES IN THE ORAL REFERENCE DOSE AND INHALATION REFERENCE CONCENTRATION

The following discussion identifies uncertainties in the derivation of the RfD and RfC for acrylamide. Uncertainties in key aspects of the AA assessment include: 1) the completeness of the database for identifying potentially adverse effects, 2) the choice of the critical effect and its relevance for humans, 3) the biological rationale supporting the choice of the dose-response model and determination of the point of departure (POD), and 4) the uncertainties in the structure and parameter values of the PBTK model relative to its use in deriving the toxicity values.

U.S. EPA has developed default uncertainty factors to account for uncertainties in an RfD or RfC due to missing or inadequate data (U.S. EPA, 2002, 1994b) and to ensure that the risk to chemicals and stressors are not underestimated. The default uncertainty factors address the following areas of uncertainty: (1) variation in susceptibility among the members of the human population (i.e., inter-individual or intraspecies variability); (2) in extrapolating animal data to humans (i.e., interspecies uncertainty); (3) in extrapolating from data obtained in a study with less-than-lifetime exposure (i.e., extrapolating from subchronic to chronic exposure); (4) in extrapolating from a LOAEL rather than from a NOAEL; and (5) associated with extrapolation when the database is incomplete. Default uncertainty factors are used in the derivation of the RfD and RfC to adjust the POD downward (i.e., to lower the acceptable exposure level) and thus reduce the potential risk of adverse effects to protect the public health.

The specific uncertainty factors used in deriving the acrylamide RfD and RfC were previously discussed in sections 5.1.3 and 5.2.3, respectively. A PBTK model was available to account for interspecies toxicokinetic differences. Default uncertainty factors were therefore used to account for toxicodynamic differences when extrapolating the dose-response relationship from test animals to humans, and to account for intrahuman variability in toxicokinetics and toxicodynamics to protect susceptible subpopulations.

In the case of AA, the uncertainties in the underlying data and methods used are similar for the RfD and the RfC since the RfC is based on the same data as the RfD. The following

discussion, therefore, addresses the main areas of uncertainty relevant to both the RfD and the RfC in Section 5.3.1. Section 5.3.2 provides a more detailed look at the uncertainty factors used in the derivation of the RfD and RfC. Key points in the discussion are summarized in Table 5-7.

5.3.1 Areas of Uncertainty

Completeness of the Database

The human data for potential non-cancer adverse effects from exposure to acrylamide are limited to occupational case reports for neurological effects following inhalation and/or dermal exposure (with no data on levels of exposure), two cross-sectional health surveillance studies of AA-exposed workers that correlate AA-hemoglobin adduct levels and measures of neurological impairment in acrylamide workers (Hagmar et al., 2001; Calleman et al., 1994), and one kinetic study in 24 human volunteers who were exposed to either a single low-level oral exposure with no observed toxicity, or to a dermal exposure with adverse effects reported for only one individual who responded with a mild reversible contact dermatitis (delayed hypersensitivity reaction) (Fennell et al., 2005). No human studies were identified on the potential for adverse reproductive or developmental effects from exposure to acrylamide via inhalation or dermal exposure, and no human repeated oral exposure studies were identified that evaluated any adverse noncancer effect.

The animal database for repeated oral exposures, however, is robust, and includes two 2-year carcinogenicity/toxicology drinking water studies in F344 rats, numerous shorter-term toxicity studies in various species, two two-generation reproductive toxicity studies (one in F344 rats and one in CD-1 mice), several single-generation reproductive toxicity studies involving prolonged prebreeding drinking water exposures (in Long-Evans rats and ddY mice), and several developmental toxicity studies with gestational exposures to dams of Sprague-Dawley rats, Wistar rats, and CD-1 mice. Animal studies for inhalation exposures are limited to three subchronic studies in cats, dogs, and rats from the mid-1950s (Hazleton Laboratories, 1954, 1953) that report neurotoxicity dependent on the dose and species tested. No chronic animal inhalation studies for exposure to AA were identified.

With respect to the route of exposure versus the observed adverse effect, animal studies indicate that acrylamide is rapidly absorbed and distributed when it enters the body from either an oral or inhalation exposure (Sumners et al., 2003). Moreover, the neurological effects reported in human occupational studies and case reports following inhalation or dermal exposure are similar to the effects observed in a broad range of oral exposure animal studies, and neurological effects appear to be the most sensitive effect (see Section 4). Thus there is good support for the hypothesis that the neurological effects observed in humans from an inhalation exposure would likely be observed from an oral exposure that produced a comparable internal level of parent acrylamide (or metabolite) at an internal target site. As a result, the absence of animal inhalation studies does not compromise the completeness of the database as it would if the spectrum of effects were very much different for different routes of exposure.

In summary, there is a substantial animal database to assess the noncancer effects of acrylamide. The oral toxicity database for laboratory animals repeatedly exposed to AA is robust and adequate to support the derivation of the RfD, and the validity of conducting a route-to-route extrapolation from the oral data to derive an RfC is well supported by the available kinetic data.

Selection of the Most Sensitive Endpoint

The available human and animal data clearly support the choice of neurotoxicity as the most sensitive endpoint. The human occupational studies and case studies report neurotoxicity, and both oral exposure animal chronic bioassays report nerve degeneration as the most sensitive adverse effect. Reproductive toxicity (e.g., reduced number of live pups per litter) has been observed in rodent studies, but the no effect level was approximately 3-5 fold higher (i.e., a less sensitive response) than observed for neurotoxicity. Heritable germ cell effects (e.g., translocations, dominant lethals) have also been reported in animal studies, and are a potentially more serious adverse event than neurotoxicity because these are effects that can occur not only in the exposed individual, but also their offspring and subsequent generations. Heritable germ cells effects, however, have only been observed at relatively high levels of acrylamide exposure in animal studies (orders of magnitude higher than the levels where neurotoxicity has been observed). Data are not available to determine if these effects would be seen at lower exposure levels in test animals, or whether they appear in humans at any level of exposure. To resolve this uncertainty as to the possibility that heritable germ cell effects are the most critical low dose effect will require more data.

Another area of uncertainty is the possibility that functional or behavioral neurotoxic endpoints might occur at lower dose levels than the morphological changes that were used as the measure of neurotoxicity in the animal chronic assays. Functional neurotoxic deficits have been observed in shorter term animal studies, and in humans occupationally exposed to acrylamide. Two precursor events have been proposed for the MOA leading to functional neurotoxicity - central nerve terminal damage and reduction in fast axonal transport. Either of these precursor events might result in serious behavioral or functional neurological deficits at doses lower than those needed to produce histologically observable morphological changes. The U.S Food and Drug Administration is conducting studies to address this issue. If adverse functional changes were, in fact, determined to occur at dose lower than those for histologically observable nerve tissue damage, the values of the RfD and RfC could potentially be lower.

Dose-Response Modeling and Determination of the Point of Departure

Benchmark dose (BMD) modeling was used to estimate the point of departure (POD) for the acrylamide RfD. BMD modeling has advantages over a POD based on a NOAEL or LOAEL because all of the data are used to characterize the dose-response relationship, and because NOAELs/LOAELs are a reflection of the particular exposure concentration or dose at which a study was conducted.

All available models in the EPA Benchmark Dose Software (BMDS version 1.3.1) were fit to the incidence data for microscopically-detected degenerative nerve changes in male and female F344 rats from the two 2-year drinking water studies (Friedman et al., 1995; Johnson et al., 1986). The benchmark response (BMR) predicted to affect 5% of the population, BMR_5 was selected for the point of departure (POD) rather than the more commonly chosen BMR of 10% for the following reasons (1) the 95% lower bound of the benchmark dose (BMD), $BMDL_5$, remained near the range of observation; (2) the 5% extra risk level is supportable given the relatively large number of animals used in the critical studies; and (3) the use of $BMDL_5$ is consistent with the technical guidance for BMD analysis (U.S. EPA, 1995).

BMD models provide empirical fits to the dose-response data, and no data or valid arguments were available to support a biological rationale for selecting one model over the other. The best model to use for estimating the POD was therefore selected based on Akaike's Information Criterion (AIC). The AIC is a measure of the goodness of fit of an estimated statistical model within the context of the complexity of the model, i.e., between models with comparable fits, the best model is the one with the lowest number of parameters (the simpler model). Once the model with the lowest AIC score for each data set is identified, the resulting PODs are compared, and the lowest POD is used to derive the RfD. For acrylamide, the log-logistic model provided the best fit for the male rat data and resulted in the lowest POD, and was thus used to derive the RfD in the current assessment. As seen in Table 5-6, all of the final POD estimates are within 2-fold of each other, supporting a relatively high degree of confidence that the estimated $BMDL_5$ in this analysis is a valid estimate of the no effect level for mild histological changes from a lifetime of exposure as a measure of acrylamide induced neurotoxicity. With respect to the impact that additional data or a new biological rationale would have on the rank ordering of the BMD models, there is no way to predict whether the revised estimate of risk to humans would go up or down.

Table 5-6. Estimated POD (mg/kg-day) from best-fitting models for doses associated with a 5% extra risk for nerve degeneration in male and female rats exposed to acrylamide in drinking water.

Model	BMD (ED_5)	BMDL (LED_5)
Johnson et al. (1986)		
Male		
Log-logistic	0.58	**0.27**
Female		
Probit	0.67	0.49
Friedman et al. (1995)		
Male		
Quantal quadratic	0.77	0.57
Female		
Gamma[a]	2.25	0.46

[a]Restrict power ≥1

Adequacy of the PBTK Model for Use in Deriving the RfD and the RfC

EPA recalibrated and parameterized a PBTK model for acrylamide (originally developed and published by Kirman et al., 2003) against more recent data (Boettcher et al., 2005; Doerge et al., 2005a,b,c; Fennell et al., 2005; Sumner et al., 2003) for use in deriving toxicity values. The recalibrated AA PBTK model was tested against the new kinetic and hemoglobin binding data in rats, mice, and humans. The model was then used to estimate the oral human equivalent concentration (i.e., extrapolate the animal dose-response relationship to humans) to derive the RfD, and to conduct a route-to-route extrapolation (oral to inhalation) to estimate the inhalation HEC to derive the RfC.

There is always some degree of uncertainty in a PBTK model's structure and estimates of internal dose, because PBTK models are simplified mathematical representations of very complex organisms. Within the context of the alternative of using default factors, however, PBTK models are increasingly offering a more scientifically supportable means to: 1) extrapolate risk as observed in studies on test animal to potential risks to humans, 2) account for the most sensitive human subpopulations, or 3) conduct route-to-route extrapolations. For acrylamide, a published and tested PBTK model was available, and in its recalibrated form, provided acceptable fits to recent and relevant data. The resulting fits to the available data were adequate to support the model's use to estimate the oral human equivalent concentration to derive the RfD, and to reduce uncertainty in that estimate compared to use of the default uncertainty factor for interspecies toxicokinetic differences. The AA PBTK model was also used to estimate the inhalation HEC based on the oral dose-response data to derive an RfC, a value

that would otherwise not have been possible since there are no credible default methods for conducting a route-to-route extrapolation. It is unknown how inaccuracies in the AA PBTK model structure or parameter values would impact the assessment; further refinements in the model or new data could increase or decrease the estimate of risks of neurotoxicity in humans.

5.3.2 Uncertainty Factors in Deriving the RfD and RfC

Uncertainty in the Completeness of the Database

As discussed above, the animal database is robust and complete by IRIS assessment standards. There is a possibility that heritable germ cell effects or functional neurotoxicity might be more sensitive endpoints, but more data are needed to resolve this issue, and meanwhile the standard suite of animal toxicity studies are available to support the derivation of toxicity values. Although the human data are limited, the predominantly neurological endpoints that have been reported are similar to those observed in the animal studies. Thus no additional reduction in the toxicity value is needed to account for uncertainty in the completeness of the database (i.e., $UF_D = 1$).

Uncertainty in the Animal to Human Extrapolation

The accuracy of extrapolating the dose-response relationship as observed in animals to the dose-response that will occur in humans is a source of uncertainty. This extrapolation is based on species differences in toxicokinetics and toxicodynamics. A PBTK model is available that accounts for the differences between rat and human toxicokinetics following exposure to acrylamide in deriving the RfD. The impact of the uncertainties in the model for each application are discussed below.

PBTK model estimate of the oral human equivalent exposure (HEC)

The acrylamide PBTK model simulated the toxicokinetics of acrylamide in the body of the Fisher 344 rat used in the chronic bioassays or in humans (an average 70 kg male). The model was exercised to estimate the external exposure in each species that would be needed to produce the same internal level of exposure at a target site or at some surrogate site. The PBTK model thus accounts for toxicokinetic differences in extrapolating the external dose at the POD for the rat to an equivalent external dose for humans based on the resulting internal dose that would be expected to result in similar level of response. For acrylamide, the internal dose metric used to estimate this human equivalent concentration (HEC) was the average concentration of acrylamide in the blood over a 24 hour period (or the area under the time-concentration curve for a 24 hour period). Since acrylamide rapidly distributes throughout the body, the acrylamide AUC in blood is considered to be a good surrogate for levels at the putative target site (brain or nerve tissue). The acrylamide PBTK model is based on sufficient animal data to support this use, however, the human data are limited contributing to some uncertainty as to the accuracy of the overall PBTK model's simulation results. It is unknown what the direction would be on risks to

humans from an inaccurate PBTK model estimate of the HEC; the actual risk to humans could be higher or lower. Additional human and animal kinetic data are being developed in research centers around the world to further support the AA PBTK model and increase the confidence in it's predictive capability.

PBTK model estimate of the inhalation HEC (route-to-route extrapolation to derive the RfC)

Unlike for interspecies extrapolation, there are no credible default approaches to conduct a route-to-route extrapolation. The AA PBTK model does so by estimating the daily inhaled intake level (mg/m^3) that would be needed to produce a human AUC for acrylamide in the blood comparable to the level estimated from an oral exposure at the POD. That daily inhaled intake level is then further adjusted with the default factors to account for uncertainty in interspecies toxicodynamic differences and human variability to derive the RfC.

A route-to-route extrapolation of the RfC from the oral dose-response data is justified based upon: (1) considerable evidence from occupational experience demonstrating that dermal and inhalation exposure results in AA-induced peripheral neuropathy (including development of the types of degenerative lesions observed in nerves of rats exposed via drinking water); (2) evidence that tissue distribution in rats is similar following i.v., i.p., oral, dermal, and inhalation exposure to AA (Sumner et al., 2003; Kadry et al., 1999; Dow Chemical Co., 1984; Miller et al., 1982; Hashimoto and Aldridge, 1970); (3) evidence that the elimination kinetics of radioactivity from either oral or i.v. administration of radiolabeled AA in rats are similar (Miller et al., 1982); and (4) lack of support for portal of entry effects. Since there are no credible default methods to estimate a safe daily inhaled intake level in the absence of inhalation study data, the level of uncertainty in the RfC based on the AA PBTK model must be compared to the complete uncertainty of having no RfC. As with uncertainty in the accuracy of the model in the derivation of the RfD, further refinements in the model or new data could ↑ or ↓ the estimate of risks to humans.

Use of default factors for the interspecies differences in toxicodynamics in conjunction with the PBTK model

The AA PBTK model replaced the default factor for interspecies toxicokinetic differences of 3 ($UF_{A\text{-}TK}$ = 3.16 without the model; $UF_{A\text{-}TK}$ = 1 with the model). A default factor of 3 was used to account for toxicodynamic difference between animals and humans ($UF_{A\text{-}TD}$ of 3; 3.16 rounded down to 3[7]). Thus the overall default factor for interspecies differences using the model was 3 (UF_A = 3 = $UF_{A\text{-}TK}$ of 1 x $UF_{A\text{-}TD}$ of 3). This compares to a default factor of 10

[7] The factor of 10 is actually split into the two toxicokinetic and toxicodynamic components by taking the square root of 10 = 3.16. For convenience when a model is used leaving only the toxicodynamic factor, it is rounded down to 3.

without the model (UF_A = 10 = $UF_{A\text{-}TK}$ of 3.16 x $UF_{A\text{-}TD}$ of 3.16). In the case of AA, using the default approach to derive the RfD[8] would result in the same value 0.003 mg/kg/day as the RfD derived with the PBTK model. One interpretation of this similarity is not that the PBTK model is deficient, rather that the interspecies differences for parent acrylamide toxicokinetics might scale roughly to the ratio of body weight to the ¾ power which for extrapolating between an average rat (350 grams) and human (70kg) is approximately a 3 fold reduction in dose on a mg/kg basis.

How much the default factor over- or underestimates interspecies differences cannot be determined.

Intrahuman Variability

Heterogeneity among humans is another source of uncertainty. In the absence of acrylamide-specific data on human variation, a default UF_H of 10 was used to account for uncertainty associated with human variation in the derivation of the RfD and RfC. How much the default factor over- or underestimates human variability cannot be determined.

Subchronic to Chronic Exposure Extrapolation

Chronic oral toxicity studies for acrylamide were available and acceptable for use in the assessment, precluding the need to use a default factor for extrapolating from a subchronic study (i.e., UF_S = 1).

[8] The RfD using the default approach is 0.003 mg/kg/day. $RfD_{default\ approach}$ = POD of 0.27 mg/kg/day ÷ UF_A of 10 ÷ UF_H of 10 = 0.0027 mg/kg/day; rounded up to 0.003 mg/kg/day.

Table 5-7. Summary of uncertainty in the acrylamide noncancer risk assessment.

Consideration/ Approach	Impact on noncancer risk estimate	Decision	Justification
Completeness of the database	Alternative endpoints not identified in the current database could ↑ the estimated risk in humans from exposure to acrylamide.	The available acrylamide database is sufficiently robust and adequate to identify commonly known endpoints for adverse effects, and to not warrant a $UF_D > 1$.	The animal database is robust and complete by IRIS assessment standards. Although the human data are limited, they clearly demonstrate neurotoxicity as the predominant observable noncancer adverse effect. The animal database for repeated oral exposures is robust and evaluates a wide spectrum of adverse effects. Although animal studies for inhalation exposures are limited, kinetic studies in animals and humans indicate no critical route specific endpoints.
Selection of the most sensitive endpoint relevance to humans	If a more sensitive endpoint than histological changes were demonstrated (e.g., functional or behavioral effects, heritable germ cell effects), there could be an ↑ in the proposed risk to humans.	The available data support neurotoxicity (as determined by histological changes) as the most sensitive endpoint.	Limited human data support neurotoxicity as the most sensitive noncancer endpoint, and this endpoint is well supported by numerous animal studies. Heritable germ cell effects have been reported in animal studies at much higher levels of exposure, and further research is warranted to evaluate the potential for these effects at lower doses. Reproductive effects have been observed in animals, but at NOAEL levels 3-5 fold higher than neurotoxic effects, and no reports were identified of reproductive effects in humans.
Dose-response modeling	Alternative approaches to determining a POD could either ↑ or ↓ the estimated risks to humans.	A BMD analysis with mulitple model choices resulted in adequate fits to the acrylamide dose-response data and provided valid estimates of the POD.	A number of BMD models provided reasonable fits to the acrylamide dose-response data from both bioassays. The model with the best AIC and the lowest POD were chosen as the basis for the RfD. There was reasonably good concordance in the estimated PODs from the best fitting models to the available chronic bioassay data supporting a relatively hgh degree of confidence in the BMD approach.

Consideration/ Approach	Impact on noncancer risk estimate	Decision	Justification
PBTK model use in estimating an oral exposure HEC to derive the RfD	An alternate approach (e.g., using default uncertainty factors) could either ↑ or ↓ the estimated risks to humans.	The PBTK model was used to estimate the human equivalent dose used in the derivation of the RfD.	The acrylamide PBTK is a published and peer reviewed model that has been further recalibrated and tested against more recent data. It provides a more scientifically supportable estimate of the HEC compared to the use of the default factor. The model's functionality and fits to the data were sufficiently good to support its use in the assessment. Additonal data sets are anticpated in the future that can be used to further test the model and reduce uncertainty in the model results.
PBTK model use in estimate the HEC (route-to-route extrapolation) to derive the RfC	An alternate method (e.g., multiple assumptions about absorption and distribution of an inhaled dose) could either ↑ or ↓ the estimated risks to humans.	PBTK model was used in a route-to-route extrapolation to derive an RfC.	Justification for deriving an RfC from the oral RfD is based on human and animal kinetic data that support a model based route-to-route extrapolation and the assumptions of comparable responses for comparable internal levels of exposure reagardless of the route of exposure.
Default uncertainty factor used to account for interspecies differences in toxicodynamics ($UF_{A\text{-}TD}$ of 3.16; rounded to 3)	The magnitude of possible over- or underestimation in the default uncertainty factor for interspecies differences in toxicodynamics could ↑ or ↓ the estimated risks to humans.	The default toxicodynamic uncertainty factor was used in conjunction with the PBTK model derived HEC in the derivation of the RfD and RfC.	The default uncertainty factor for toxicodynamic differences was used in the absence of an adequately developed and tested PBTD model, or other chemical or species specific data to support a more informed extrapolation. In keeping with the EPA's goal of protecting public health and the environment, the default factor for intraspecies differences in toxicodynamics is used to ensure that the risk to chemicals and stressors are not underestimated.

Consideration/ Approach	Impact on noncancer risk estimate	Decision	Justification
Default uncertainty factor used to account for intrahuman variability: $UF_H = 10$	The magnitude of possible over- or underestimation in the default factor for intrahuman differences could ↑ or ↓ the estimated risks to humans.	The default uncertainty factor for human variability was used.	The default factor for intrahuman variability was used in the absence of an adequately developed and tested PBPK/PD model (or other chemical and human data) that would support a more informed estimate of intrahuman variability. As above, the default factor for intrahuman variability is used to ensure that the risk to chemicals and stressors are not underestimated.

5.4. CANCER ASSESSMENT

5.4.1. Choice of Study/Data—with Rationale and Justification

As summarized in Section 4.6.1, acrylamide is likely to be carcinogenic to humans based on findings of increased incidences of thyroid follicular cell tumors (combined adenomas and carcinomas in either sex), scrotal sac mesotheliomas (males), mammary gland tumors (females) in two chronic drinking water exposure bioassays with F344 rats, and CNS tumors (Friedman et al., 1995; Johnson et al., 1986); increased incidences of skin tumors in SENCAR and Swiss-ICR mice given oral, i.p., or dermal initiating doses of AA followed by tumor-promoting doses of TPA (Bull et al., 1984a,b); and increased incidences of lung tumors in strain A/J mice following i.p. injection of AA (Bull et al., 1984a). Evidence from available human studies is judged to be limited to inadequate. No statistically significant increased risks for cancer-related deaths were found in two cohort mortality studies of AA, with the exception of the finding in one study of an increased risk of pancreatic cancer in a subgroup of workers with the highest cumulative AA exposure (Marsh et al., 1999; Collins et al., 1989). Most of the available epidemiology studies on increased risk of cancer from AA in food have been conducted by Mucci and colleagues including three case-control studies for increased risk of cancers of the large bowel, bladder, kidneys, renal cell or breast (Mucci et al., 2003, 2004, 2005), and one prospective study for colorectal cancers (Mucci et al., 2006). In another large case-control study, Pelucchi et al. (2006) evaluated the relation between dietary AA intake and cancers of the oral cavity and pharynx, esophagus, large bowel, rectum, larynx, breast, ovary and prostate. None of these studies report a significant increased incidence of cancer associated with increased intake of AA in food at the levels of intake observed.

The mechanisms by which AA induces cancer in animals are not fully understood, however, the weight of the scientific evidence strongly supports a mutagenic mode of action (MOA), as discussed in Section 4.7.3.1. An alternative MOA has been proposed for the development of AA-induced thyroid follicular cell tumors, scrotal sac mesotheliomas, and mammary gland tumors in rats (see Section 4.7.3.2); however, the available evidence is limited or nonexistent, and is not informative concerning the relevance of these tumors (or the other tumors observed in animals) to humans[9]. Therefore, the cancer dose-response relationships for

[9] As discussed in detail in Section 4.7.3, the evidence that acrylamide-induced mesotheliomas in male Fischer 344 rats may not be relevant to humans includes observations that acrylamide caused decreased circulating levels of prolactin in male Fischer 344 rats (presumably through dopamine agonist activity at the D2 dopamine receptor); that chemicals that induce Leydig cell tumors in rats are generally not considered relevant to humans because, unlike rat Leydig cells, human Leydig cells do not decrease luteinizing hormone receptors in response to decreased prolactin; and the extent of Leydig cell neoplasia has been linked to the development of malignant

tumors with statistically significantly elevated incidences in both of the available rat bioassays (thyroid tumors in both sexes, mammary gland tumors in females and tunica vaginalis mesotheliomas in males) are the best available basis for deriving an oral cancer slope factor and inhalation unit risk for AA. The Johnson et al. (1986) cancer rat bioassay reported increased tumor incidence at other sites in females (CNS, oral cavity, pituitary gland); however, this study had abnormally high CNS and oral cavity tumors in control males and possible confounding effects from a viral infection. The Friedman et al. (1995) study did not reproduce the CNS or oral tumors and was designed to address some of the deficiencies in the Johnson et al. (1986) study. These improvements included different dose spacing to support better characterization of the dose-response relationship, and a substantially larger control group (n=204) and 0.1 mg/kg-day male rat group (n = 204) to increase the statistical power in the study to detect significantly increased tumor incidence. The resulting data further supported selection of the tumor types that were replicated in the second study (thyroid, mammary, and testicular tumors) for development of the oral slope factor.

5.4.2. Dose-Response Data

Incidences of tumors with statistically significant increases in both of the 2-year bioassays with F344 rats exposed to AA in drinking water are shown in Table 5-8. As discussed in the previous section, incidence data for thyroid tumors in male and female rats, tunica vaginalis mesotheliomas in male rats, and mammary gland tumors in female rats from the Friedman et al. (1995) bioassay were chosen for oral slope factor development to support reliable characterizations of dose-response relationships.

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mesotheliomas in control and acrylamide-exposed male Fischer 344 rats. However, additional support for this proposal, such as the lack of mesotheliomas in other animal species exposed to acrylamide, is not currently available. In the absence of additional support for this proposal, the male rat mesotheliomas are assumed to be relevant to humans.

Table 5-8. Incidence of tumors with statistically significant increases in a 2-year bioassay 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**
Friedman et al., 1995/males[a]								
Follicular cell adenoma/carcinoma	3/100	2/102[c]	–	12/203	5/101	–	17/75[d]	–
Tunica vaginalis mesothelioma[b]	4/102	4/102	–	9/204	8/102	–	13/75[d]	–
Friedman et al., 1995/females[a]								
Follicular cell adenoma/carcinoma	1/50	1/50	–	–	–	10/100	–	23/100[e]
Mammary malignant/benign	7/46	4/50	–	–	–	21/94[e]	–	30/95[e]

[a]Two control groups were included in the study design to assess variability in background tumor responses.
[b]Incidences reported herein are those originally reported by Friedman et al. (1995) and not those reported in the reevaluation study by Iatropoulos et al. (1998).
[c]The data reported in Table 4 in Friedman et al. (1995) lists 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.
[d]Statisically significant ($p < 0.05$).
[e]Statisically significant ($p < 0.001$).

Source: Friedman et al. (1995).

5.4.3. Dose Adjustments and Extrapolation Method(s)

The current EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) indicate that the method used to characterize and quantify cancer risk from a chemical is determined by what is known about the MOA of the carcinogen and the shape of the cancer dose-response curve. The dose response is assumed to be linear in the low dose range, when evidence supports a mutagenic MOA because of DNA reactivity, or if another MOA that is anticipated to be linear is applicable. The linear approach is used as a default option if the MOA of carcinogenicity is not understood. (U.S. EPA, 2005). In the case of acrylamide, there are data available that support a mutagenic mode of carcinogenic action. Thus, a linear-low-dose extrapolation approach was used to estimate human carcinogenic risk associated with acrylamide exposure.

Data for both the individual incidence and the incidence of tumor bearing animals in the Friedman et al. (1995) drinking water bioassays were modeled to derive potential points of departure for an oral slope factor and inhalation unit risk. For males, the tumor types were tunica vaginalis mesotheliomas or thyroid follicular cell (adenoma/carcinoma). For females, the tumor types were mammary gland tumors (malignant and benign combined) or thyroid follicular cell (adenoma/carcinoma).

Details of the modeling are described in Appendix D. Briefly, the female data were fit with the multistage model to estimate the BMD, which is the same as the effective dose (ED), and the 95% lower confidence limit on the BMD, the BMDL (or 95% lower bound of the ED

[LED]). Because male rats in the highest dose group in the Friedman et al. (1995) study showed early mortalities (75% vs. 53% and 44% in control groups 1 and 2; statistical analysis not reported), the multistage-Weibull model—which adjusted for early mortality—was fit to the data for tunica vaginalis mesotheliomas and thyroid follicular cell adenoma and carcinoma, using the licensed software MULTI-WEIB (KS Crump and Company, Ruston LA). The model includes two explanatory variables, dose and time to death with tumor, for predicting probability of tumor occurrence; the mathematical function for dose is a polynomial exponential (i.e., multistage) function and time to death is described as a Weibull function. Pathology reports for individual rats in the study (Tegeris Laboratories, 1989) were examined to extract time-to-death and tumor occurrence data for each animal. The incidence of mortality rate in female rats between the high dose (49%) and the two control groups (40 and 28%) was similar. Consequently, it was judged that the multistage-Weibull model would not provide an appreciably different estimate of risk for either tumor site, and a time-to-tumor modeling approach was not applied.

The POD results for modeling the female mammary tumor and thyroid tumor incidence data separately are presented in Table 5-9. In addition, the results for considering female rats with either tumor are also presented in Table 5-9. The rat slope factors corresponding to mammary tumors and to follicular cell thyroid tumors in female F344 rats were very similar, 0.13 vs. 0.11 $(mg/kg\text{-}day)^{-1}$. The BMR was selected so as to use a low benchmark response level as a point of departure for a cancer response while maintaining the BMD close to the empirical data. For the female rat data, the BMR of 10% was chosen for both tumor types when analyzed separately. Given that there was more than one tumor site, basing the unit risk on one tumor site may underestimate the carcinogenic potential of AA. The EPA cancer guidelines (U.S. EPA, 2005) suggest two approaches for calculating the risks when there are multiple tumor sites in a data set to assess the total risk. The simpler approach suggested in the cancer guidelines would be to estimate cancer risk from the incidence of tumor-bearing animals. EPA traditionally used this approach until the NRC (1994) *Science and Judgment* document indicated that evaluating tumor-bearing animals would tend to underestimate overall risk when tumor types occur in a statistically independent manner. The NRC-recommended approach involves adding distributions of the individual tumor incidence to obtain a distribution of the summed risk for all etiologically different tumor types. Consistent with the 2005 cancer guidelines, both approaches were considered for this assessment (see Table D-3 for the summed risk of mammary or thyroid tumors in female F344 rats). The point of departure for the combined incidence approach was based on 20% extra risk, because 20% was the lowest extra risk consistent with the lower end of the observed data range. The BMD_{20} is 1.2 mg/kg-day, and the $BMDL_{20}$ is 0.88 mg/kg-day. For linear low-dose extrapolation, the rat slope factor associated with this combined

risk is 0.2/0.88 (mg/kg-day)$^{-1}$, or 0.23 (mg/kg-day)$^{-1}$, approximately two-fold higher than either of the risks estimated from the individual sites (see Appendix D for more details). Both approaches yielded a similar result when rounded to one significant digit, 0.2 (mg/kg-day)$^{-1}$.

Table 5-9. Points of departure from multistage model fits and rat slope factors derived from incidences of mammary tumors alone, thyroid tumors alone, or combined incidence of mammary or thyroid tumors in female rats exposed to acrylamide in drinking water

Incidence modeled	BMD_R[a] (mg/kg-day)	$BMDL_R$[a] (mg/kg-day)	Rat Slope factor [risk level/BMDL] (mg/kg-day)$^{-1}$
Mammary tumors	1.2	0.78	1.3×10^{-1}
Follicular cell thyroid tumors	1.3	0.94	1.1×10^{-1}
Mammary or thyroid tumors[b]	1.2	0.88	2.3×10^{-1}

[a]R = 10% extra risk for mammary tumors, thyroid tumors; 20% for the incidence of either tumor type.
[b]Tumor-bearing animal method: Individual rats that had more than one of the tumor types were counted only once (see Table D-1 for incidences). For the NRC (1994) approach, the rat slope factor was 0.24 (see Appendix D).

Data source: Friedman et al. (1995).

Because of mortality issues in the male rat data, time-to-tumor modeling was used (see Appendix D). The time-to-tumor results for the male tunica vaginalis mesothelioma (TVM) and thyroid tumor incidence data evaluated separately or combined are presented in Table 5-10. For the male rat data, the BMDs and BMDLs were linear with risk in the range of 1–10% risk (see model output in Appendix D). Consequently, the BMR of 10% was chosen for estimating rat slope factors. As with the female rats, two methods were considered for estimating total cancer (see Table D-5 for the summed risk of tunica vaginalis mesotheliomas or thyroid tumors in male F344 rats). Both approaches (tumor-bearing and summed risk) yielded a similar result for risks from multiple tumor sites when rounded to one significant digit, 0.3 (mg/kg-day)$^{-1}$.

Table 5-10. Predictions from time-to-tumor model for doses associated with 10% extra risk for TVM alone, thyroid tumors alone, or combined TVM or thyroid tumors in male rats exposed to acrylamide in drinking water, with associated rat cancer slope factors

Incidence modeled	BMD_R[a] (mg/kg-day)	$BMDL_R$[a] (mg/kg-day)	Rat Slope factor [risk level/BMDL] $(mg/kg\text{-}day)^{-1}$
TVM	1.2	0.75	1.3×10^{-1}
Follicular cell thyroid tumors	0.71	0.45	2.2×10^{-1}
TVM or thyroid tumors[b]	0.70	0.30	3.3×10^{-1}

[a]R = 10% extra risk.
[b]Tumor-bearing animal method: Individual rats that had more than one of the tumor types were counted only once (see Table D-1 for incidences). For the NRC (1994) approach, the rat slope factor was 0.34 (see Appendix D).

Data source: Friedman et al. (1995).

For linear low-dose extrapolation, the rat slope factor associated with the $BMDL_{10}$ of 0.3 mg/kg-day for combined TVM and thyroid tumor incidence is 0.1/(0.3 mg/kg-day), or $0.33\ (mg/kg\text{-}day)^{-1}$, approximately 50% higher than the risk for just thyroid tumors, $0.22\ (mg/kg\text{-}day)^{-1}$ and 2.5-fold higher than for testicular tumors, $0.13\ (mg/kg\text{-}day)^{-1}$ (see Appendix D for more details).

Based on the analyses discussed above, the recommended upper bound estimate on human extra cancer risk from continuous, lifetime oral exposure to AA (see Sections 5.3.4 and 5.3.5 for derivation) should be based on a rat slope factor $0.3\ (mg/kg\text{-}day)^{-1}$ as derived from the male rat data for the risk of TVM or thyroid tumors. The human equivalent slope factor (derived below) should not be used with human equivalent exposures greater than those corresponding to the highest exposure in the male rat bioassay (2.0 mg/kg-day) because above this level the dose-response relationships of the observed tumor types are not likely to continue linearly and there are no data to indicate where the nonlinearity would begin to occur.

5.4.4. Human Equivalent Concentration Using the PBTK Model

The recalibrated Kirman et al. (2003) PBTK model (discussed in Section 3.5) was used to simulate the rat internal exposure from the external BMDLs, then to simulate the external HEC that would be needed to result in that same internal exposure. Both PBTK model variations were used: enzyme-catalyzed glutathione binding of AA and GA and passive glutathione binding of AA and GA. The most appropriate measure of internal dose was considered to be the AUC in blood for the GA metabolite. The choice of AUC as the dose metric is based on a mutagenic MOA for a GA-induced carcinogenic endpoint and the assumption that total amount of this active metabolite would more strongly correlate to incidence of tumors than other dose metric

choices such as peak concentration, time above peak, or average concentration. The choice of AUC in the blood compartment is based on the available blood level (or hemoglobin adduct) data that support model calibration and simulation of the blood compartment compared to the relatively sparse data for tissue levels of glycidamide or the most tumor susceptible tissues in humans.

Table 5-11 presents the model simulation results of the GA AUC for the male BMD_{10} and $BMDL_{10}$ and the human equivalent daily oral intake that would produce that same AUC in humans from a drinking water exposure and the drinking water concentration, assuming a 70 kg person who drinks 2 L/day. For the drinking water simulations in humans, the PBTK model used an ingestion pattern that divided the total daily intake among five daily water bolus ingestions, three of which were at meal times (8 am, 1 pm, and 6 pm), each amounting to 25% of the total daily intake, and two that were in between meals (10:30 am and 3:30 pm), each amounting to 12.5% of the total daily intake.

Table 5-11. PBTK model simulation results for HEC based on male rat carcinogenicity data

BMD analysis results (mg/kg-day)[a]	**BMD_{10}**	**$BMDL_{10}$**
	0.7	0.3
PBTK Model 1—enzyme catalyzed glutathione binding		
AUC for GA in rat blood (μM-hour)	35.3	15.1
Human equivalent intake (mg/kg-day) for a comparable GA AUC in blood, based on a drinking water exposure	0.52	**0.22**
Drinking water concentration that would result in the HEC daily intake (mg/L) (70 kg person, 2 L/day)	18.3	7.8
PBTK Model 2—passive glutathione binding		
AUC for GA in rat blood (μM-hour)	36.0	15.4
Human equivalent intake (mg/kg-day) for a comparable GA AUC in blood, based on a drinking water exposure	0.53	**0.23**
Drinking water concentration that would result in the HEC daily intake (mg/L) (70 kg person, 2 L/day)	18.5	7.9

[a]See Tables D-4 and D-5 for BMD_{10} and $BMDL_{10}$ derivations for male TVM or thyroid tumors.

Data source: Friedman et al. (1995).

5.4.5. Oral Slope Factor and Inhalation Unit Risk

5.4.5.1. *Oral Slope Factor*

A linear extrapolation approach is taken based on the assumption that AA likely induces cancer through a mutagenic MOA at dose levels below the POD. Support for this approach includes observations of: (1) strong evidence of mutagenicity in somatic cells and male germ cells from in vivo assays; (2) male-mediated dominant lethal mutations following subchronic oral exposure at dose levels (2.8 to 13.3 mg/kg-day) in the vicinity of chronic oral dose levels

that induced carcinogenic effects in rats (0.5 to 3 mg/kg-day); (3) initiation of skin tumors (presumably via a genotoxic action) in mice by short-term exposure to oral doses as low as 12.5 mg/kg-day followed by TPA promotion; (4) metabolism of AA by CYP2E1 to the DNA-reactive metabolite, GA; (5) following an i.p. dose of AA or GA, DNA adducts of GA observed in all tissues where tumors have been observed in rats and mice.

The values used to derive an HEC POD as the basis for cancer risks in humans orally exposed to acrylamide, are the male rat BMD_{10} of 0.7 mg/kg-day and $BMDL_{10}$ of 0.3 mg/kg-day for the combined risk of male rats bearing TVM or thyroid tumors. The PBTK model was used to extrapolate to the HEC-BMD_{10} of 0.52 mg/kg-day for the model with enzyme-catalyzed glutathione binding and 0.53 mg/kg-day for the model with passive glutathione binding. The PBTK model yielded an HEC-$BMDL_{10}$ of 0.22 mg/kg-day for the model with enzyme-catalyzed glutathione binding and 0.23 mg/kg-day for the model with passive glutathione binding. There is little difference between results from the two variations of the PBTK model, and, in light of the uncertainty as to which type of binding occurs in humans, the lower value of 0.22 mg/kg-day is used as the POD. The human oral slope factor is derived by linear extrapolation from the POD to the origin, corrected for background. The slope of the linear extrapolation from the $HEC_{PBTK\ model}$-BMD_{10} (a central estimate) is 0.19 $(mg/kg\text{-}day)^{-1}$ (response rate/BMD_{10}; 0.1/[0.52 mg/kg-day] = 0.19 $[mg/kg\text{-}day]^{-1}$) and the oral slope factor derived from the linear extrapolation from the $HEC_{PBTK\ model}$-$BMDL_{10}$ is 0.45 $(mg/kg\text{-}day)^{-1}$ (response rate/$BMDL_{10}$; 0.1/0.22 [mg/kg-day] = 0.45 $[mg/kg\text{-}day]^{-1}$).

With rounding to one significant figure, the human oral slope factor based on the $HEC_{PBTK\ model}$-$BMDL_{10}$ is 0.5 $[mg/kg\text{-}day]^{-1}$.

The human slope factor for acrylamide should not be used with exposures exceeding the POD (LED_{10}), because above this level the fitted dose-response model better characterizes what is known about the carcinogenicity of acrylamide. Additionally, ADAFs combined with age specific exposure estimates should be applied to this slope factor when assessing cancer risks to individuals <16 years old or for lifetime exposures that begin in less than 2-year-olds (U.S. EPA, 2005b) [see Section 5.4.6].

5.4.5.2. *Inhalation Unit Risk*

A PBTK model is used to conduct a route-to-route extrapolation from the acrylamide oral cancer bioassay results to an equivalent inhalation exposure, i.e., inhalation exposures that result in comparable internal doses as those obtained from an administered oral dose. Support for use of PBTK model in the derivation of an inhalation unit risk value based on oral data comes from: (1) evidence that tissue distribution in rats is similar following i.v., i.p., oral, dermal, and

inhalation exposure to AA (Sumner et al., 2003; Kadry et al., 1999; Dow Chemical Co., 1984; Miller et al., 1982; Hashimoto and Aldridge, 1970); (2) the widespread distribution of AA and the formation of GA adducts in diverse tissue throughout the body (Doerge et al., 2005a); (3) evidence that the elimination kinetics of radioactivity from oral or i.v. administration of radiolabeled AA in rats was similar (Miller et al., 1982); and (4) lack of support for portal-of-entry effects. The PBTK model also accounts for first-pass metabolism, one of the main factors that can lead to different distributional profiles between an oral and an inhalation exposure.

The inhalation unit risk for AA is based on adult exposures and is derived from the HEC of the rat bioassay $BMDL_{10}$, which is the 95% lower bound on the exposure associated with a 10% extra cancer risk, by dividing the risk (as a fraction) by the $BMDL_{10}$. The inhalation unit risk represents an upper bound risk estimate for continuous lifetime exposure without consideration of increased early life susceptibility due to AA's mutagenic MOA.

The PBTK model is used to derive an inhalation HEC-BMD_{10} and HEC-$BMDL_{10}$ based on comparable levels achieved of the AUC for GA in blood to those from the oral exposure BMD_{10} of 0.7 mg/kg-day and the $BMDL_{10}$ of 0.3 mg/kg-day in male F344 rats for combined incidence of animals bearing tunica vaginalis mesotheliomas or thyroid tumors (Friedman et al., 1995). The PBTK model predicted an oral $HEC_{PBTK\ model}$-BMD_{10} of 0.53 mg/kg-day and an HEC-$BMDL_{10}$ of 0.22 mg/kg-day. The air concentrations required to achieve these daily intake levels, assuming a continuous 24-hour inhalation exposure for a 70 kg person who breathes 20 m^3/day air are 1.84 mg/m^3 for the $HEC_{PBTK\ model}$-BMD_{10} and 0.79 mg/m^3 for the $HEC_{PBTK\ model}$-$BMDL_{10}$ (see Table 5-12). The slope of the linear extrapolation from the HEC-BMD_{10} (a central estimate) of the equivalent air concentration as a POD is 5×10^{-5} $(\mu g/m^3)^{-1}$ and the inhalation unit risk based on the HEC-$BMDL_{10}$ equivalent air concentration as a POD is 1.3×10^{-4} $(\mu g/m^3)^{-1}$.

$HEC_{PBTK\ model}$-$BMDL_{10}$ equivalent air concentration, lower 95% bound on exposure at 10% extra risk—0.79 mg/m^3

$HEC_{PBTK\ model}$-BMD_{10} equivalent air concentration, central estimate of exposure at 10% extra risk—1.84 mg/m^3

Inhalation unit risk based on the $HEC_{PBTK\ model}$-$BMDL_{10}$

$= 0.1/0.79\ mg/m^3 = 1.3 \times 10^{-4}\ (\mu g/m^3)^{-1}$;

With rounding to one significant figure, the inhalation unit risk is 1×10^{-4} $(\mu g/m^3)^{-1}$.

As noted in the discussion on the oral slope factor, age-dependent adjustment factors (ADAFs) combined with age-specific exposure estimates should be applied to this inhalation unit risk when assessing cancer risks to individuals <16 years old or for lifetime exposures that begin in less than 2-year-olds (U.S. EPA, 2005b) [see Section 5.3.6].

This derivation of the inhalation unit risk is not suitable to derive MLEs or 95% UCLEs for lifetime inhalation exposure concentrations that would be associated with various levels of risks. Appendix D provides an alternate derivation for MLEs and 95% UCLEs with similar results.

Table 5-12. PBTK model simulation results for HEC to derive the inhalation unit risk based on male rat oral exposure cancer data

	BMD_{10}	$BMDL_{10}$
BMD_{10} analysis results (mg/kg-day)[a]	0.7	0.3
PBTK Model 1—enzyme catalyzed glutathione binding AUC for GA in rat blood (μM-hour)	35.3	15.1
Human equivalent intake (mg/kg-day) for a comparable GA AUC in blood, based on a continuous air exposure	0.53	0.22
Air concentration that would result in the HEC daily intake (mg/m^3) (70 kg person, 20 m^3/day)	1.84	0.79
PBTK Model 2—passive glutathione binding AUC for GA in rat blood (μM-hour)	36.0	15.4
Human equivalent intake (mg/kg-day) for a comparable GA AUC in blood, based on a continuous air exposure	0.54	0.23
Air concentration that would result in the HEC daily intake (mg/m^3) (70 kg person, 20 m^3/day)	1.89	0.81

[a]See Tables D-4 and D-5 for BMD and $BMDL_{10}$ derivations.

Data source: Friedman et al. (1995).

5.4.6 Application of Age-Dependent Adjustment Factors

Because a mutagenic MOA for AA carcinogenicity is sufficiently supported in laboratory animals and relevant to humans (Section 3.4.1), and in the absence of chemical-specific data to evaluate differences in susceptibility, increased early-life susceptibility is assumed and the age-dependent adjustment factors (ADAFs) should be applied, as appropriate, in accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). The oral slope factor of 0.5 per mg/kg-day and the inhalation unit risk of 1×10^{-4} per $\mu g/m^3$, calculated from data for adult exposures, do not reflect presumed early-life susceptibility for this chemical. Example evaluations of cancer risks based on age at exposure are given in Section 6 of the *Supplemental Guidance*.

The *Supplemental Guidance* establishes ADAFs for three specific age groups. The current ADAFs and their age groupings are 10 for <2 years, 3 for 2 to <16 years, and 1 for 16 years and above (U.S. EPA, 2005b). The 10-fold and 3-fold adjustments in slope factor are to be combined with age specific exposure estimates when estimating cancer risks from early life (<16 years age) exposure to AA. These ADAFs and their age groups were obtained from the 2005 *Supplemental Guidance*, and they may be revised over time. The most current information on the application of ADAFs for cancer risk assessment can be found at www.epa.gov/cancerguidelines/. In estimating risk, EPA recommends using age-specific values for both exposure and cancer potency; for AA, age-specific values for cancer potency are estimated using the appropriate ADAFs. A cancer risk is derived for each age group, and these are summed across age groups to obtain the total risk for the exposure period of interest.

Oral exposure

To illustrate the use of the ADAFs established in the 2005 *Supplemental Guidance* (U.S.EPA, 2005b), some sample calculations are presented for three exposure duration scenarios, including full lifetime, assuming a constant AA exposure of 1 μg/kg-day.

70-year exposure to 1 μg/kg-day AA from ages 0-70:

Age group	ADAF	unit risk (per μg/kg-day)	exposure concentration (μg/kg-day)	duration adjustment	partial risk
0 - < 2 years	10	0.5×10^{-3}	1	2 years/70 years	1.4×10^{-4}
2 - < 16 years	3	0.5×10^{-3}	1	14 years/70 years	3.0×10^{-4}
≥ 16 years	1	0.5×10^{-3}	1	54 years/70 years	3.9×10^{-4}
					Total risk = 8×10^{-4}

Note that the partial risk for each age group is the product of the values in columns 2-5 [e.g., $10 \times (0.5 \times 10^{-3}) \times 1 \times 2/70 = 1.4 \times 10^{-4}$], and the total risk is the sum of the partial risks. Thus, a 70-year risk estimate for a constant exposure of 1 μg /kg-day is equivalent to a lifetime unit risk estimate of 8×10^{-4} per μg/kg-day, adjusted for early-life susceptibility, assuming a 70-year lifetime and constant exposure across age groups.

If calculating the cancer risk for a 30-year exposure to a constant AA exposure level of 1 μg /kg-day from ages 0-30, the duration adjustments would be 2/70, 14/70, and 14/70, and the partial risks would be 1.4×10^{-4}, 3.0×10^{-4}, and 1.0×10^{-4}, resulting in a total risk estimate of 5×10^{-4}.

If calculating the cancer risk for a 30-year exposure to a constant AA exposure level of 1 μg /kg-day from ages 20-50, the duration adjustments would be 0/70, 0/70, and 30/70, and the partial risks would be 0, 0, and 2.1×10^{-4}, resulting in a total risk estimate of 2×10^{-4}.

Inhalation Exposure

To illustrate the use of the ADAFs established in the 2005 *Supplemental Guidance* (U.S.EPA, 2005b), some sample calculations are presented below for three exposure duration scenarios assuming a constant AA exposure of 1 $\mu g/m^3$.

70-year exposure to 1 $\mu g/m^3$ AA from ages 0-70:

Age group	ADAF	unit risk (per $\mu g/m^3$)	exposure concentration ($\mu g/m^3$)	duration adjustment	partial risk
0 - < 2 years	10	1×10^{-4}	1	2 years/70 years	2.9×10^{-5}
2 - < 16 years	3	1×10^{-4}	1	14 years/70 years	6.0×10^{-5}
≥ 16 years	1	1×10^{-4}	1	54 years/70 years	7.7×10^{-5}
					Total risk = 2×10^{-4}

Note that the partial risk for each age group is the product of the values in columns 2-5 [e.g., $10 \times (1x10^{-4}) \times 1 \times 2/70 = 2.9 \times 10^{-5}$], and the total risk is the sum of the partial risks. This 70-year risk estimate for a constant exposure of 1 μg /m^3 is equivalent to a lifetime unit risk estimate of 2×10^{-4} per $\mu g/m^3$, adjusted for early-life susceptibility, assuming a 70-year lifetime and constant exposure across age groups.

If calculating the cancer risk for a 30-year exposure to a constant AA exposure level of 1 $\mu g/m^3$ from ages 0-30, the duration adjustments would be 2/70, 14/70, and 14/70, and the partial risks would be 2.9×10^{-5}, 6.0×10^{-5}, and 2.0×10^{-5}, resulting in a total risk estimate of 1×10^{-4}.

If calculating the cancer risk for a 30-year exposure to a constant AA exposure level of 1 $\mu g/m^3$ from ages 20-50, the duration adjustments would be 0/70, 0/70, and 30/70, and the partial risks would be 0, 0, and 4.3×10^{-5}, resulting in a total risk estimate of 4×10^{-5}.

Other subgroups that may be more or less susceptible to AAs carcinogenic effects include people with DNA repair deficiencies (increased sensitivity to mutagenic events), or who have lower levels or activity of CYP2E1 enzymes due to genetic polymorphisms or age related developmental differences. Those with lower enzyme activity levels could have potentially decreased susceptibility to carcinogenicity due to the lower production of the putative mutagen, the GA active metabolite (see Section 4.8). At present, there are no methods to develop quantitative adjustments in risk for these potential subpopulations.

5.4.7. Uncertainties in Cancer Risk Values

The following discussion identifies uncertainties associated with the estimated risk of cancer in humans from exposure to acrylamide, specifically the cancer oral slope factor (CSF) and the inhalation unit risk (IUR). These uncertainties arise either from incomplete knowledge about acrylamide's toxic effects and mode of action in humans, or because of insufficient or absent data to support key steps in the quantitation of risks.

Uncertainties in the AA cancer risk assessment include: 1) the completeness of the database for identifying AA carcinogenic potential, 2) the choice of the tumor types and their relevance for humans, 3) the choice of methods for modeling the dose-response relationship and estimating the cancer risks, 4) the structure and parameter values of the PBTK model relative to its use in deriving the oral slope factor and the inhalation unit risk (IUR), and 5) the choice of the low-dose linear method of extrapolation from the POD to estimate the CSF and IUR.

In the case of AA, the uncertainties in the underlying data and methods used to derive the CSF and IUR are similar since the IUR is based on the same oral dose-response data used to derive the CSF. The following discussion on uncertainty is therefore applicable to both the CSF and IUR values. The discussion is accompanied by a summary of the main points in Table 5-13.

5.4.7.1. Areas of Uncertainty

Completeness of the Database

Uncertainty in the risk assessment due to lack of completeness of the database is primarily due to the lack of human data. The available human epidemiology studies as of 2007 provide limited to inadequate support for definitive statements. Animal bioassays, however, clearly demonstrate multi-site carcinogenicity, and provide good support for classifying acrylamide as "likely to be carcinogenic to humans" (U.S. EPA, 2005a). The uncertainty in the database is being actively addressed in on-going studies sponsored by US FDA and other

national and international public and private sector organizations. The impact of new data could be to either increase or decrease the estimate of risks of acrylamide induced cancer in humans.

Selection of Bioassay(s), Tumor Types, and Relevance to Humans (i.e., the MOA).

In the absence of direct human data, the most appropriate animal bioassays to use in the derivation of cancer risk values are chronic (i.e., lifetime) studies in two species of rodents for the most relevant route of exposure. Only two chronic bioassays were available for acrylamide exposure via the drinking water, both in the F344 rats (Friedman et al., 1995; Johnson et al., 1986). The Friedman et al. (1995) study addressed some of the deficiencies in the Johnson et al. (1986) study by improving the dose spacing to better characterize the dose-response relationship, and by substantially increasing the size of the control group (n=204) and a 0.1 mg/kg-day male rat group (n = 204) to increase the statistical power to detect increased incidence of tumors. Uncertainty in the choice of bioassay arises because there was only one species tested, data are only available for the oral route of exposure (albeit the most relevant to humans), and the two studies were not conducted by completely independent laboratories (i.e., the primary author of the Friedman et al. [1995] study was also an author for the Johnson et al. [1986]). On-going National Toxicology Program (NTP) studies at US FDA research laboratories will add considerable new chronic bioassay data in rats and mice for both acrylamide and glycidamide (U.S. FDA [2006b]). The impact of these new data could be either to increase or decrease the estimate of risks of acrylamide induced cancer in humans

Tumor types that were consistently observed to increase in both of the available chronic rat drinking water bioassays included statistically significant increases in thyroid follicular cell adenomas or carcinomas in male and female rats, tunica vaginalis testis (i.e., scrotal sac) mesotheliomas in male rats, and mammary gland tumors (adenomas, fibroadenomas or fibromas) in female rats. These were the tumor types used in the derivation of the CSF and IUR. Uncertainty in the selection of tumor type arises because Johnson et al. (1986) reported a variety of other tumor types in females (CNS, oral cavity, pituitary gland). Although the Johnson et al. (1986) study had abnormally high CNS and oral cavity tumors in control males and possible confounding effects from a viral infection including, the CNS tumors are of concern considering acrylamide's known neurotoxicity. Rice (2005) has raised an issue of under-reporting of CNS tumors in the Friedman et al (1995) study, and this is a significant source of uncertainty. The impact of the new data to be reported from the NTP studies may resolve this issue of types of animal tumors consistently induced, however it is not known whether the incidence data will increase or decrease the estimate of risks of acrylamide induced cancer in humans.

The relevance of the tumor types observed in animals to humans based on a proposed mode of action was considered in Section 4.8.3. The available limited human data do not provide any support for acrylamide induced thyroid, mammary, scrotal sac, or brain tumors in humans. The precise mechanism(s) by which the multi-site carcinogenicity occurs in animal models is not well-established, however, currently available information indicate that AA and GA covalently bind and modify proteins, and that the mutagenic events that lead to tumors from exposure to AA are most likely produced by GA via direct alkylation of DNA. The basic biology of DNA adduct formation and subsequent perturbation of gene structure and function is believed to be similar between test animals and humans. Thus, a mutagenic MOA is considered a biologically relevant MOA in humans. Qualitatively, there is considerable evidence in test animal and mammalian cells to support the relevance of a mutagenic MOA for AA in humans. Quantitative data are only available in one in vitro assay that measured mutagenicity directly in human bronchial epithelial cells (Besaratinia and Pfeifer, 2004). The uncertainty in the MOA and significance of the animal tumor types to humans will require additional data to resolve. Additional data are also needed to resolve why only hormonally responsive tissues were observed to have increased tumors in the Friedman et al. (1995) chronic rat bioassay, whereas GA-DNA adducts have been observed in a much wider array of tissues.

Methods for the Dose-Response Modeling and Estimate of Cancer Risks

For acrylamide, there is a lack of knowledge about the underlying biology, but extensive guidance (U.S. EPA, 2005a, 1995) and expert judgment to support a BMD analysis, the choice of the most appropriate model, BMR, and approach for calculating risks when there are multiple tumor types. The male rat incidence data (tunica vaginalis mesotheliomas and/or thyroid tumors) were fit with the multistage-Weibull model that accounts for early mortality because the highest male dose group in the Friedman et al. (1995) study had increased early mortalities compared with controls. Mortality rates among high dose and control female rats were similar, so the female incidence data (mammary gland and/or thyroid tumors) were fit with the multistage model. For the benchmark response level (BMR) as a point of departure for the cancer dose-response, the lowest BMR was selected consistent with a resulting bench mark dose (BMD) that remained close to the empirical data (U.S. EPA 1995).

Model and parameter uncertainty at the BMD can be assessed by comparing the BMD, a central estimate of risk, with the BMDL, which corresponds to the lower statistical confidence limit of a one-sided 95% confidence interval on the BMD. The multistage-Weibull modeling of the Friedman et al. (1995) male rat data yielded a combined incidence BMD_{10} of 0.7 mg/kg-day, and $BMDL_{10}$ of 0.3 mg/kg-day, an approximately 2.3 fold difference. The multistage modeling

of the female rat data yielded a combined incidence BMD_{20} of 1.2 mg/kg-day, and $BMDL_{20}$ of 0.88 mg/kg-day, an approximately 1.4 fold difference. These numbers reflect a relatively low level of uncertainty in the model results for these data sets. The use of the BMD central estimate would decrease the estimated risk of cancer by decreasing the value of the slope factor.

EPA cancer guidelines (U.S. EPA, 2005a) suggest two approaches for calculating the risks when there are multiple tumor sites in a data set to assess the total risk: 1) estimate cancer risk from the incidence of tumor-bearing animals; and 2) adding distributions of the individual tumor incidence to obtain a distribution of the summed risk for all etiologically different tumor types. Both approaches were considered in this assessment. For the male rat data, both approaches (tumor-bearing and summed risk) yielded a similar result for risks from multiple tumor sites when rounded to one significant digit, 0.3 $(mg/kg\ day)^{-1}$. Analysis of the female rat data with both approaches also yielded a similar result for the cancer slope factor when rounded to one significant digit, 0.2 $(mg/kg\text{-}day)^{-1}$. These relatively similar results from the female or male rat data for different approaches to calculating total risk increases the confidence in the results. The impact of additional knowledge about the underlying biological processes or availability of other data sets on the estimated risks of cancer in humans is unknown, and could either increase or decrease the estimated risks.

Adequacy of the PBTK Model for Use in Deriving the CSF and IUR

EPA recalibrated and parameterized a PBTK model for acrylamide (originally developed and published by Kirman et al., 2003) against more recent data (Boettcher et al., 2005; Doerge et al., 2005a,b,c; Fennell et al., 2005; Sumner et al., 2003) for use in deriving toxicity values. The recalibrated AA PBTK model was tested against the new kinetic and hemoglobin binding data in rats, mice, and humans. The model was then used to estimate the oral human equivalent concentration (i.e., extrapolate the animal dose-response relationship to humans) to derive the cancer slope factor, and to conduct a route-to-route extrapolation (oral to inhalation) to derive the inhalation HEC to derive the inhalation unit risk.

There is always some degree of uncertainty in a PBTK model's structure and estimates of internal dose, because PBTK models are simplified mathematical representations of very complex organisms. Within the context of the alternative of using default factors, however, PBTK models are increasingly offering a more scientifically supportable means to: 1) extrapolate risk as observed in studies on test animal to potential risks to humans, 2) account for the most sensitive human subpopulations, or 3) conduct route-to-route extrapolations. For acrylamide, a published and tested PBTK model was available, and in its recalibrated form, provided acceptable fits to recent and relevant data. The resulting fits to the available data were adequate to support the model's use in the assessment. The model was especially important to

estimating the oral human equivalent concentration in the CSF derivation because the putative toxin was the acrylamide metabolite, glycidamide, the internal levels of which the PBTK model did account for, while the default uncertainty factor for interspecies toxicokinetic differences would not. The AA PBTK model was also used to derive an inhalation HEC based on the oral dose-response data, a value that would also otherwise not have been possible since there are no credible default methods for conducting a route-to-route extrapolation.

To estimate the oral exposure HEC, the acrylamide PBTK model first simulated the toxicokinetics of acrylamide and its metabolite, glycidamide, in the Fisher 344 rat used in the chronic bioassays and then in an average male human (i.e., a 70 kg male). The model was exercised to estimate the external exposure in each species that would be needed to produce the same internal level of exposure at a target site or at some surrogate site. The PBTK model thus accounted for toxicokinetic differences in extrapolating the external dose at the POD for the rat to an equivalent external dose for humans based on the resulting internal dose that would be expected to result in a similar level of response. For acrylamide, the internal dose metric used to estimate the oral human equivalent concentration (HEC) was the average concentration of glycidamide in the blood over a 24 hour period (or the area under the time-concentration curve for a 24 hour period). Glycidamide is the putative toxin for carcinogenic effects.

The AA PBTK model was also used to derive an inhalation HEC based on the oral dose-response data, a value that would otherwise not have been possible since there were no available inhalation data to directly derive an IUR, and no credible alternate method for conducting a route-to-route extrapolation. The AA PBTK model does so by estimating the daily inhaled intake level (mg/m^3) that would be needed to produce a human AUC for glycidamide in the blood comparable to the level estimated from an oral exposure at the POD. That daily inhaled intake level is then used as the POD to derive the IUR. The use of the AA PBTK model to estimate the inhalation HEC in the derivation of the IUR based on the oral dose-response data is justified because the model accounts for differences in acrylamide internal disposition following exposure from the oral or the inhalation route of exposure.

Overall, it is unknown how inaccuracies in the AA PBTK model structure or parameter values would impact the risks; further refinements in the model or new data could increase or decrease the estimate of risks of cancer in humans

Choice of Low-dose Extrapolation Approach.

The mode of action discussion in Section 4.8.3 concludes that at present, the mechanistic sequence of events by which AA induces the tumor types observed in the animal studies is not completely defined, however, the majority of the data, support a mutagenic MOA for AA carcinogenicity. An alternative MOA has been proposed for some of the tumors observed in the

animal bioassays (i.e., disruption of hormone levels or activity), but data supporting this MOA are limited or lacking.

In accordance with the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), a mutagenic MOA prompts the use of a linear low-dose extrapolation from the POD to estimate the risk of cancer in humans. The value of the cancer slope factor is accompanied with the caveat that it should not be used with human equivalent exposures greater than those corresponding to the highest exposure in the male rat bioassay (2.0 mg/kg-day) because above this level the dose-response relationships of the observed tumor types are not likely to continue linearly and there are no data to indicate where the nonlinearity would begin to occur. If a new data or a re-analysis of the extant data were to conclude that the MOA for AA carcinogenicity was not a mutagenic MOA or that there were nonlinearities (i.e., specifically sublinearities) in the low level dose-response than the estimated risk of cancer to humans would be decreased. Conversely, if new cancer incidence data supported a steeper dose-response and a linear low dose-response relationship, then the estimate of risk would increase.

Human Population Variability and Sensitive Subpopulations

Neither the extent of interindividual variability in acrylamide metabolism nor human variability in response to acrylamide has been well characterized. Factors that could contribute to a range of human response to acrylamide include variations in CYP450, epoxide hydrolase, or glutathione transferase levels (or activity) because of age-related, gender, or genetic differences or other factors including exposure to other chemicals that induce or inhibit enzyme levels, nutritional status, alcohol consumption, or the presence of underlying disease that could alter metabolism of acrylamide or antioxidant protection systems. Incomplete understanding of the potential differences in metabolism and susceptibility across exposed human populations represents a considerable source of uncertainty. The uncertainties associated with this lack of data and knowledge about human variability can, at present, only be discussed in qualitative terms, however, EPA has developed age-dependent adjustment factors (ADAFs) to quantitatively account for some of the potential differences in age-dependent response to carcinogens with a mutagenic MOA. ADAFs are to be applied to the slope factors when assessing cancer risks for less than 16-year-old subpopulations or for lifetime exposures that begin in less than 2-year-olds (U.S. EPA, 2005b, also see Section 5.4.6).

Table 5-13. Summary of uncertainty in the acrylamide cancer risk assessment.

Consideration/ Approach	Impact on cancer risk estimate	Decision	Justification
Completeness of the database	New data could ↑ or ↓ the estimate of risks for acrylamide induced cancer in humans.	Based on the currently available data, EPA classified AA as "likely to be carcinogenic to humans" (U.S. EPA, 2005a)	The available human epidemiology studies as of 2007 provide limited to inadequate support for definitive statements. Animal bioassays, however, clearly demonstrate multi-site carcinogenicity, and provide good support for acrylamide being classified as likely to be carcinogenic to humans.
Selection of bioassay	Analysis based on alternative bioasssys or human data could ↑ or ↓ the estimated risks of acrylamide related cancer in humans.	The Friedman et al (1995) and Johnson et al. (1986) studies were chosen for use in the derivation of the CSF and IUR.	In the absence of direct human data, the Friedman et al. (1995) and the Johnson et al. (1986) chronic rat drinking water studies were the only available cancer bioassays. Uncertainty in the risk values based on these bioassay arises because there was only one species tested, data are only available for the oral route of exposure (albeit the most relevant to humans), and the two studies were not conducted by completely independent laboratories (i.e., the primary author of the Friedman et al. [1995] study was also an author for the]). On-going National Toxicology Program (NTP) studies will add considerable new chronic bioassay data on tumor types in rats and mice for both acrylamide and glycidamide (U.S. FDA [2006b]).

Consideration/ Approach	Impact on cancer risk estimate	Decision	Justification
Selection of tumor types, and relevance to humans	A different selectionof tumor types from the Johnson et al (1986) study could ↑ or ↓ the estimated risks of acrylamide related cancer in humans.	Tumor types used in the derivation of the CSF and IUR included reproducible and statistically significant increases in thyroid and testicular tumors in male rats, and thyroid and mammary gland tumors in female rats.	The choice of tumor types used in the analysis was based on those tumor that were consistently observed to increase in both of the available chronic rat drinking water bioassays. As to relevance to humans, currently available information indicate that GA directly alkylates DNA, which is the most likely mutagenic event leading to tumorigenicity. The basic biology of DNA adduct formation and subsequent perturbation of gene structure and function is believed to be similar between test animals and humans. Thus, a mutagenic MOA for AA related carconogenicity is considered likely, and is a biologically relevant MOA in humans.
Methods used for the dose-response modeling and estimate of cancer risks.	Alternative approaches to determining a POD could either ↑ or ↓ the estimated risks of acrylamide related cancer in humans.	A BMD analysis was used to fit to the acrylamide dose-response data and provided valid estimates of the POD.	The BMD approach used to develop the POD is in accordance with EPA guidance (U.S. EPA, 2005a, 1995). Model and parameter uncertainty at the BMD was assessed by comparing the BMD with the BMDL, and indicated a relatively low level of uncertainty in the model results. EPA cancer guidelines (U.S. EPA, 2005a) was followed to calculate risks for multiple tumor sites. The relatively similar results from the female or male rat data for different approaches to calculating total risk increased confidence in the results.

Consideration/ Approach	Impact on cancer risk estimate	Decision	Justification
Use of a PBTK model in the derivation of the CSF and the IUR	Alternative methods could ↑ or ↓ the estimate of risks to humans.	A PBTK model for acrylamide was used to estimate the oral human equivalent concentration in the derivation of the CSF, and the inhalation HEC in the derivation of the IUR	The AA PBTK model was especially important to estimating the oral human equivalent concentration in the CSF derivation because the putative toxin was the acrylamide metabolite, glycidamide. The default uncertainty factor for interspecies toxicokinetic differences would not account for differences in the internal levels of GA, while PBTK model could and did. The AA PBTK model was also used to derive an inhalation HEC based on the oral dose-response data, a value that would otherwise not have been possible since there were no available inhalation data to directly derive an IUR, and no credible alternate method for conducting a route-to-route extrapolation.
Choice of low-dose extrapolation approach	An low-dose extrapolation that assumed a nonlinear dose-respopnse relationship at lower doses would likely ↓ the estimated risks.	A linear low-dose extrapolation from the POD was used to estimate the risk of cancer in humans.	In accordance with the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), a mutagenic MOA prompts the use of a linear low-dose extrapolation from the POD. The mode of action discussion in Section 4.8.3 concludes that the majority of the data support a mutagenic MOA for AA carcinogenicity. An alternative MOA has been proposed for some of the tumors observed in the animal bioassays (i.e., disruption of hormone levels or activity), but data supporting this MOA are limited or lacking.

Consideration/ Approach	Impact on cancer risk estimate	Decision	Justification
Method used to protect sensitive subpopulations	Alternative methods could ↑ or ↓ the estimated risk for susceptible subpopulations.	ADAFs are to be applied to the slope factors when assessing cancer risks for less than 16-year-old subpopulations or for lifetime exposures that begin in less than 2-year-olds. ADAF's should only be applied as appropriate and in conjunction with site specific exposure information.	Neither the extent of interindividual variability in acrylamide metabolism nor human variability in response to acrylamide has been well characterized. The uncertainties associated with this lack of data and knowledge about human variability can, at present, only be discussed in qualitative terms, however, EPA has developed age-dependent adjustment factors (ADAFs) to quantitatively account for some of the potential differences in age-dependent response to carcinogens with a mutagenic MOA (U.S. EPA, 2005b).

5.4.8. Previous Cancer Assessment

A cancer assessment for AA was previously entered into the IRIS database on September 26, 1998. Using the EPA cancer classifications at that time, AA was classified as Group B2, a probable human carcinogen, based on inadequate human data and sufficient evidence of carcinogenicity in animals (significantly increased incidences of benign and/or malignant tumors at multiple sites in both sexes of rats and carcinogenic effects in a series of one-year limited bioassays in mice by several routes of exposure). The classification was supported by positive genotoxicity data, adduct formation activity, and structure-activity relationships to vinyl carbamate and acrylonitrile. An oral slope factor of 4.5 $(mg/kg\text{-}day)^{-1}$ and a drinking water unit risk of 1.3×10^{-4} $(\mu g/L)^{-1}$ were derived using a linearized multistage procedural analysis (extra risk) of combined incidence data for tumors in the CNS, mammary and thyroid glands, uterus, and oral cavity in female F344 rats exposed to AA in drinking water for 2 years (Johnson et al., 1986), with the external AA exposure as the dose metric. The current derivation of the oral slope factor of 0.45 $[mg/kg\text{-}day]^{-1}$ (rounded to 0.5 $[mg/kg\text{-}day]^{-1}$) is based on different cancer incidence data (combined incidence of thyroid tumors and tunica vaginalis mesotheliomas in male rats), linear extrapolation from a point of departure determined by a benchmark dose analysis, and the use of a PBTK model to estimate the human equivalent internal levels of GA, the AA metabolite, as the dose metric. Glycidamide is considered to be the putative toxin for the mutagenic MOA leading to carcinnogenicity, and thus a better internal dose metric to correlate to response than the internal (or external) level of AA.

The previous inhalation unit risk of 1.3×10^{-3} $(\mu g/m^3)^{-1}$ was calculated from the oral data and an external exposure level of AA, based on the assumption that the tissue distribution of AA appeared to be quantitatively the same regardless of route of exposure (Dearfield et al., 1988). This assumption was supported by the data on the distribution of AA following oral or i.v. administration in rats (Miller et al., 1982). The current inhalation unit risk of 1.3×10^{-4} $(\mu g/m^3)^{-1}$ is based on EPA's subsequent methodology for inhalation dosimetry (U.S. EPA, 1994, 1989), an improved understanding of the toxicokinetics of acrylamide, and the use of a PBTK model to conduct a route-to-route extrapolation (oral to inhalation) of the dose-response relationship derived from the oral data, and to derive a human equivalent dose based on the internal level of glycidamide.

5.5. QUANTITATING RISK FOR HERITABLE GERM CELL EFFECTS

U.S. EPA's *Guidelines for Mutagenicity Risk Assessment* (1986) describe procedures for the qualitative and quantitative assessment of risk of heritable mutations in human germ cells. Although no studies that directly reported the effects of AA on human germ cells were identified to support a definitive statement about AA's heritable mutagenic effects, there are sufficient animal toxicity data and other supporting data (e.g., toxicokinetics, mechanistic studies in germ and somatic cells) to support the hypothesis that AA is a potential human germ-cell mutagen. In accordance with the Guidelines, the data is sufficient to prompt both a qualitative and quantitative assessment of risk. The qualitative assessment of AA's heritable germ cell effects has been previously discussed in Section 4.4. Presented in Section 5.5 are the results of different approaches to quantitate AA's potential heritable germ cell effects in humans, along with the uncertainties in the underlying assumptions. With the caveat concerning the overall uncertainty in the quantitation, there is further discussion of the estimated incidence of heritable effects given different exposure scenarios including exposure at the levels of the proposed IRIS reference values. Finally, there is a discussion of the data needed to reduce uncertainties in the qualitative and quantitative risk assessment of risk of AA's heritable effects.

5.5.1. Quantitative Approaches

In 1993, a European Commission (EC)/ U.S. EPA workshop was convened to identify the methodology, data requirements, and mechanistic research that was being used to understand and quantitate the human health risk for germ cell mutagens from exposure to genotoxins. The workshop results were published in a special edition of Mutation Research (EC/US EPA Workshop, 1995), and included four case studies, one of which addressed AA's effects (Dearfield et al., 1995). Acrylamide has, perhaps, more quantitative data on genetic and heritable germ cell effects than any other chemical under evaluation in the IRIS Program, yet important data gaps remain that add considerable uncertainty to the human quantitative risk assessment. Dearfield et al. (1995) summarized the data up to 1995, and evaluated several approaches to quantitate the human dose-response for AA induced heritable germ cell effects, including a parallelogram approach, a modified direct approach, and a doubling dose approach. A discussion of each approach are provided below along with the results, key assumptions, and uncertainties in those assumptions.

Parallelogram Approach

The parallelogram approach was originally formulated by F.H. Sobels (1977, 1982, 1989) to derive an estimate (corrected by DNA adduct dosimetry) of the risk of chemically-induced heritable effects in human germ cells. The method consisted of first measuring a common endpoint in human and test animal somatic cells (such as gene mutation in lymphocytes), and in test animal germ cells; then extrapolating the test animal somatic to germ cell mutation rate ratio to estimate the "analogous" mutation rate in human germ cells (which are not directly measurable). A schematic of the original concept is presented in Figure 5-1. The key assumption in this approach is that the ratio of the somatic to germ cell mutation rate in the test animal is the same as the ratio in man for a specified dose range (Waters and Nolan, 1995).

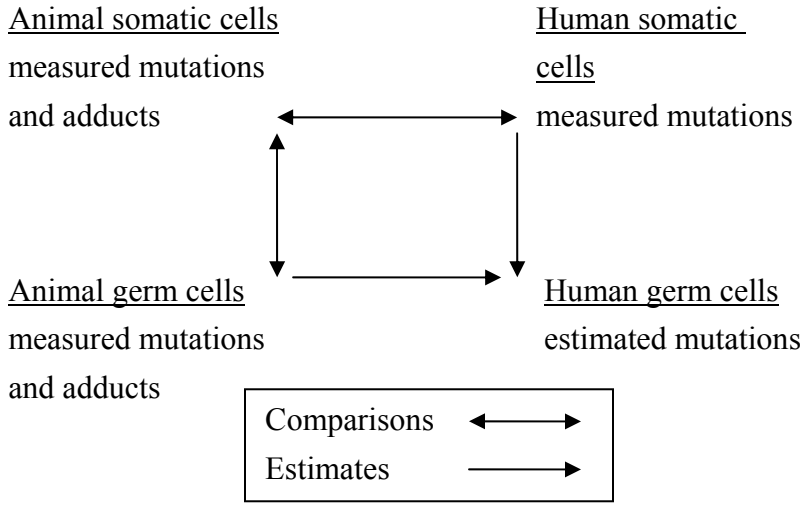

Figure 5-1: Original parallelogram approaches for estimating risk of heritable germ cell effects.

Dearfield et al. (1995) evaluated two modification to the original parallelogram approach for use in quantitating the risk for AA, as presented in Figure 5-2. The first modification (Figure 5-2a) incorporates somatic in vivo data into the parallelogram approach, since by 1995, it was possible to measure mutations in somatic cells in vivo, and to determine the relationship between specific DNA adducts (or other alterations) and outcomes, and whether these relationships are the same among somatic and germ cells treated in vitro and between in vitro and in vivo exposures. The technology was also available to determine the relationship between the applied dose and specific DNA adduct production. A representation of the modifications is shown in Figure 5-2a. The EC/US EPA workshop particpants who evaluated this case study concluded, however, that the modified parallelogram approach in Figure 5-2a was not relevant for AA,

because AA appeared to act primarily via a clastogenic mechanism (e.g., aneuploidy or via protein [e.g., protamine] adduction), and aside from specific-locus mutations suggestive of a point mutation mechanism, there were very few other related data to implement

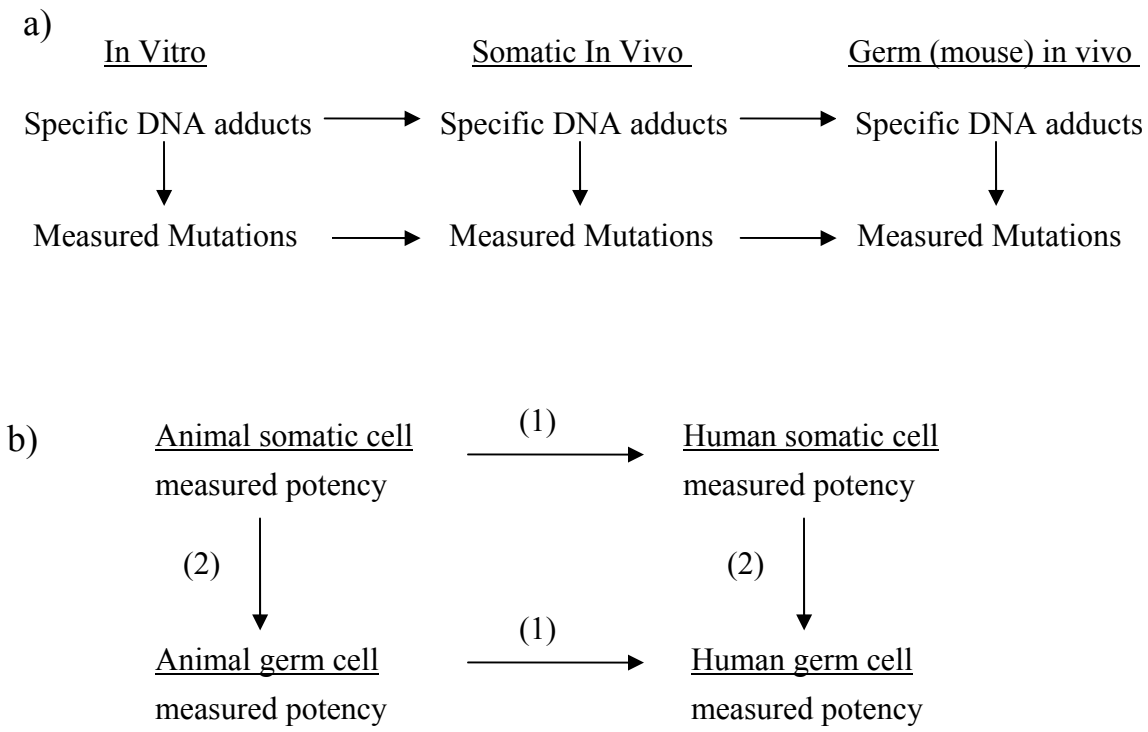

Figure 5-2: Two modifications in the parallelogram approach for estimating risk of heritable germ cell effects from exposure to AA.

the parallelogram approach in Figure 5-2a. Furthermore, there is no representation of human germ cell effects in this modification, nor was information available at the time that related specific DNA-adduct formation to a measured mutational outcome, which remains true as of mid-2007.

A second parallelogram approach shown in Figure 5-2b addresses effects in human germ cells, and assumes that the mathematical relationship "(2)" between the somatic cell and the germ cell effect is the same in rodents and humans. It further assumes that the mouse-to-human somatic cell outcome relationship "(1)" is the same as the mouse-to-human germ cell outcome relationship, and that all three measures of potencies are equivalent. The measured potency, in each case, is derived from a dose-effect relationship, and for example, could be based on specific

DNA adduct formation. As with the approach in Figure 5-2a, however, the types of data needed to implement the approach in Figure 5-2b are not available for AA. Specifically, the only information on AA's effects in human somatic cells, is hemoglobin adduction (Bergmark et al., 1993; Fennell et al, 2005; Boettcher et al., 2005), and GA induced unscheduled DNA synthesis in human epithelial cells in vitro (Butterworth et al., 1992). Other deficiencies in the AA database that preclude implementation of the Figure 5-2b parallelogram approach include: 1) no comparative endpoints in germ cells to establish a similar biological endpoint dosimetry, and 2) no standardized procedures to measure potency of effects in human germ cells following chemical exposure. The parallelogram approach also does not provide a means to estimate increased incidence of genetic disease(s).

As an alternative to the parallelogram approach, the workshop participants determined that enough information was available on AA's heritable effects in mice, and dose-response relationships to chemical mutagens in general, to support quantitation of heritable germ cell effects in humans using either a direct approach (or modified direct approach) or a doubling dose approach.

Uncertainty in the Quantitation of Heritable Germ Cell Effects

Both of the approaches discussed below are based on a number of assumptions about the similarity or differences between mice and human responses and variability of critical processes in the MOA leading to heritable disease. The assumptions as discussed by Ehling (1988) include:

> 1) The amount of genetic damage induced by a given type of exposure under a given set of conditions is the same in the germ cells of mice and humans.
> 2) The various biological and application factors affect the magnitude of the induced mutation frequency in similar ways and to similar extents in mice and in humans.

The parallelogram approach (i.e., relationships "(1)" and "(2)" in Figure 5-2b) was then used to identify data to support estimates of the extrapolation factors for key events in the MOA leading to genetic diseases that could be used to extrapolate from a mouse dose-response to a human dose-response. An International Commission for Protection Against Environmental Mutagens and Carcinogens (ICPEMC) Workgroup in 1993 developed risk extrapolation factors (REFs) to quantitate risk from exposure to acrylamide, and to extrapolate risk from rodent (e.g., mice) experimental models to humans (ICPEMC, 1993a):

Parameter	REF*
Locus specificity	2
DNA repair variability	0.1
Metabolic variability	1
Dose rate variability	1
Exposure route	1
Germ cell stage specificity	1
Dose-response kinetics	1
Overall REF	**0.2**

*An REF of 1 indicates equivalency between the animal and human.

There is considerable uncertainty in the above assumptions and risk extrapolation factors. It was assumed in 1995 that any effects seen in germ cells represented an integration of effects from both the parent AA and its metabolite GA. Although GA has been reported to be as effective as AA in inducing dominant lethal mutations for similar germ cell stage sensitivity (post-meiotic), more recent research has demonstrated that GA is a much more potent inducer of dominant lethal mutations in germ cells (Generoso et al., 1996; Adler et al., 2000) compared to AA, and is also the primary inducer of DNA-adducts in somatic cells (Besaratinia and Pfeifer, 2005). The acrylamide REFs specified above of 1 for metabolic variability and dose-response kinetics (i.e., indicating equivalency), therefore, may not accurately reflect interspecies toxicokinetic differences for GA production and the resulting estimated interspecies extrapolation of the external dose to mutation rate relationship. These uncertainties in the assumptions and data gaps warrant further research to improve the usefulness of the following quantitative estimates of risk for AA induced heritable effects.

Direct and Modified Direct Approach

In the "direct approach" to estimating genetic disease rates based on mutation rates, a dominant mutation and endpoint, such as dominant skeletal or cataract alteration is used. In contrast, the "modified direct approach" uses a recessive mutation rate to predict dominant disease rates. A modified direct approach was used for AA based on an estimate of the per locus mutation rate in the mouse relative to the number of loci in humans capable of mutating to dominantly expressed disease alleles. Although the value for the number of human loci capable of mutating to dominantly expressed disease alleles is critical to the derivation of the estimated risk to exposed humans, this number is not known and was assumed to be 1000 for dominant single gene diseases, and 10 for dominant chromosomal diseases (i.e., this assumption represents

another source of uncertainty). The modified direct approach incorporates these estimates into the following equation to derive the number of new diseases in offspring descendent from exposed parents (ICPEMC, 1993a,b):

Number of new diseases in the offspring descendent from exposed parents

= REF x M_{mouse} x L_{human} x D x N where:

M_{mouse} = induced per locus mutation rate per unit dose exposure estimated in the mouse; L_{human} = number of loci in humans that mutate to dominant disease alleles; D = exposure dose; N = number of offspring descendent from exposed parents; REF = risk extrapolation factor (see above for AA).

Doubling Dose Approach.

The doubling dose approach does not require a specific estimate of the number of human loci that mutate to dominant disease alleles as does the modified direct approach. Instead, the doubling dose approach is based on an estimate of the overall spontaneous mutation frequency in humans that leads to dominant disease alleles. The doubling dose (DD) is the dose which induces a mutation rate equal to the spontaneous mutation rate. This dose can be evaluated in animal studies and extrapolated to humans based on the assumptions discussed above. Dearfield et al. (1995) state that data for spontaneous mutation rates in humans are more available than the number of disease associated loci in humans thus making the doubling dose approach preferable to (i.e., less uncertain than) the modified direct approach. For an estimate of the spontaneous mutation rate and the spontaneous chromosomal aberration rate in humans, Dearfield et al. (1995) used numbers developed by UNSCEAR (1986) and Sankaranarayanan (1982) of 1.5 x 10^{-3} and 6.2 x 10^{-8}, respectively. These mutation frequencies in humans were used in the following equation (ICPEMC, 1993a,b) to derive the number of new diseases in the offspring descendent from exposed parents:

Number of new diseases in the offspring =

REF X $Spon_{humans}$ x D/DD x N

where: REF = risk extrapolation factor (see above discussion of REFs); $Spon_{humans}$= overall spontaneous mutation rate to dominant disease alleles in humans; D = exposure dose; DD = doubling dose estimated in the mouse (the DD is calculated as the mouse spontaneous rate per unit dose); and N = number of offspring descendent from exposed parents.

Dearfield et al. (1995) derive a doubling dose in mice based on four data sets (Ehling and Neuhauser-Klaus, 1992; Shelby et al., 1987; Adler et al., 1994; Adler, 1990) using the following equation:

$$DD = \frac{\text{Spontaneous mutation rate}}{\text{Induced mutation rate / unit exposure}}$$

As an example using data from Ehling and Neuhauser-Klaus (1992):

$$DD = \frac{22 / 248{,}413}{[(6/23{,}489) - (22/248{,}413)] / 100 \text{ mg/kg}} = 53.1 \text{ mg/kg}$$

The other estimates were 1.8 mg/kg, 3.3 mg/kg, and 0.39 mg/kg for the Shelby et al. (1987), Adler et al. (1994), and Adler (1990) data, respectively. Aside from the wide range of values derived from the different data sets, a major assumption in these calculations is that the doubling doses increases linearly with dose. The gene mutation rates are based on a single data point and no other dose-response data were available in 1995 to suggest a non-linear response. Dearfield et al. (1995) note that from an empirical examination of AA data at doses of 100 mg/kg and lower, most of the data from the dominant lethal studies have a linear component (e.g., based on data from the dermal dominant lethal study), and that the Adler et al. (1994) data from the control and the 50 and 100 mg/kg doses could be fitted with a linear equation. As an alternate model, Adler et al.(1994) combined both of their data sets and fit the resulting dose-response curve with a Weibull model to derive a human DD estimate of about 25 mg/kg based on a human background translocation frequency of 1.9 per 1000 newborns (Lyon et al., 1983).

Quantitative Assessment for Various Exposure Routes and Levels

The results of the Dearfield et al. (1995) quantitative analysis for risk of heritable germ cell effects from different routes and levels of exposure are presented in Table 5-14. In these derivations, N is set at one million (1×10^6), the total REF is set to 0.2, and a range of values are presented using the two approaches (modified direct and doubling dose) for each of four mouse data sets (Ehling and Neuhauser-Klaus, 1992; Shelby et al., 1987; Adler et al., 1994; Adler, 1990). For example, the estimated risk for heritable mutations that could potentially lead to induced genetic disease in offspring from fathers exposed to 1.3×10^{-5} µg AA/kg-day in drinking water range from $7.3 \times 10^{-5} / 10^6$ offspring for gene mutations leading to disease (using the doubling dose approach and the Ehling and Neuhauser-Klaus [1992] data) to $3 \times 10^{-2} / 10^6$ for chromosomal alterations (using the modified direct approach and the Shelby et al.[1987] data). The oral exposure level that Dearfield et al. (1995) used was derived from estimates of drinking water consumption and AA levels in drinking water. By using the Fennell et al. (2005) updated upper estimate of daily oral exposure to an average adult male based on background hemoglobin

adduct levels (i.e., 1.26 µg/kg-day instead of Dearfield et al.'s [1995] estimate of 1.3×10^{-2} µg/kg-day), the upper range of the estimated risk for heritable mutations potentially leading to induced genetic disease would be $3/10^6$ offspring for chromosomal alterations using the modified direct approach and the Shelby et al.(1987) data. Table 5-14 also presents risk for induced genetic disease in offspring from fathers exposed via inhalation or dermal exposures in occupational settings that are considerably higher.

Conclusions on the Utility of the Quantitation of Heritable Germ Cell Effects and Identification of Data Needs

The quantitation of heritable germ cell effects described in Dearfield et al. (1995) is based primarily on male translocation data and one gene mutation study, and accounts only for dominant genetic diseases induced by either gene mutations or chromosomal alterations. The estimates do not take into account other potential genotoxic mechanisms such as effects in spermatogonia stem cells, effects in female germ cells, or induction of recessive mutations that would not appear in the first generation, but could lead to additional adverse effects in subsequent generations. Thus, the Dearfield et al. (1995) risk estimates may be an underestimate of the total effects on heritable germ cells.

The uncertainties in the assumptions used to quantitate risks for heritable germ cell effects (discussed above), however, reduce the utility of the Dearfield et al. (1995) quantitative results for risk assessment purposes. A National Toxicology Program (NTP) expert panel (NTP/CERHR, 2004), charged with evaluating the evidence for acrylamide's adverse reproductive and developmental effects, reviewed the Dearfield et al. (1995) quantitation of heritable germ cell effects, and concluded that little weight could be placed on the estimated risks due to the uncertainties associated with the assumptions employed in the quantitation.

The lack of knowledge about the timing of an AA exposure relative to the most affected germ cell stage also confound how the results would be used for risk assessment. For example, short-term exposures that induce mutations in spermatogonia stem cells could result in potential adverse outcomes (increased risk) for the remainder of a male's reproductive life, while comparable exposures that induce damage only during the post-meiotic stages of the germ cell cycle (as reported in most of the studies to-date), would increase risks levels only while the affected sperm are viable, i.e., before they are reabsorbed and replaced by unaffected sperm. In this scenario, exposures at earlier stages would result in little, if any, risks. Continuous exposures would result in some weighted combination of risk depending on the sensitivity of each germ cell stage to damage.

Given the uncertainties in the current quantitative characterization of heritable germ cell effects, EPA does not consider the quantitative results from Dearfield et al. (1995) sufficient to support derivation of a toxicity value. EPA does, however, agree with the NTP Expert Panel conclusion that, "considering the incidence in treated and control animals of the response detected for heritable translocations at the lowest dose level tested (40 mg/kg bw/day × 5 days), it is likely that such effects would occur at lower dose levels" (NTP/CERHR, 2004). Thus, further research and data are clearly needed to fill the critical data gaps and reduce uncertainties in the characterization of risks for heritable germ cell effects including gaps in the interspecies extrapolation factors, in the quantitative relationship between genetic alterations in germ cells and heritable disease, and in the shape of the low-dose response relationship.

Table 5-14. Heritable genetic risk estimates for humans exposed to acrylamide

				Number of induced genetic diseases/10^6 offspring					
				Ingestion	Inhalation			Dermal	
Endpoint	Mouse dose, mg/kg (dose schedule)	Approach	Doubling dose, mg/kg	1.3×10^{-5} mg/kg-day	0.027 mg/kg-day OSHA PEL	0.00072 mg/kg-day grout worker	0.011 mg/kg-day grout worker	0.016 mg/kg-day grout worker	0.13 mg/kg-day grout worker
Gene mutation	100[a] (single)	Doubling dose	53.1	7.3×10^{-5}	0.15	0.004	0.062	0.09	0.73
		Modified direct		4.3×10^{-3}	9.0	0.24	3.7	5.3	43.4
Chromosomal alterations	200[b] (5 × 40)	Doubling dose	1.8	3.0×10^{-2}	6.3	0.17	2.6	3.7	30.3
		Modified direct		3.1×10^{-2}	64.4	1.7	26.3	38.2	310
	50[c] (single)	Doubling dose	3.3	1.7×10^{-3}	3.4	0.09	1.4	2.0	16.5
		Modified direct		2.7×10^{-3}	6.0	0.15	2.3	3.3	27.0
	250[d] (5 × 50)	Doubling dose	0.39	1.4×10^{-2}	29.1	0.78	11.8	17.2	140
		Modified direct		2.3×10^{-2}	47.2	1.3	19.2	28.0	227
	Combined[c,d]	Doubling dose	25	2.2×10^{-4}	0.45	0.01	0.18	0.27	2.2

[a]Ehling and Neuhäuser-Klaus (1992).
[b]Shelby et al. (1987).
[c]Adler et al. (1994).
[d]Adler (1990).

Source: Dearfield et al. (1995).

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Acrylamide (CASRN 79-06-1) has the chemical formula C_3H_5NO (structural formula CH_2=CH-$CONH_2$) and a molecular weight of 71.08. Acrylamide (AA) is an odorless, white, crystalline solid at room temperature with a melting point of 84.5°C. It is soluble in water (2.155 g/mL at 30°C) and is used in photopolymerization systems, adhesives and grouts, and polymer cross-linking. The primary commercial use of AA is in the production of polyacrylamides, which are used in the coagulation process of water treatment; as thickening agents for agricultural sprays, papermaking, textile printing paste, and consumer products; and as water retention aids. Release of AA to the environment can occur during the manufacturing process and from polyacrylamide materials containing residual AA. Acrylamide forms during the high-temperature heating of starchy foods. Acrylamide is expected to be highly mobile in water and soils but is not expected to accumulate in the environment due to fairly rapid physical and biological degradation.

Neurological impairment (including peripheral neuropathy involving nerve tissue damage) has been repeatedly observed in case reports, and health surveillance studies, as well as extensive laboratory animal studies clearly establishing this endpoint as a potential human health hazard associated with acute and repeated occupational exposure via inhalation of airborne AA or dermal contact with AA-containing materials. There are only a few case reports of similar effects in humans orally exposed to AA, and the human data are inadequate to develop a quantitative characterization of the dose-response, however there are many laboratory animal studies that have quantitatively examined the general toxicity, neurotoxicity, reproductive toxicity, and developmental toxicity of chronic and less-than-lifetime oral exposure to AA. The animal studies indicate that microscopically-detected degenerative peripheral nerve changes are the most sensitive health effect from repeated oral exposure to AA, with LOAELs in chronic rat studies in the 1–2 mg/kg-day range. Early animal research associated AA functional neurotoxicity with central and peripheral distal axonopathy and, more specifically, with histopathologic findings of neurofilamentous accumulations in distal paranodal regions of large peripheral nerve fibers that appeared to cause local axon swelling and subsequent degeneration of myelin. Axon degeneration was observed to progress proximally toward the cell body region, a process known as "dying back." Based on these findings, neurofilaments were thought to be a target for AA toxicity. Other potential pathways for AA-induced axonopathy include interference with nerve cell body metabolism and delivery of nutrients to the axon, interruption of axonal protein transport, disruption of axon cytoskeleton, diminished axolemma Na^+/K^+-ATPase activity, and reduction of fast anterograde axonal transport capacity.

Impaired male reproductive performance (i.e., male-mediated implantation losses) has been observed in laboratory animals orally exposed to AA, but the lowest dose levels associated with this effect (~3–13 mg/kg-day) are generally higher than the lowest doses associated with degenerative nerve changes. To date, associations between human exposure to AA and reproductive effects have not been reported.

Two recent reviews of studies in mice for heritable germ cell effects from exposure to AA have both concluded that AA induces transmissible genetic damage in male germ cells of mice in the form of reciprocal translocations and gene mutations. No experiments have studied the potential for AA to induce heritable mutations in the female germ line. The heritable germ cell effect in male mice is consistent with the extensive evidence supporting dominant lethal effects in male murine test animals. The main adverse effects are summarized as follows: (1) AA is mutagenic in spermatozoa and spermatid stages of the male germ line; (2) in these spermatogenic stages AA is mainly or exclusively a clastogen; (3) per unit dose, i.p. exposure is more effective than dermal exposure; and (4) per unit dose, GA is more effective than AA. Since stem cell spermatogonia persist and may accumulate mutations throughout the reproductive life of males, assessment of induced mutations in this germ cell stage is critical for the assessment of genetic risk associated with exposure to a mutagen.

Mechanistic proposals have been made for a common MOA for neurotoxic and male fertility effects (e.g., effects on mounting, sperm motility, and intromission) involving modifications of kinesin and sulfhydryl groups of other proteins by AA and/or GA and a separate mechanism for male dominant lethal mutations involving clastogenic effects from AA and/or GA interactions with protamine or spindle fiber proteins in spermatids and/or direct alkylation of DNA by GA.

In accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), acrylamide is "likely to be carcinogenic to humans" based on findings of: (1) increased incidences of thyroid tumors in male and female rats, scrotal sac mesotheliomas in male rats, and mammary gland tumors in female rats in two drinking water bioassays; (2) AA initiation of skin tumors following oral, i.p., or dermal exposure to AA and tumor promotion by TPA in two strains of mice; and (3) increased incidence of lung adenomas in another mouse strain following i.p. injection of AA. Evidence from available human studies is judged to be limited to inadequate. No statistically significant increased risks for cancer-related deaths were found in either of two cohort mortality studies of AA workers with the exception that, in an exploratory dose-response analysis of the most comprehensive study, an increased risk for pancreatic cancer was found in a subgroup with the highest cumulative AA exposure. In one case-control study, no statistically significant associations were found between increased risks for large bowel, kidney, or bladder cancer and frequent consumption of foods containing high or moderate levels of AA.

Although the precise mechanism(s) by which the multi-site carcinogenicity occurs in animal models cannot be well-established, currently available information indicates that mutagenicity plays an important role in AA-induced carcinogenicity. The evidence consists of AA induced genotoxicity in somatic and germ cells of rodents in vivo and cultured cells in vitro including gene mutations and some types of chromosomal aberrations (i.e., translocations), formation of GA-DNA in mammalian somatic cells, the positive mouse lymphoma assay response, and mutagenicity of glycidamide in short-term bacterial assays. The available data indicate that the major genotoxic effects of AA may involve covalent modifications of proteins by AA and GA, and that the mutagenic events that lead to tumors from exposure to AA are most likely produced by GA via direct alkylation of DNA. Errors in base sequence during DNA replication, especially for the DNA adduct component, may be involved in the MOA.

An alternative MOA involving altered hormonal responses has also been proposed for the carcinogenicity of AA, but the available data are insufficient to make a determination as to the likelihood of this MOA. It should be noted that that AA-induced carcinogenicity may have a mixed MOA involving a mutagenic component and another component, such as an altered hormonal response or some as yet unknown MOA.

On-going Studies at the US Food and Drug Administration

The US Food and Drug Administration's National Center for Toxicological Research (NCTR) under the auspices of the National Toxicology Program (NTP), are conducting long-term carcinogenicity bioassays of carcinogenicity for AA and GA in male and female F344 rats and male and female B6C3F1 mice. The proposed schedule for completion of these studies is as follows: in-life phase complete by August 2007; pathology complete February 2008; Pathology Working Group review by May 2008 (data will then become available on the NTP website); statistics complete by August 2008; BSI Report preparation by May 2009; NTP Technical Report Subcommittee approval by November 2009. NCTR is also conducting a developmental neurotoxicity study of AA in F344 rats under the auspices of the NTP Program. EPA will continue to monitor new science to inform future directions.

Suggestions for Additional Studies

To further resolve if there is dose-concordance and temporal sequence in the mutagenic MOA, a study could be conducted with the same regimen as in a cancer bioassay with measurement of gene mutations in the tumor target tissues, employing sampling times that would establish the temporal induction of mutation. A study that would help resolve the difference between AA and GA mutagenicity leading to tumors would breed wild type lacI mice with knockouts for CypIIE1, and evaluate mutations in the target tissue. Additional studies to identify

the types of mutations in oncogenes or tumor suppressor genes from the tumors induced in rodents by AA (or GA) are needed.. A treatment-specific tumor mutational spectrum that matched the mutational signature of AA/GA would be powerful evidence of a mutagenic MOA, especially if the mutational signature were developed in the tumor target tissue (e.g., using the lacI transgene).

Additional studies are warranted to evaluate the potential for hormonal disruption, and the interaction of hormonal disruption and increased levels of DNA adducts in the tumor bearing tissues observed in the animal studies. Additional studies are also warranted to further evaluate the low-dose response relationship for heritable germ cell effects, to reduce the uncertainty in the interspecies extrapolation factors for the dynamic events in the MOA for heritable effects, and to improve estimates of the quantitative relationship between genetic alterations in germ cells and heritable disease.

Estimates of Risks from Other Organizations

Estimates of risk for acrylamide derived by other organizations are compiled by the National Libraries of Medicine and can be found on the TOXNET webpage at http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?iter . Additionally, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) information on acrylamide risk and toxicity is available at: http://www.who.int/foodsafety/chem/chemicals/acrylamide/en/ .

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

Increased incidence of degenerative lesions of peripheral nerves was selected as the critical effect for derivation of the RfD for AA, because the doses associated with this effect in subchronic and chronic drinking water studies with rats were lower than the lowest doses associated with other AA-induced noncancer effects in animals, including male-mediated implantation losses. The two 2-year drinking water bioassays with F344 rats were selected as co-principal studies for deriving an RfD (Friedman et al., 1995; Johnson et al., 1986), and the final quantitative RfD value is based on the dose-response data from only the Johnson et al. study. A BMD analysis of the incidence data for microscopically-detected degenerative nerve lesions in rats indicated that male rats were slightly more sensitive than female rats in these studies. The 95% lower confidence limits on estimates of doses associated with 5% extra risk ($BMDL_5$) for nerve lesions were 0.49 and 0.46 mg/kg-day for female rats and 0.27 and 0.57 mg/kg-day for male rats in the Johnson et al. (1986) and Friedman et al. (1995) studies, respectively. The lowest of the BMDLs from the Johnson et al. (1986) study (0.27 mg/kg-day for 5% extra risk for mild-to-moderate lesions) reflects the most sensitive response, and was

selected as the POD for deriving the RfD. A PBTK model was used to derive an HEC based on an internal dose metric of total AUC for AA in the blood and for a simulated drinking water exposure. The HEC was 0.076 mg/kg-day, and was used as the POD. The POD was then divided by a total UF of 30 (3 for animal-to-human extrapolation to account for toxicodynamic differences; 10 for intra-individual variability in human toxicokinetics and toxicodynamics) to derive the RfD of 0.003 mg/kg-day.

The overall confidence in this RfD assessment is medium to high based on medium to high confidence in the studies and medium to high confidence in the database. The animal database is robust and complete by IRIS assessment standards. Although no data were available to characterize the neurotoxic dose-response relationships from chronic oral exposure in humans, neurotoxicity from inhaled or dermal occupational exposures to acrylamide are well documented. Two co-principal studies provide adequate characterization of the dose-response relationship for degenerative nerve lesions from a chronic-duration oral exposure, and for neurotoxicity as the most sensitie endpoint. There might, however, be behavioral or functional effects that were not evaluated in these bioassays that could have lower NOAELs than the histological effects used to derive the RfD. There is also uncertainty as to low-does reponse relationship for heritable germ cell effects. These two issues lower the confidence in the overall RfD to medium to high. Some of these data needs are being addressed in on-going studies sponsored by the NTP.

6.2.2. Noncancer/Inhalation

An inhalation RfC for acrylamide was derived by application of a PBTK model to extrapolate the internal dose metric (AUC for AA in the blood) from an oral exposure in rat to an oral exposure in humans and then to an equivalent inhalation exposure in humans, assuming continuous inhalation exposure over 24 hours. Results from studies of occupationally exposed workers are sufficient to firmly establish neurological impairment as a potential health hazard from inhalation and dermal exposure to AA but are insufficient to describe dose-response relationships for inhalation exposure. Justification for deriving an RfC from the oral RfD comes from: (1) considerable evidence from occupational experience involving dermal and inhalation exposure that AA-induced peripheral neuropathy (including development of the types of degenerative lesions observed in nerves of rats exposed via drinking water) is a well-established human health hazard; (2) evidence that tissue distribution in rats is similar following i.v., i.p., oral, dermal, and inhalation exposure to AA (Sumner et al., 2003; Kadry et al., 1999; Dow Chemical Co., 1984; Miller et al., 1982; Hashimoto and Aldridge, 1970); (3) evidence that the elimination kinetics of radioactivity from oral or i.v. administration of radiolabeled AA in rats was similar (Miller et al., 1982); and (4) lack of support for portal-of-entry effects.

The oral $BMDL_5$ was converted to a human equivalent daily intake, and then to an air concentration that would result in comparable internal level assuming a 70 kg person who breathes 20 m^3/day air. The resulting air concentration of 0.25 mg/m^3 was used as a POD. The POD was then divided by a total UF of 30 (3 for animal-to-human extrapolation to account for toxicodynamic differences; and 10 for intra-individual variability in human toxicokinetics and toxicodynamics) to derive the RfC of 0.008 mg/m^3.

Since the RfC is based on a route to route extrapolation of the oral exposure data using the same PBTK model to develop an inhalation HEC, the overall confidence in the RfC is similar to that for the RfD, namely medium to high. The AA PBTK model provided a scientifically supportable estimate of the inhalation HEC as the basis for the RfC, a reference value that would otherwise have been unobtainable in the absence of adequate inhalation bioassay data.

6.2.3. Cancer/Oral

Two methods (incidence of tumor-bearing animals and summed risk) were considered for estimating human cancer risk from male F344 rats bearing scrotal sac mesotheliomas or thyroid follicular cell tumors (adenoma and carcinoma); or females bearing mammary gland tumors (malignant and benign) or thyroid follicular cell tumors (adenoma and carcinoma), from a 2-year drinking water rat bioassay (Friedman et al., 1995) in male. Both approaches yielded a similar result for risks from multiple tumor sites when rounded to one significant digit. A linear extrapolation to the origin, corrected for background, from a BMDL (as a POD) was used to derive the oral slope factor. Support for a linear extrapolation comes from evidence of a mutagenic MOA for AA, including observations of: (1) strong evidence of mutagenicity in in vitro assays and somatic cells from in vivo assays; (2) male-mediated dominant lethal effects following subchronic oral exposure at dose levels (2.8 to 13.3 mg/kg-day) in the vicinity of chronic oral dose levels that induced carcinogenic effects in rats (0.5 to 3 mg/kg-day); (3) initiation of skin tumors (presumably via a mutagenic action) in mice by short-term exposure to oral doses as low as 12.5 mg/kg-day followed by TPA promotion; (4) metabolism of AA by CYP2E1 to the DNA-reactive metabolite, GA; and (5) DNA adducts of GA in various tissues in rats and mice exposed to AA. Although proposals have been made that AA induction of scrotal sac mesotheliomas in male rats and mammary gland tumors in female rats may be caused by hormonally based MOAs that may not be relevant to humans, the available evidence in support of these hypotheses is judged to be inadequate to rule out human relevance.

The POD for the derivation of the oral slope factor for cancer risks in humans exposed to AA was the lowest $BMDL_{10}$ for male rats bearing TVM or thyroid tumors which was converted to the human equivalent $BMDL_{10}$ by using a PBTK model. The human equivalent internal dose is based on a metric of total AUC of GA in the blood. Glycidamide has been shown to be the primary reactive mutagenic agent, and the total amount in blood is the most appropriate and

supportable dose metric to use as a correlate to increased incidence of tumors. The resulting HEC-$BMDL_{10}$ (0.22 mg/kg-day) was used to derive a human oral slope factor of 0.5 $(mg/kg\text{-}day)^{-1}$.

Because a mutagenic MOA for AA carcinogenicity is sufficiently supported in laboratory animals and relevant to humans (Section 3.4.1), and in the absence of chemical-specific data to evaluate differences in susceptibility, increased early-life susceptibility is assumed and the age-dependent adjustment factors (ADAFs) should be applied to the slope factor, as appropriate, in accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b).

The overall confidence in the oral cancer slope factor is medium based on medium confidence in the study and medium confidence in the database. The principal 2-year study (Friedman et al., 1995) corroborates earlier tumor incidence data (Johnson et al., 1986) for most, but not all tumor types, and was a larger and better designed study. There remain, however, uncertainties concerning the differences between the two study tumor types and incidence data, in particular for the CNS tumors, and some controversy over the histopathological interpretation of the male tunica vaginalis mesotheliomas. The database is also incomplete with only one animal species tested, and little human data to support acrylamide's carcinogenic potential in humans. At this time, the preponderance of evidence supports a mutagenic MOA with insufficient evidence to confidently rule out the human relevance of the acrylamide-induced tumors observed in the F344 rat bioassays (thyroid, mammary gland, and scrotal sac). Although an alternate nonmutagenic MOA has been proposed involving hormonal pathway disruption for tumors specific to Fischer 344 rats, supporting data are limited or nonexistent. Additional oral cancer bioassay data and research into acrylamide's MOA(s) are needed to resolve these issues and data needs.

6.2.4. Cancer/Inhalation

No animal or human cancer data were available to directly derive an inhalation unit risk. An AA PBTK model is available that simulates both oral and inhalation exposures and provides the means to conduct a route-to-route extrapolation of the dose-response relationship from the oral exposure data to what one might expect from an inhalation exposure in humans. The basis of the extrapolation is an exposure (oral or inhalation) that yields a comparable internal dose using the internal dose metric of area under the time-concentration curve for GA in blood. The AA metabolite, GA, is considered to be the putative mutagen and most directly related to AA's carcinogenicity.

Support for the extrapolation of an inhalation unit risk from the oral data for AA comes from: (1) evidence that tissue distribution in rats is similar following i.v., i.p., oral, dermal, and inhalation exposure to AA (Sumner et al., 2003; Kadry et al., 1999; Dow Chemical Co., 1984; Miller et al., 1982; Hashimoto and Aldridge, 1970); (2) the widespread distribution of AA and the formation of GA adducts in diverse tissue throughout the body (Doerge et al., 2005a); (3) evidence that the elimination kinetics of radioactivity from oral or i.v. administration of radiolabeled AA in rats was similar (Miller et al., 1982); and (4) lack of support for portal-of-entry effects. The AA PBTK model was especially important in estimating the internal levels of GA, and in accounting for first-pass metabolism, one of the main factors that could lead to different distributional profiles between an oral and an inhalation exposure.

An inhalation unit risk, calculated from data for adult exposures, is derived from the $BMDL_{10}$, the 95% lower bound on the exposure associated with an 10% extra cancer risk, by dividing the risk (as a fraction) by the $BMDL_{10}$. The inhalation unit risk thus represents an upper bound risk estimate for continuous lifetime exposure without consideration of increased early life susceptibility due to AA's mutagenic MOA.

The inhalation unit risks for AA are based on PBTK model simulations of the HEC for intake from a continuous inhalation exposure that are comparable (based on an internal dose metric of GA AUC in blood) to the oral exposure BMD_{10} of 0.7 mg/kg-day and the $BMDL_{10}$ of 0.3 mg/kg-day. The PBTK model-derived oral exposure HEC-$BMDL_{10}$ is 0.22 mg/kg-day. The air concentrations required to achieve these intake levels, assuming a continuous 24-hour inhalation exposure for a 70 kg person who breathes 20 m^3/day air are 1.84 mg/m^3 for the HEC-BMD_{10} and 0.79 mg/m^3 for the HEC-$BMDL_{10}$. The resulting inhalation unit risk based on the HEC-$BMDL_{10}$ air concentration as the POD is 1×10^{-4} $(\mu g/m^3)^{-1}$.

As noted above, because a mutagenic MOA for AA carcinogenicity is sufficiently supported in laboratory animals and relevant to humans (Section 3.4.1), age-dependent adjustment factors (ADAFs) should be applied to the inhalation unit risk, as appropriate, in accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b).

The overall confidence in the inhalation unit risk is similar to that for the the oral slope factor (see discussion in the previous section) since it is based upon the same study (medium confidence) and database (medium confidence), and the same AA PBTK model to estimate an inhalation HEC.

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APPENDIX A. Summary of External Peer Review and Public Comments and Disposition

APPENDIX B. MUTAGENICITY TEST RESULTS

Table B-1. Results of acrylamide mutagenicity testing

Assay	Test system[a]	Dose/Concentration	HID or LED[b]	Result	Reference
Bacterial gene mutation assays					
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA98, TA100	10–10,000 µg/plate ± S9 activation	100	Weakly positive in TA98, TA100 only with activation; others negative	Zeiger et al., 1987
	S. typhimurium TA1535, TA97, TA98, TA100	100–10,000 µg/plate ± S9 activation	10,000	Negative	
	S. typhimurium TA1535, TA1537, TA98, TA100, TA102	1–100 mg/plate ± S9 activation	100	Negative	Knaap et al., 1988
	S. typhimurium TA1535, TA1537, TA98, TA100 *E. coli* WP2 *uvrA*$^-$	0.5–50 mg/plate ± S9 activation	50	Negative in both systems	Tsuda et al., 1993
	S. typhimurium TA1535	Up to 5 mg/plate ± S9 activation	5	Negative	Jung et al., 1992; Müller et al., 1993
	S. typhimurium TA1535, TA1537, TA1538, TA98, TA100	Up to 1 mg/plate ± S9 activation	1	Negative	Lijinsky and Andrews, 1980
	S. typhimurium TA1535, TA1537, TA1538, TA98, TA100	0.5–5000 µg/plate ± S9 activation	5000	Negative	Hashimoto and Tanii, 1985
Fluctuation test	*K. pneumoniae* ur$^-$ pro$^-$	2–10 mg/mL	10	Negative	Knaap et al., 1988
Nonmammalian gene mutation assays in vivo					
Sex-linked recessive lethal	*D. melanogaster*	40–50 mM abdominal injection	50	Negative	Knaap et al., 1988
	D. melanogaster	0.24–5 mM larvae feeding	1.0	Positive	Tripathy et al., 1991
Somatic mutation, recombination	*D. melanogaster*	1–1.5 larvae feeding (unit unspecified)	1	Weakly positive	Knaap et al., 1988
	D. melanogaster	1–1.5 mM larvae feeding	1	Positive	Batiste-Alentorn et al., 1991
	D. melanogaster	0.25–5 mM larvae feeding	1.0	Positive	Tripathy et al., 1991

Table B-1. Results of acrylamide mutagenicity testing

Assay	Test system[a]	Dose/Concentration	HID or LED[b]	Result	Reference
Mammalian gene mutation assays in vitro					
	Mouse lymphoma L5178Y $TK^{+/-}$, tk locus	10 mM	10	Positive (more pronounced without activation)	Barfknecht et al., 1988
	Mouse lymphoma L5178Y $TK^{+/-}$, tk locus	0–0.85 mg/mL without activation	0.5	Positive	Moore et al., 1987
	Mouse lymphoma L5178Y $TK^{+/-}$, tk and HPRT loci	0.5–7.5 mg/mL with or without metabolic activation		Equivocal, increases only at cytotoxic concentrations	Knaap et al., 1988
	Mouse lymphoma L5178Y $TK^{+/-}$, HPRT locus	0.1–0.5 mg/mL with cocultivated mammalian cells	0.3	Positive	Knaap et al., 1988
	Chinese hamster V79H3 cells, HPRT locus	1–7 mM no activation	7	Negative	Tsuda et al., 1993
Mammalian gene mutation assays in vivo					
Transgenic mouse liver cII, lymphocyte HPRT	Big Blue Mouse (M, F)	100, 500 mg/L AA or GA Drinking water for 3–4 weeks	100 (est. 19–25 mg/kg-day)	Positive	Manjanatha et al., 2006
Transgenic mouse *lacZ*	Muta® Mouse	5 × 50 mg/kg-day i.p. injection	5 × 50	Weakly positive, no statistical analysis	Hoorn et al., 1993
	Muta® Mouse	50–100 mg/kg i.p. injection	100	Negative	Krebs and Favor, 1997
Mouse spot test	Mouse embryos $(T \times HT)F_1$	1 × 50 or 75 mg/kg 3 × 50 or 75 mg/kg i.p. injection	50 3 × 50	Positive Positive	Neuhäuser-Klaus and Schmahl, 1989
Morphological specific locus	Mouse $(C3H/R1 \times 101/R1)F_1$ (M)	5 × 50 mg/kg i.p. injection	50	Positive (postspermatogonia)	Russell et al., 1991
	Mouse $(102/E1 \times C3H/E1)F_1$ (M)	100–125 mg/kg i.p. injection	100	Positive (postspermatogonia; spermatogonia)	Ehling and Neuhäuser-Klaus, 1992
Chromosomal alterations in mammalian cells in vitro					
Chromosomal aberrations	Chinese hamster cells	0.5–5 mM no activation used	2	Positive	Tsuda et al., 1993
	Chinese hamster cell line (V79)	0.1–3 mg/mL ± S9 activation	1	Positive, with or without metabolic activation	Knaap et al., 1988

Table B-1. Results of acrylamide mutagenicity testing

Assay	Test system[a]	Dose/Concentration	HID or LED[b]	Result	Reference
	Mouse lymphoma L5178Y $TK^{+/-}$ -3.7.2 cells	0.65–0.85 mg/mL without activation	0.75	Positive	Moore et al., 1987
Cell division aberration	Chinese hamster lung cell line DON:Wg3h	0.2–1 mg/mL	0.2	Positive	Warr et al., 1990
	Chinese hamster lung fibroblast LUC2 p5	0.01–1 mg/mL	0.01	Positive	Warr et al., 1990
Chromosome enumeration	Chinese hamster lung fibroblast LUC2 p5	0.0125–0.5 mg/mL	0.5	Positive	Warr et al., 1990
Polyploidy	Chinese hamster cell line (V79)	0.5–5 mM	1	Positive	Tsuda et al., 1993
Spindle disturbances	Chinese hamster cell line (V79)	0.01–1 mg/mL	0.01	Positive	Adler et al., 1993
Micronucleus	Seminiferous tubular segments (spermatids from SD rats)	5–50 μg/mL	50	Negative	Lähdetie et al., 1994
Chromosomal alterations in mammalian cells in vivo					
Chromosomal aberrations	Mouse (101/E1 × C3H/E1)F_1 (bone marrow cells)	50–150 mg/kg i.p. injection	50	Positive	Adler et al., 1988
	Mouse (ICE-SPF) (bone marrow cells)	100 mg/kg i.p. injection	100	Positive	Čihák and Vontorková, 1988
	Mouse (ddY) (bone marrow cells)	100–200 mg/kg i.p. injection	200	Negative	Shiraishi, 1978
	Mouse (ddY) (bone marrow cells)	500 ppm in diet for 7 to 21 days (78 mg/kg-day)	78	Negative	Shiraishi, 1978
	Rat (bone marrow cells)	100 mg/kg i.p. injection	100	Negative	Krishna and Theiss, 1995
	Mouse (C57BL/6J) (spleen lymphocytes)	50–125 mg/kg i.p. injection	125	Negative	Backer et al., 1989
	Mouse (C57BL/6) (splenocytes)	100 mg/kg i.p. injection	100	Negative	Kligerman et al., 1991
	Mouse (101/E1 × C3H/E1)F_1 (spermatogonia)	50–150 mg/kg i.p. injection	150	Negative	Adler et al., 1988
	Mouse (C57BL/6J) (spermatogonia)	50–125 mg/kg i.p. injection	125	Negative	Backer et al., 1989
	Mouse (102/E1 × C3H/E1)F_1 (spermatogonia)	5 × 50 mg/kg-day i.p. injection	5 × 50	Negative	Adler, 1990

Table B-1. Results of acrylamide mutagenicity testing

Assay	Test system[a]	Dose/Concentration	HID or LED[b]	Result	Reference
	Mouse (102/E1 × C3H/E1)F_1 (spermatocytes)	100 mg/kg i.p. injection	100	Positive	Adler, 1990
	Mouse (CF_1) (first cleavage embryos)	150 mg/kg i.p. injection	150	Positive in embryos from which the males had mated 6–8 days following treatment (early spermatozoa stage)	Valdivia et al., 1989
	Mouse (B6C3F1) (M) (first cleavage one-cell zygotes, examined after mating)	75 and 125 mg/kg or 5 × 50 mg/kg-day i.p. injection	75	Positive	Pacchierotti et al., 1994
Polyploidy or aneuploid	Mouse bone marrow cells	100–200 mg/kg i.p. injection	100	Positive	Shiraishi 1978
	Mouse bone marrow cells	500 ppm in the diet for 7 to 21 days (78 mg/kg-day)	78	Positive	Shiraishi 1978
Spindle disturbances	Mouse (102/E1 × C3H/E1) bone marrow cells	120 mg/kg i.p. injection	120	Negative	Adler et al., 1993
Micronucleus	Mouse (101/E1 × C3H/E1)F_1 bone marrow cells (M,F)	50–125 mg/kg i.p. injection	50	Positive	Adler et al., 1988
	Mouse (ICR-SPF) bone marrow cells (M)	100 mg/kg i.p. injection	100	Positive	Čihák and Vontorková, 1988
	Mouse (ICR-SPF) bone marrow cells (M)	25–100 mg/kg-day for 2 days i.p. injection	25	Positive	Čihák and Vontorková, 1988
	Mouse (Swiss NIH) bone marrow cells (M,F)	136 mg/kg i.p. injection	136	Positive	Knaap et al., 1988
	Mouse (ICR-SPF) bone marrow cells (M,F)	42.5–100 mg/kg-day (1, 2, or 3 days) i.p. injection	M: 42.5 F: 55	Positive	Čihák and Vontorková, 1990
	Rat (Sprague-Dawley) bone marrow cells (M)	100 mg/kg i.p. injection	100	Negative	Paulsson et al., 2002
	Rat bone marrow cells	100 mg/kg i.p. injection	100	Negative	Krishna and Theiss, 1995
	Mouse (BALB/c) reticulocytes	50–100 mg/kg i.p. injection	50	Positive	Russo et al., 1994
	Mouse (CBA) reticulocytes	25–50 mg/kg i.p. injection	25	Positive, but results were not analyzed statistically	Paulsson et al., 2002

Table B-1. Results of acrylamide mutagenicity testing

Assay	Test system[a]	Dose/Concentration	HID or LED[b]	Result	Reference
	Mouse (CBA) reticulocytes	0.18, 0.35, 0.70 mmol/kg; i.p. injection	0.35	Positive, but results were not analyzed statistically	Paulsson et al., 2003
	Rat (Sprague-Dawley) reticulocytes	0.70, 1.4 mmol/kg i.p. injection	0.7	Positive, but nonmonotonic, probably due to toxicity at high dose	Paulsson et al., 2003
	Mouse (C57BL/6J) (M) spleen lymphocytes	50–125 mg/kg i.p. injection	50	Positive	Backer et al., 1989
	Mouse (C57BL/6) (M) splenocytes	100 mg/kg i.p. injection	100	Positive	Kligerman et al., 1991
	Mouse (C57BL/6J) spermatids	10–100 mg/kg i.p. injection	50	Positive	Collins et al., 1992
	Mouse (BALB/c) spermatids	50–100 mg/kg or 4 × 50 mg/kg-day i.p. injection	50	Positive	Russo et al., 1994
	Rat (Lewis) spermatids	50–100 mg/kg or 4 × 50 mg/kg-day i.p. injection	100	Positive	Xiao and Tates, 1994
	Rat (Sprague-Dawley) spermatids	50–100 mg/kg or 4 × 50 mg/kg-day i.p. injection	4 × 50	Positive	Lähdetie et al., 1994
Synaptonemal complex aberrations	Mouse (C57BL/J6) (M) germ cells	50–150 mg/kg i.p. injection	150	Negative	Backer et al., 1989
Synaptonemal complex irregularities	Mouse (C57BL/J6) (M) germ cells	50–150 mg/kg i.p. injection	50	Weakly positive, asynapsis in meiotic prophase	Backer et al., 1989
Heritable translocations	Mouse (C3H × 101)F_1 (M)	5 × 40–50 mg/kg-day i.p. injection	40	Positive	Shelby et al., 1987
	Mouse (C3H/E1) (M)	50–100 mg/kg i.p. injection	50	Positive	Adler et al., 1994
	Mouse (C3H/E1) (M)	5 × 50 mg/kg-day dermal	50	Positive	Adler et al., 2004
Reciprocal translocations	Mouse (C3H/E1) (M)	5 × 50 mg/kg-day i.p. injection	50	Positive	Adler, 1990
DNA damage and repair and DNA adduct formation					

Table B-1. Results of acrylamide mutagenicity testing

Assay	Test system[a]	Dose/Concentration	HID or LED[b]	Result	Reference
Spore rec assay	*Bacillus subtilis* H17 (rec^+) and M45 (rec^-)	1–50 mg/disk	10	Positive	Tsuda et al., 1993
DNA breakage	Mouse (C3H × C57BL/10)F_1 (M)	25–125 mg/kg i.p. injection	25	Positive	Sega and Generoso, 1990
In vitro UDS	Rat primary hepatocytes	5–20 mM	17.5	Weakly positive	Barfknecht et al., 1988
	Rat (F344) (M) primary hepatocytes	0.01–1 mM	1	Negative	Butterworth et al., 1992
	Human mammary epithelial cells	1–10 mM	1	Positive	Butterworth et al., 1992
In vivo/in vitro UDS	Rat (F344)(M) hepatocytes	1 × 100 mg/kg 5 × 30 mg/kg-day gavage	1 × 100 5 × 30	Negative	Butterworth et al., 1992
	Rat (F344)(M) spermatocytes	1 × 100 mg/kg 5 × 30 mg/kg-day gavage	5 × 30	Positive	Butterworth et al., 1992
In vivo UDS	Mouse (C3H × 101)F_1 and (C3H × BL10)F_1 (M) germ cells	7.8–125 mg/kg i.p. injection	7.8	Positive	Sega et al., 1990
DNA adducts	Mouse (C3H × BL10)F_1 testis	46 mg/kg i.p. injection	46	Positive	Sega et al., 1990
	Mouse (C3H × BL10)F_1 (M) liver	46 mg/kg i.p. injection	46	Positive	Sega et al., 1990
	Rat (Sprague-Dawley) liver, lung, kidney, brain, testis	46 mg/kg i.p. injection	46	Positive	Segerbäck et al., 1995
	Mouse (BALB/c) liver, kidney, brain	53 mg/kg i.p. injection	53	Positive	Segerbäck et al., 1995
Sister chromatid exchange					
In vitro	Chinese hamster V79 cells	0.1–1 mg/mL ± S9 activation	0.3	Positive at 0.3 mg/mL without S9 and 1.0 mg/mL with S9	Knaap et al., 1988
	Chinese hamster V79 cells	0.5–2.5 mM no activation used	1	Positive	Tsuda et al., 1993
In vivo	Mouse (C57BL/6J) (M) spleen lymphocytes	50–125 mg/kg i.p. injection	50	Positive	Backer et al., 1989
	Mouse (C57BL/6) (M) splenocytes	100 mg/kg i.p. injection	100	Positive	Kligerman et al., 1991

Table B-1. Results of acrylamide mutagenicity testing

Assay	Test system[a]	Dose/Concentration	HID or LED[b]	Result	Reference
	Mouse (BALB/c) differentiating spermatogonia	50–100 mg/kg	50	Positive	Russo et al., 1994
Cell transformation					
	Mouse C3H/10T1/2 clone 8 cells	25–200 µg/mL	50	Positive	Banerjee and Segal, 1986
	Mouse NIH/3T3 cells	2–200 µg/mL	0.0125	Positive	Banerjee and Segal, 1986
	Mouse C3H/10T1/2 cells	0.01–0.3 mg/mL	0.3	Negative	Abernethy and Boreiko, 1987
	Mouse BALB/c 3T3 cells	0.5–2 mM	1	Positive	Tsuda et al., 1993
	Syrian hamster embryo cells	0.1–0.7 mM	0.5	Positive	Park et al., 2002
	Syrian hamster embryo cells	0.001–10 mM	10	Negative	Kaster et al., 1993
Germ cell effects					
Sperm head DNA alkylation	Mouse (C3H × 101)F_1	125 mg/kg i.p. injection	125	Weakly positive	Sega et al., 1989
Sperm head protamine alkylation	Mouse (C3H × 101)F_1	125 mg/kg i.p. injection	125	Positive	Sega et al., 1989
Sperm head abnormalities	Mouse (ddY)	0.3–1.2 mM in drinking water for 4 weeks	1.2	Positive	Sakamoto and Hashimoto, 1986

[a]M = male, F = female.
[b]HID, highest ineffective dose/concentration for negative tests; LED, lowest effective dose/concentration for positive tests.

APPENDIX C. DOSE-RESPONSE MODELING FOR DERIVING THE RfD

All available models in the EPA Benchmark Dose Software (BMDS version 1.3.2) were fit to incidence data for microscopically detected degenerative nerve changes in male and female F344 rats from the two 2-year drinking water studies (Friedman et al., 1995; Johnson et al., 1986). The data that were modeled are shown below in Table C-1. The benchmark response predicted to affect 5% of the population (BMR_5) was selected for the point of departure. A BMR lower than a 10% extra risk was selected for the following reasons (1) the $BMDL_5$ remained near the range of observation; (2) the 5% extra risk level is supportable given the relatively large number of animals used in the critical studies; and (3) the use of $BMDL_5$ is consistent with the technical guidance for benchmark dose analysis, which states that "while it is important to always report ED_{10}s and LED_{10}s for comparison purposes, the actual 'benchmark dose' used as a POD may correspond to response levels below (or sometimes above) 10%, although for convenience standard levels of 1%, 5%, or 10% have typically been used."

Table C-1. Incidence data for degenerative changes detected by light microscopy in nerves of male and female F344 rats exposed to acrylamide in drinking water for 2 years

	Dose (mg/kg-day)							
Reference	**0**	**0**	**0.01**	**0.1**	**0.5**	**1.0**	**2.0**	**3.0**
Johnson et al., 1986 (incidence of rats with changes in tibial nerves: see Table 4.9)								
Males (moderate to severe)	9/60	–	6/60	12/60	13/60	–	16/60[b]	–
Females (slight to moderate)	3/60	–	7/60	5/60	7/60	–	16/61[c]	–
Friedman et al., 1995[a] *(incidence of rats with minimal to mild changes in sciatic nerves: see Table 4.12)*								
Males	30/83	29/88	–	21/65	13/38	–	26/49[c]	–
Females	7/37	12/43	–	–	–	2/20	–	38/86[c]

[a]Two control groups were included in the study design to assess variability in background tumor responses.
[b]Statistically significant trend test.
[c]Statistically significantly different from control incidences.

All models provided adequate fits to the data for changes in tibial nerves in male and female rats in the Johnson et al. (1986) study, as assessed by a chi-square goodness-of-fit test (see Tables C-2 and C-3 and following plots [Figures C-1 and C-2] of observed and predicted values from the various models). The log-logistic model provided the best fit for the male rat data as assessed by Akaike's Information Criterion (AIC) and was thus selected to estimate a benchmark dose (BMD) from the Johnson et al. (1986) data. The probit model provided the best fit of the female rat data. Table C-4 lists the predicted doses associated with 10, 5, and 1% extra risk for nerve degeneration in female and male rats in the Johnson et al. (1986) study.

Table C-2. Predictions (mg/kg-day) from models for doses associated with a 10% extra risk for nerve degeneration in male rats exposed to acrylamide in drinking water

Model	BMD (ED_{10})	BMDL	χ^2 *p*-value	AIC
Log-logistic[a]	1.22	0.57	0.49	288.59
Gamma[b]	1.28	0.64	0.48	288.65
Multistage[c]	1.28	0.64	0.48	288.65
Quantal linear	1.28	0.64	0.48	288.65
Weibull[b]	1.28	0.64	0.48	288.65
Probit	1.45	0.87	0.45	288.85
Logistic	1.48	0.90	0.44	288.88
Quantal quadratic	1.75	1.19	0.34	289.57
Log-probit[a]	1.72	1.06	0.33	289.67

[a]Slope restricted to >1.
[b]Restrict power ≥1.
[c]Restrict betas ≥0, degree of polynomial = 4.

Data source: Johnson et al. (1986).

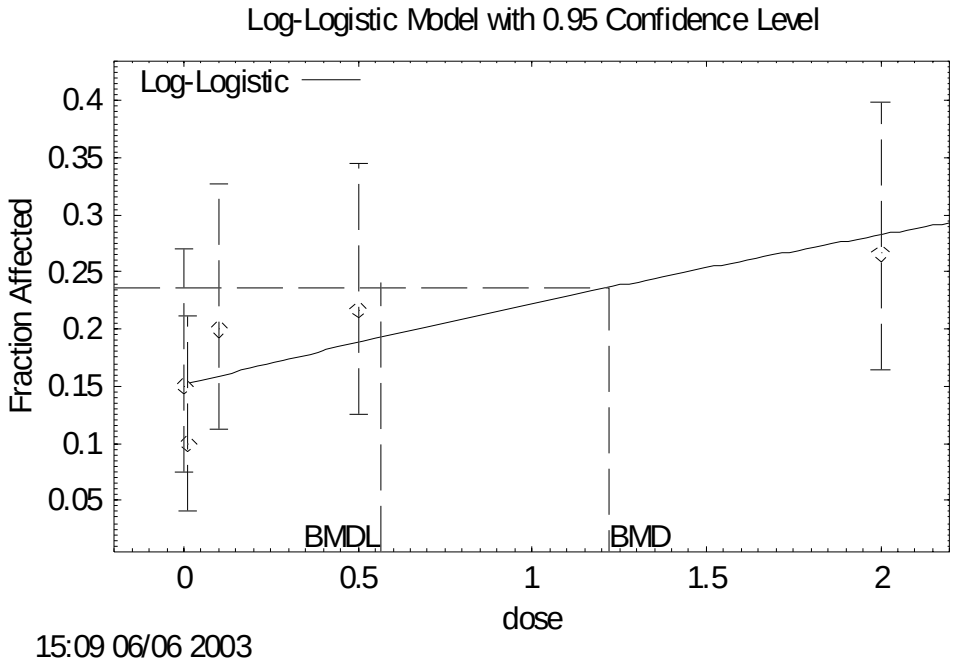

Figure C-1. Observed and predicted incidences for nerve changes in male rats exposed to acrylamide in drinking water for 2 years.

Data source: Johnson et al. (1986).

Table C-3. Predictions (mg/kg-day) from models for doses associated with a 10% extra risk for nerve degeneration in female rats exposed to acrylamide in drinking water

Model	BMD (ED_{10})	BMDL (LED_{10})	χ^2 *p*-value	AIC
Probit	1.19	0.88	0.62	220.68
Logistic	1.24	0.93	0.62	220.69
Quantal quadratic	1.40	1.07	0.59	220.92
Quantal linear	0.98	0.59	0.59	220.75
Log-probit[a]	1.31	0.91	0.59	220.94
Gamma[b]	1.10	0.60	0.41	222.69
Multistage[c]	1.19	0.60	0.41	222.68
Weibull[b]	1.11	0.60	0.41	222.69
Log-logistic[a]	1.10	0.54	0.41	222.69

[a]Slope restricted to >1.
[b]Restrict power ≥1.
[c]Restrict betas ≥0, degree of polynomial = 3.

Data source: Johnson et al. (1986).

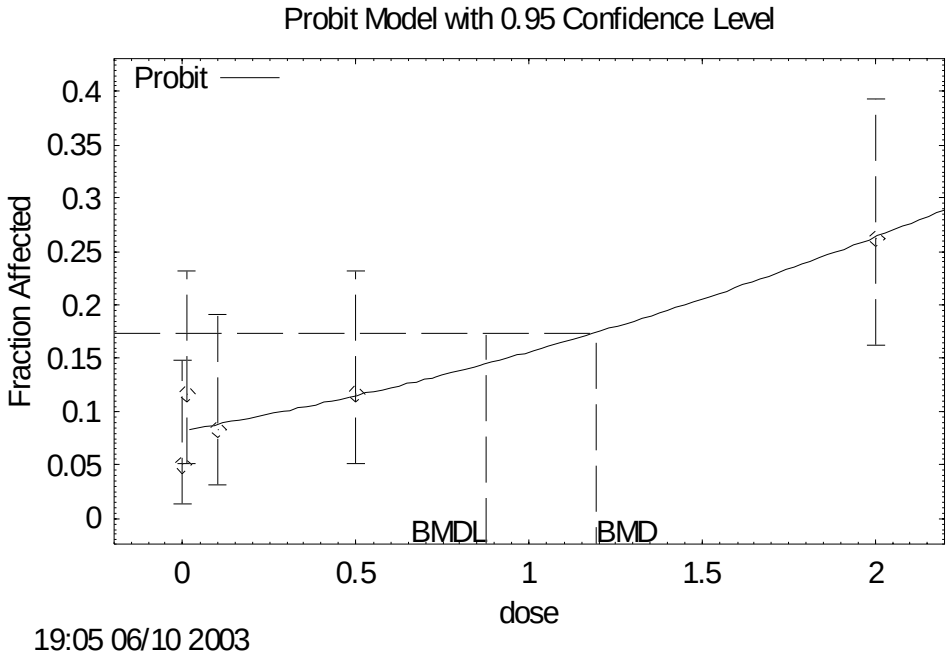

Figure C-2. Observed and predicted incidences for nerve changes in female rats exposed to acrylamide in drinking water for 2 years.

Data source: Johnson et al. (1986).

Table C-4. Predictions (mg/kg-day) from best-fitting models for doses associated with a 10, 5, and 1% extra risk for nerve degeneration in male and female rats exposed to acrylamide in drinking water

Model	BMD (ED_{10})	BMDL (LED_{10})	BMD (ED_5)	BMDL (LED_5)	BMD (ED_1)	BMDL (LED_1)
Male						
Log-logistic	1.22	0.57	0.58	0.27	0.11	0.05
Female						
Probit	1.19	0.88	0.67	0.49	0.15	0.11

Data source: Johnson et al. (1986).

Several models in the software provided adequate fits to the data for minimal to mild changes in sciatic nerves in male and female rats in the Friedman et al. (1995) study, as assessed by a chi-square goodness-of-fit test (see Tables C-5 and C-6 and following plots [Figures C-3 and C-4] of observed and predicted values from the best-fitting models). The quantal quadratic model provided the best fit to the male rat data as assessed by AIC and was selected to estimate a BMD. The BMD associated with a 10% extra risk for minimal to mild changes in sciatic nerves for male rats was 1.1 mg/kg-day and its lower 95% confidence limit (BMDL) was 0.8 mg/kg-day. Table C-7 lists the predicted doses associated with 10%, 5%, and 1% extra risk for sciatic nerve changes in female and male rats in the Friedman et al. (1995) study.

Table C-5. Predictions (mg/kg-day) from models for doses associated with a 10% extra risk for sciatic nerve changes in male rats exposed to acrylamide in drinking water

Model	BMD (ED_{10})	BMDL (LED_{10})	χ^2 *p*-value	AIC
Quantal quadratic	1.11	0.82	0.96	422.84
Logistic	0.73	0.46	0.89	423.15
Probit	0.73	0.45	0.89	423.16
Gamma[a]	1.30	0.37	0.86	424.82
Multistage[b]	1.39	0.37	0.86	424.82
Quantal linear	0.65	0.35	0.86	423.28
Weibull[a]	1.38	0.13	0.86	424.82
Log-logistic[c]	NA[d]			
Log-probit[c]	NA			

[a]Restrict power ≥1.
[b]Restrict betas ≥0, degree of polynomial = 4.
[c]Slope restricted to >1.
[d]NA = failed to generate a model.

Data source: Friedman et al. (1995).

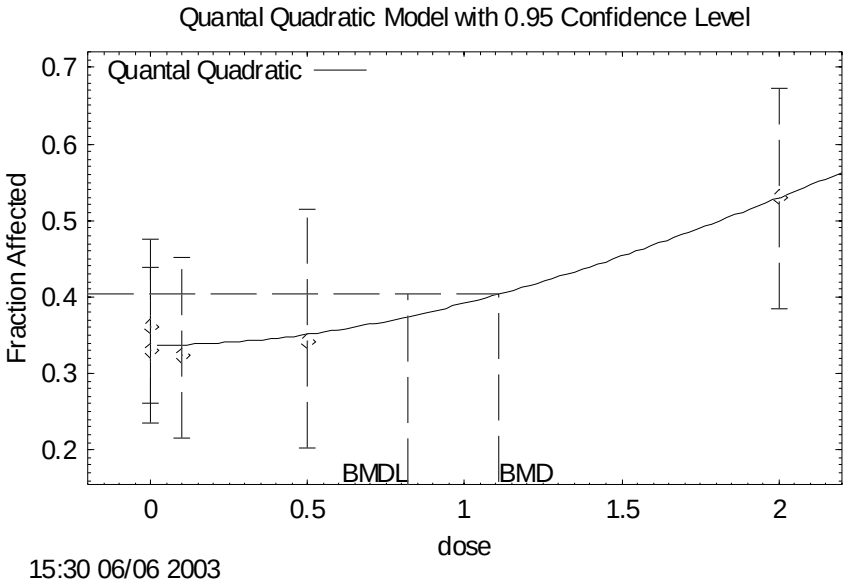

Figure C-3. Observed and predicted incidences for nerve changes in male rats exposed to acrylamide in drinking water for 2 years.

Data source: Friedman et al. (1995).

Table C-6. Predictions (mg/kg-day) from models for doses associated with a 10% extra risk for sciatic nerve changes in female rats exposed to acrylamide in drinking water

Model	BMD (ED_{10})	BMDL (LED_{10})	χ^2 *p*-value	AIC
Gamma[a]	2.48	0.93	0.25	224.85
Multistage[b]	2.02	0.86	0.22	225.12
Quantal quadratic	1.68	1.35	0.18	225.69
Probit	1.20	0.88	0.11	226.92
Logistic	1.23	0.91	0.11	226.85
Quantal linear	1.04	0.65	0.09	227.46
Weibull[a]	2.75	0.93	0.09	226.85
Log-probit[c]	NA[d]			
Log-logistic[c]	NA			

[a]= Restrict power ≥1.
[b]= Restrict betas ≥0, degree of polynomial = 4.
[c]= Slope restricted to >1.
[d]NA = failed to generate a model.

Data source: Friedman et al. (1995).

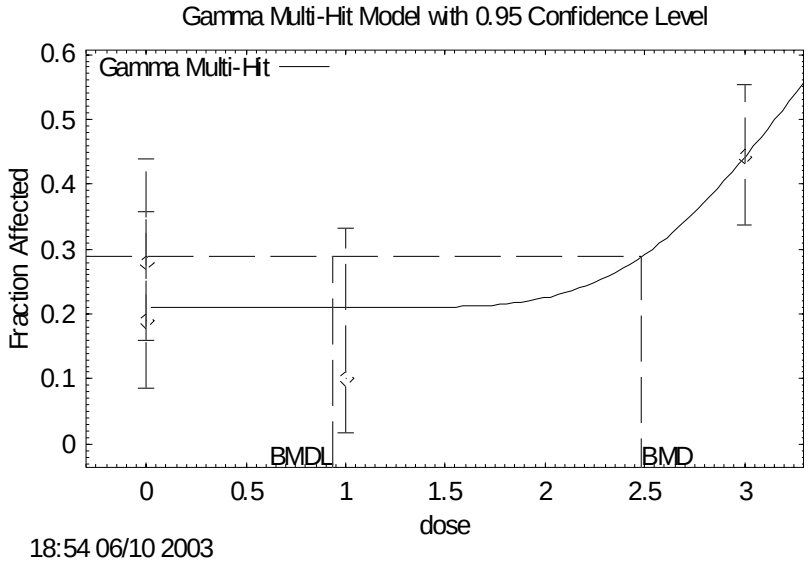

Figure C-4. Observed and predicted incidences for nerve changes in female rats exposed to acrylamide in drinking water for 2 years.

Data source: Friedman et al. (1995).

Table C-7. Predictions (mg/kg-day) from best-fitting models for doses associated with 10, 5, and 1% extra risk for sciatic nerve changes in male and female rats exposed to acrylamide in drinking water

Model	BMD (ED_{10})	BMDL (LED_{10})	BMD (ED_5)	BMDL (LED_5)	BMD (ED_1)	BMDL (LED_1)
Male						
Quantal quadratic	1.11	0.82	0.77	0.57	0.34	0.25
Female						
Gamma[a]	2.48	0.93	2.25	0.46	1.86	0.09

[a]Restrict power ≥1.

Data source: Friedman et al. (1995).

APPENDIX D. DOSE-RESPONSE MODELING FOR CANCER

METHODS

Data: Tumor data from the latest 2-year bioassay with F344 rats (Friedman et al., 1995) were modeled to obtain potential points of departure for deriving an oral slope factor and inhalation unit risk (Table D-1). An earlier study (Johnson et al., 1986) had abnormally high CNS and oral cavity tumors in control males, and possible confounding effects from a viral infection. The new cancer bioassay was subsequently conducted with a larger number of rats (increased statistical power) and dose spacing that improved the characterization of the dose-response relationships for thyroid tumors, mammary gland tumors, and TVM. Tumor data from Friedman et al. (1995) were expected to provide a more reliable characterization of dose-response relationships and were selected for dose-response modeling analysis.

Table D-1. Incidence of tumors with statistically significant increases in the second 2-year bioassay with F344 rats exposed to acrylamide in drinking water

	Dose (mg/kg-day)						
Reference/tumor type	**0**	**0**	**0.1**	**0.5**	**1.0**	**2.0**	**3.0**
Friedman et al., 1995/males[a]							
Follicular cell adenoma/carcinoma	3/100	2/102[e]	12/203	5/101	–	17/75[i]	–
Tunica vaginalis mesothelioma[b]	4/102	4/102	9/204	8/102	–	13/75[i]	–
Friedman et al., 1995/females[a]							
Follicular cell adenoma/carcinoma	1/50	1/50	–	–	10/100	–	23/100[j]
Mammary malignant/benign[c]	7/46	4/50	–	–	21/94[j]	–	30/95[j]
Combined mammary or thyroid tumor[d]	8/46	4/50[f]	–	–	27/94[g,j]	–	48/95[h,j]

[a]Two control groups were included in the study design to assess variability in background tumor responses.
[b]Incidences reported herein are those originally reported by Friedman et al. (1995) and not in the reevaluation study by Iatropoulos et al. (1998).
[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]Mammary tissue was not available for testing in four animals in one control group, six animals in the 1 mg/kg-day dose group and five animals in the 3 mg/kg-day dose group; these animals were not counted for either tumor type, and subtracted from the total number of animals in the group.
[e]The data reported in Table 4 in Friedman et al. (1995) lists 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 (zero) and the total number (two) of combined adenomas and carcinomas in the second control group are used in the tables of this assessment.
[f]One animal had both a mammary and a thyroid tumor; this animal was only counted once in the combined total.
[g]Three animals had both a mammary and a thyroid tumor; these animals were only counted once in the combined total.
[h]Five animals had both a mammary and a thyroid tumor; these animals were only counted once in the combined total.
[i]Statistically significant ($p < 0.05$).
[j]Statistically significant ($p < 0.001$).

Source: Friedman et al. (1995).

Adenoma and carcinoma incidences within each site were combined by counting animals with either of the responses, under the assumption that the tumor types represent different realizations along a continuum of effects resulting from the same mechanism, as recommended by the cancer guidelines (U.S. EPA, 2005).

***Extrapolation Models*:** When there are no biologically based models suitable for modeling the available data, EPA has generally used one dose-response model to promote consistency across cancer assessments. The multistage model (and the related multistage-Weibull model) has been used by EPA in the vast majority of quantitative cancer assessments because it is thought to reflect the multistage carcinogenic process and it fits a broad array of dose-response patterns. Occasionally the multistage model does not fit the available data, in which case an alternative model should be considered. The related multistage-Weibull model has been the preferred model when individual data are available for time-to-tumor modeling, which considers more of the observed response than does the simpler dichotomous response model.

The multistage model is given by:

$$P(d) = 1 - exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)],$$

where $P(d)$ represents the lifetime risk (probability) of cancer at dose d, and q_i (for i = 0, 1, ..., 6) are parameters estimated in fitting the model. The multistage model in BMDS (Benchmark Dose Software, version 1.3.2; U.S. EPA, 2001) was used for all multistage model fits.

The multistage-Weibull model is given by:

$$P(d,t) = 1 - exp[-(q_0 - q_1 d - q_2 d^2 . \ldots - q_k d^k)\ (t - t_0)^j]$$

where $P(d)$ represents the lifetime risk (probability) of cancer at dose d, t is the time to observation of the tumor, t_0 is the time from initiation of the tumor to the time it is observed, and j and q_i (for i = 0, 1, ..., 6) are parameters estimated in fitting the model. Most often there are not sufficient data to estimate t_0, which would at least involve interim sacrifice data at multiple intervals. Without data which help identify times of tumor initiation from the concurrent study or other studies, t_0 is set to 0. The model was fit using the licensed software, MULTI-WEIB (KS Crump and Company, Ruston, LA).

RESULTS

Female Rat Tumor Modeling. For mammary gland tumors (benign or malignant), the two female control groups were combined for modeling, obtaining incidences of 11/96, 21/94, and 30/95 for the 0, 1, and 3 mg/kg-day groups. A one-stage multistage model provided an

adequate fit (p = 0.47) (see Figure D-1). The POD was based on 10% extra risk because this was the lowest level of extra risk that is consistent with the lower end of the observed data range. The BMD_{10} was estimated to be 1.2 mg/kg-day, with a $BMDL_{10}$ of 0.78 mg/kg-day. For linear low-dose extrapolation, the slope factor associated with this site is 0.1/(0.78 mg/kg-day), or 0.13 $(mg/kg\text{-}day)^{-1}$ (see Table D-2).

For thyroid follicular cell adenomas or carcinomas, the two female control groups were combined for modeling, obtaining incidences of 2/100, 10/100, and 23/100 for the 0, 1, and 3 mg/kg-day groups. A one-stage multistage model provided an adequate fit (p = 0.90; see Figure D-2). The POD was based on 10% extra risk which represented the lowest extra risk consistent with the lower end of the observed data range. The BMD_{10} was estimated to be 1.3 mg/kg-day, with a $BMDL_{10}$ of 0.94 mg/kg-day. For linear low-dose extrapolation, the slope factor associated with this site is 0.1/(0.94 mg/kg-day), or 0.11 $(mg/kg\text{-}day)^{-1}$ (see Table D-2).

Table D-2. Risk estimate derived from separate and combined incidence of mammary or thyroid tumors in female F344 rats exposed to acrylamide in drinking water

Tumor site	BMD_R (mg/kg-day)	$BMDL_R$ (mg/kg-day)	Slope factor[a] $(mg/kg\text{-}day)^{-1}$
Mammary (benign and malignant)	1.2	0.78	0.13
Thyroid (adenomas and carcinomas)	1.3	0.94	0.11
Mammary or thyroid tumors (tumor-bearing animals)	1.2	0.88	0.23

[a]Slope factor is the upper bound on lifetime extra risk, calculated using $R/BMDL_R$, where R = 0.1 for mammary tumors or for thyroid tumors and 0.2 for the combination mammary or thyroid tumors.

Data source: Friedman et al. (1995).

Despite a few early mortalities, there were no statistically significant incidences of early mortalities in female rats exposed to acrylamide. Consequently, it was judged that the multistage-Weibull model would not provide an appreciably different estimate of risk compared to the multistage model for either tumor site.

The slope factors corresponding to mammary tumors and to follicular cell thyroid tumors in female F344 rats were very similar, 0.13 vs. 0.11 $(mg/kg\text{-}day)^{-1}$. Given that there was more than one tumor site, basing the unit risk on one tumor site may underestimate the carcinogenic potential of acrylamide.

The EPA cancer guidelines (U.S. EPA, 2005) suggest two approaches for calculating risks when there are multiple tumor sites in a data set to assess the total risk from multiple tumor sites. The simpler approach suggested in the cancer guidelines would be to estimate cancer risk from the combined incidence of tumor-bearing animals. EPA traditionally used this approach

until the NRC (1994) *Science and Judgment* document made a case that evaluating tumor-bearing animals would tend to underestimate overall risk when tumor types occur in a statistically independent manner. The NRC-recommended approach involves adding distributions of the individual tumor incidence to obtain a distribution of the summed incidence for all tumor types. Both approaches were considered for this assessment.

Following the combined incidence approach, the combined incidence of female rats bearing thyroid or mammary tumors from exposure to acrylamide in the drinking water (Tegeris Laboratories, 1989) were considered for dose-response modeling. The data that were modeled are shown in Table D-1, with the control groups combined as above. A one-stage multistage model provided an adequate fit ($p = 0.85$) (see Figure D-3). The POD was based on 20% extra risk because this was the lowest level of extra risk that is consistent with the lower end of the observed data range, yielding a BMD_{20} of 1.2 mg/kg-day and a $BMDL_{20}$ of 0.88 mg/kg-day. For linear low-dose extrapolation, the slope factor associated with this site is 0.2/(0.88 mg/kg-day), or 0.23 $(mg/kg\text{-}day)^{-1}$, approximately two-fold higher than either of the risks estimated from the individual sites.

Following the other recommendation of the EPA cancer guidelines for summing risks from multiple tumor sites (U.S. EPA, 2005; NRC, 1994), etiologically different tumor types—that is, tumors in different organs—are not combined across sites prior to modeling, to allow for the possibility that different tumor types can have different dose-response relationships. Consequently, the modeling carried out separately for the two tumor types was used as a basis for estimating a statistically appropriate upper bound on total risk. Note that this estimate of overall risk describes the risk of developing any combination of the tumor types considered, not just the risk of developing both simultaneously. The estimate involved the following steps:

1. It was assumed that the tumor types associated with acrylamide exposure were statistically independent—that is, that the occurrence of mammary tumors was not dependent on whether there were thyroid follicular cell adenomas/carcinomas. This assumption cannot currently be verified and if not correct could lead to an overestimate of risk from summing across tumor sites. NRC (1994) argued that a general assumption of statistical independence of tumor-type occurrences within animals was not likely to introduce substantial error in assessing carcinogenic potency from rodent bioassay data.

2. The models previously fitted to estimate the BMCs and BMCLs were used to extrapolate to a low level of risk (R), as low as 10^{-6} risk, in order to reach the region of each estimated dose-response function where the slope was reasonably constant and upper bound estimation was still numerically stable.[10] The unit risk for each site was then estimated by $R/BMDL_R$, as for the estimates for each tumor site above.

3. The central tendency or maximum likelihood estimates of unit potency (i.e., risk per unit of exposure), estimated by R/BMD_R, were summed across the two sites for female F344 rats.

4. An estimate of the 95% upper bound on the summed unit risk was calculated by assuming a normal distribution for the individual risk estimates, and deriving the variance of the risk estimate for each tumor site from its 95% upper confidence limit (UCL), according to the formula

$$95\%\ UCL = MLE + (1.645 \times SD)$$

where 1.645 is the t-statistic corresponding to a one-sided 95% confidence interval and >120 degrees of freedom, and the standard deviation (SD) is the square root of the variance of the MLE. The variances were summed across tumor sites to obtain the variance of the sum of the MLE. The 95% UCL on the sum of the individual MLEs was calculated from the variance of the sum of the MLE.

Table D-3 lists the site-specific risk estimates derived via multistage model extrapolation to low exposures and the summed risks for female rats. First note that the individual unit risks are virtually the same as those estimated using the POD approach above. Specifically, the model-extrapolated slope factor for mammary tumors is 0.14 $(mg/kg\text{-}day)^{-1}$ compared with 0.13 $(mg/kg\text{-}day)^{-1}$ using the POD approach (Table D-2), and both methods lead to the same slope factor for thyroid tumors, 0.11 $(mg/kg\text{-}day)^{-1}$.

There is some potential for greater model uncertainty in the model-extrapolated estimates because it is unknown whether the multistage model adequately characterizes the underlying dose-response relationship in this low-exposure range; however, it appears to be minimal for

[10] Although this step appears to differ from the explicit recommendation of the cancer guidelines (U.S. EPA, 2005) to estimate cancer risk from a point of departure near the lower end of the observed range, without significant extrapolation to lower doses, this method is recommended in the guidelines as a method for combining multiple extrapolations. For this purpose, a quantitative combination of individual risks within the range of observation is not generally practicable. More significantly, numerical combination of risks in the range of observation does not generally lead to a numerically unique result, due to different dose-response relationships. When risk is expected to be low-dose linear, the approach followed here leads to the most reliable estimate of the summed risk.

these data. Consequently, the multistage model extrapolations introduce little additional uncertainty into summing risks across these tumor sites.

The resulting 95% UCL on the summed risk of mammary tumors or thyroid follicular cell adenomas/carcinomas for female F344 rats was 0.23 $(mg/kg\text{-}day)^{-1}$, and the summed central tendency was 0.17 $(mg/kg\text{-}day)^{-1}$, about a 1.4-fold difference (Table D-3). The estimated risk for mammary tumors was more variable, contributing about 70% of the overall variability in the summed risk. As was the case with the tumor-bearing approach, the summed upper bound risk is nearly two-fold higher than either of the individual risks. For these data, the two approaches yield very similar results.

Table D-3. Risk estimates derived from separate and summed dose-response modeling of mammary and thyroid tumors in female F344 rats exposed to acrylamide in drinking water

Tumor site	BMD_R (mg/kg-day)	$BMDL_R$ (mg/kg-day)	Central tendency oral potency[a] $(mg/kg\text{-}day)^{-1}$	Upper bound on lifetime extra risk $(mg/kg\text{-}day)^{-1}$
Mammary (benign and malignant)	1.1×10^{-4}	7.4×10^{-5}	8.9×10^{-2}	0.14
Thyroid (adenomas and carcinomas)	1.2×10^{-4}	8.9×10^{-5}	8.1×10^{-2}	0.11
Risk of either mammary or thyroid tumors			0.17	0.23[b]

[a]Central tendency oral potency = R/BMD_R, where $R = 1 \times 10^{-5}$. The combined central tendency risk is the sum of the individual oral potencies.
[b]The slope factor for the combination of tumor sites is the 95% UCL on the sum of the central tendency unit potencies, not the sum of the individual slope factors; see the preceding text for derivation. This slope factor should not be used with exposures greater than 3 mg/kg-day, because above this level the dose-response relationship is likely to be nonlinear.

Data source: Friedman et al. (1995).

Male Rat Tumor Modeling

As was done with the female rat control groups, the two male rat control groups were combined into one control group: 5/202 males had thyroid follicular cell adenomas or carcinomas, and 8/202 had tunica vaginalis mesotheliomas.

Because male rats in the highest dose group in the Friedman et al. (1995) study showed early mortalities, models that adjusted for early mortality were fit to the data for tunica vaginalis mesotheliomas and thyroid follicular cell adenomas and carcinoma. Pathology reports for individual rats in the study (Tegeris Laboratories, 1989) were examined to extract time-to-death and tumor occurrence data for each animal. Outputs from the computer program follow.

For TVM, MULTI-WEIB provided a model fit with a one-degree polynomial. The dose associated with 10% extra risk (ED_{10}) at 108 weeks (i.e., full lifetime) was 1.2 mg/kg-day, with a lower 95% confidence limit (LED_{10}) of 0.75 mg/kg-day. For linear low-dose extrapolation, the

slope factor associated with this site, using the POD approach, is 0.1/(0.75 mg/kg-day), or 0.13 $(mg/kg\text{-}day)^{-1}$ (see Table D-4).

For thyroid follicular cell adenomas or carcinomas, MULTI-WEIB provided a model fit with a one-degree polynomial. The dose associated with 10% extra risk (ED_{10}) at 108 weeks (i.e., full lifetime) was 0.71 mg/kg-day, with an LED_{10} of 0.45 mg/kg-day. For linear low-dose extrapolation, the slope factor associated with this site, using the POD approach, is 0.1/(0.45 mg/kg-day), or 0.22 $(mg/kg\text{-}day)^{-1}$ (see Table D-4).

Table D-4. Risk estimates for separate and combined incidence of TVMs or thyroid tumors in male rats exposed to acrylamide in drinking water

Incidence modeled	BMD_R[a] (mg/kg-day)	$BMDL_R$[a] (mg/kg-day)	Slope factor (risk level/BMDL) $(mg/kg\text{-}day)^{-1}$
TVM	1.2	0.75	1.3×10^{-1}
Follicular cell thyroid tumors	0.71	0.45	2.2×10^{-1}
TVM or thyroid tumors[b]	0.70	0.30	3.3×10^{-1}

[a]R = 10% extra risk.
[b]Tumor-bearing animal method: Individual rats that had more than one of the tumor types were counted only once (see Table D-1 for incidences). For the NRC (1994) approach, the slope factor was 0.34 (see discussion below).

Data source: Friedman et al. (1995).

The first recommended method in the EPA cancer guidelines for assessing total risk from multiple tumor sites (U.S. EPA, 2005; NRC, 1994) does not combine data from etiologically different tumor types prior to modeling to allow for the possibility that different tumor types can have different dose-response relationships. Note that the multistage-Weibull model yielded distinctly different values of *j*, the parameter that describes the relationship of incidence with increasing age, for the two tumor sites. For TVM, *j* was 1, indicating no difference between the groups regarding incidence increasing with increasing age. For thyroid tumors, *j* was 3.7, indicating relatively greater tumor incidence with increasing exposure as age increases. Consequently, keeping the dose-response assessments separate maintains a better correspondence with the observed biological events. The risks from the individual sites were summed using the statistical approach as described for female rats above.

Table D-5 lists the site-specific risk estimates derived via multistage-Weibull model extrapolation to low exposures, and the summed risks. First note that these individual unit risks are virtually the same as those estimated using the POD approach above. Specifically, the model-extrapolated slope factor for TVM is 0.14 $(mg/kg\text{-}day)^{-1}$ compared with 0.13 $(mg/kg\text{-}day)^{-1}$, using the POD approach (Table D-4), and the model-extrapolated factor for thyroid tumors is 0.23 $(mg/kg\text{-}day)^{-1}$ compared with 0.22 $(mg/kg\text{-}day)^{-1}$, using the POD approach (Table D-2). While there is some potential for greater model uncertainty in the model-extrapolated estimates, because it is unknown whether the multistage model adequately characterizes the

underlying dose-response relationship in this low-exposure range, it appears to be minimal for these data. Consequently, the multistage model extrapolations introduce little additional uncertainty into summing risks across these tumor sites.

Table D-5. Risk estimates derived from modeling separate and summed incidence of TVM and thyroid tumors in male F344 rats exposed to acrylamide in drinking water

Tumor site	BMD_R (mg/kg-day)	$BMDL_R$ (mg/kg-day)	Central tendency oral potency[a] $(mg/kg\text{-}day)^{-1}$	Upper bound on lifetime extra risk $(mg/kg\text{-}day)^{-1}$
TVM	1.1×10^{-5}	7.1×10^{-6}	8.8×10^{-2}	0.14
Thyroid (adenomas and carcinomas)	6.7×10^{-6}	4.3×10^{-6}	0.15	0.23
Risk of either TVM or thyroid tumors			0.24	0.34[b]

[a]Central tendency oral potency = R/BMD_R, where $R = 1 \times 10^{-6}$. The combined central tendency risk is the sum of the individual oral potencies.
[b]The slope factor for the combination of tumor sites is the 95% UCL on the sum of the central tendency unit potencies, not the sum of the individual slope factors; see the preceding text for derivation. This slope factor should not be used with exposures greater than 2 mg/kg-day, because above this level the dose-response relationship is likely to be nonlinear.

Data source: Friedman et al. (1995).

The resulting 95% UCL on the summed risk of TVM or thyroid follicular cell adenomas/carcinomas for male F344 rats was 0.34 $(mg/kg\text{-}day)^{-1}$, and the summed central tendency was 0.24 $(mg/kg\text{-}day)^{-1}$, about a 1.4-fold difference (Table D-5). The estimated risk for thyroid tumors was the more variable, contributing about 70% of the overall variability in the summed risk. The upper bound on the summed risks is about 1.4-fold higher than the risk of thyroid tumors alone, the higher of the two individual risks.

Based on the analyses discussed above, the recommended upper bound estimate on human extra cancer risk from continuous, lifetime oral exposure to acrylamide is 0.3 $(mg/kg\text{-}day)^{-1}$, rounding the summed risk for male rats above to one significant digit.[11] The slope factor can be used to estimate cancer risks from doses up to approximately 2.0 mg/kg-day due to the approximate linear dose-response throughout the observable range. This slope factor should not be used with exposures greater than 2.0 mg/kg-day, the highest exposure in the male rat bioassay, because above this level the cancer dose-response relationships are not likely to continue linearly, and there are no data to indicate where this nonlinearity would begin to occur.

[11] For comparison, the tumor-bearing animal approach applied to the combined incidence of thyroid or TVM tumors (see Table D-1 for data) led to a multistage-Weibull model with a three-stage polynomial, and $j = 5.4$. The dose associated with a 10% extra risk (ED_{10}) at 108 weeks (i.e., full lifetime) was 0.70 mg/kg-day, with an LED_{10} of 0.30 mg/kg-day (see the last output). For linear low-dose extrapolation, the slope factor associated with this combination, using the point of departure approach, is 0.1/(0.30 mg/kg-day), or 0.33 per mg/kg-day, virtually identical to that estimated above using the NRC (1994) approach.

As in most risk assessments, extrapolation of study data to estimate potential risks to human populations from exposure to acrylamide has engendered some uncertainty in the results. The uncertainty falls into two major categories: model uncertainty and parameter uncertainty. Model uncertainty refers to a lack of knowledge needed to determine which is the correct scientific theory on which to base a model, whereas parameter uncertainty refers to a lack of knowledge about the values of a model's parameters (U.S. EPA, 2005). In the absence of a biologically based model, a multistage model was the preferred model because it has some concordance with the multistage theory of carcinogenesis and serves as a benchmark for comparison with other cancer dose-response analyses. That said, it is unknown how well this model or the linear low-dose extrapolation predicts low-dose risks for acrylamide. Also, while the female rats did not appear to have as strong a carcinogenic response as the male rats, it is not known which species is more relevant for extrapolation of risk to humans.

Parameter uncertainty can be assessed through confidence intervals and probabilistic analysis. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. Uncertainty in the animal dose-response data can be assessed through the ratio of BMDs to their BMDLs. For the tumor sites evaluated here, the ratios were below a factor of 2, which is typical in similarly designed bioassays.

FEMALE RATS, MALIGNANT AND BENIGN MAMMARY TUMORS, ACRYLAMIDE
DATA SOURCE: Tegeris Laboratories, 1989

```
 ================================================================
     Multistage Model. (Version: 2.5;  Date: 10/17/2005)
     Input Data File: G:\_BMDS\PCE\ACRYLAMIDE_FRIEDMAN_F.(d)
     Gnuplot Plotting File:  G:\_BMDS\PCE\ACRYLAMIDE_FRIEDMAN_F.plt
                                    Mon Jun 05 11:32:19 2006
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the probability function is:

  P[response] = background + (1-background)*[1-EXP(
            -beta1*dose^1-beta2*dose^2)]

  The parameter betas are restricted to be positive

  Dependent variable = mamm
  Independent variable = mg_kg_d

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

            Default Initial Parameter Values
              Background =    0.131573
                 Beta(1) =   0.0827018
                 Beta(2) =           0

        Asymptotic Correlation Matrix of Parameter Estimates

       ( *** The model parameter(s)  -Beta(2)
            have been estimated at a boundary point, or have been specified by the user,
            and do not appear in the correlation matrix )

         Background    Beta(1)

Background        1       -0.71

  Beta(1)      -0.71         1


                     Parameter Estimates

                                        95.0% Wald Confidence Interval
     Variable      Estimate      Std. Err.    Lower Conf. Limit  Upper Conf. Limit
    Background      0.124597      0.0835445       -0.0391471        0.288342
      Beta(1)      0.0887157      0.0565531       -0.0221264        0.199558
      Beta(2)              0             NA

NA - Indicates that this parameter has hit a bound
    implied by some inequality constraint and thus
```

has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-143.354	3			
Fitted model	-143.609	2	0.51136	1	0.4746
Reduced model	-149.278	1	11.8483	2	0.002674

AIC: 291.219

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.1246	11.961	11	96	-0.297
1.0000	0.1989	18.698	21	94	0.595
3.0000	0.3292	31.270	30	95	-0.277

Chi^2 = 0.52 d.f. = 1 P-value = 0.4713

Benchmark Dose Computation

Specified effect = 0.1 Risk Type = Extra risk Confidence level = 0.95 BMD = 1.18762 BMDL = 0.776448	Specified effect = 0.0001 Risk Type = Extra risk Confidence level = 0.95 BMD = 0.00112725 BMDL = 0.000736981
Specified effect = 1e-005 Risk Type = Extra risk Confidence level = 0.95 BMD = 0.00011272 BMDL = 7.36948e-005	Specified effect = 1e-006 Risk Type = Extra risk Confidence level = 0.95 BMD = 1.1272e-005 BMDL = 1.12717e-005

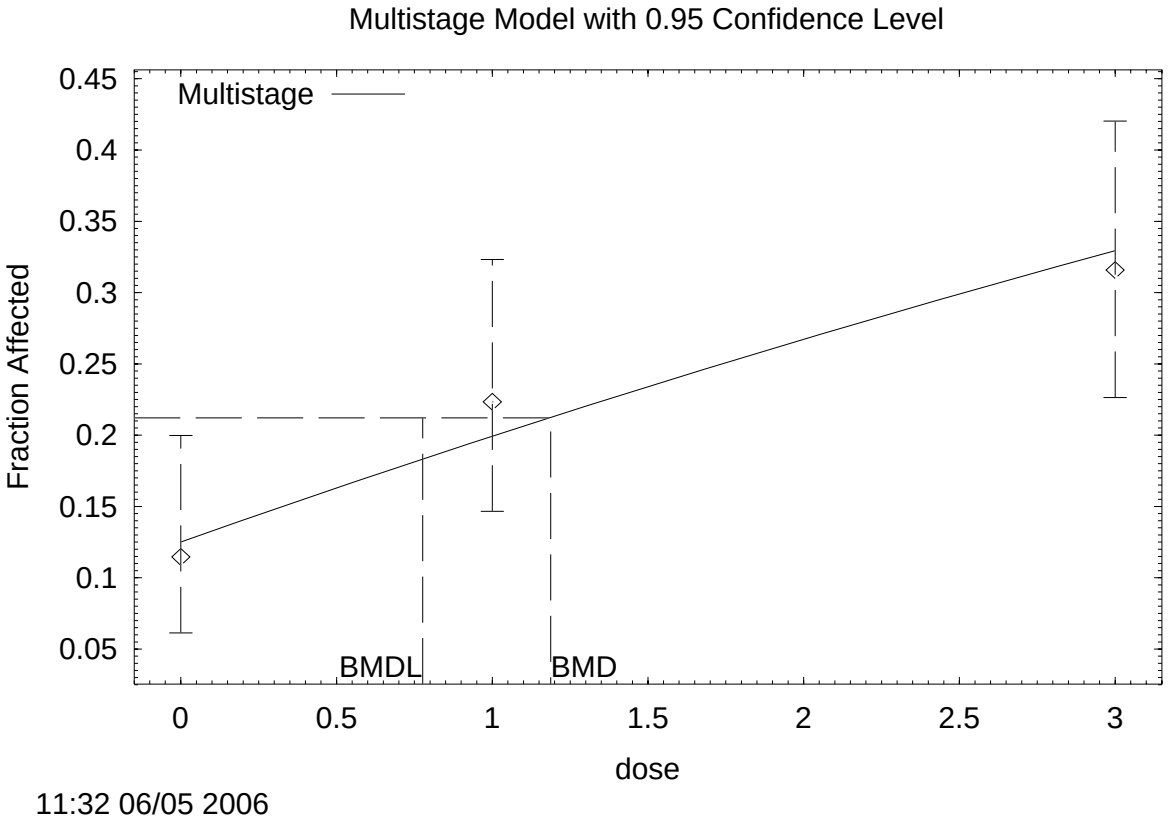

Figure D-1. Observed and predicted incidences for mammary gland tumors in female rats exposed to acrylamide in drinking water for 2 years.

Source: Friedman et al. (1995).

```
 ================================================================
     Multistage Model. (Version: 2.5;  Date: 10/17/2005)
     Input Data File: G:\_BMDS\PCE\ACRYLAMIDE_FRIEDMAN_F.(d)
     Gnuplot Plotting File:  G:\_BMDS\PCE\ACRYLAMIDE_FRIEDMAN_F.plt
                                      Mon Jun 05 11:38:01 2006
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the probability function is:

  P[response] = background + (1-background)*[1-EXP(
           -beta1*dose^1-beta2*dose^2)]

  The parameter betas are restricted to be positive


  Dependent variable = thyroid
  Independent variable = mg_kg_d

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



            Default Initial Parameter Values
              Background =   0.0220015
                 Beta(1) =   0.0800466
                 Beta(2) =         0


        Asymptotic Correlation Matrix of Parameter Estimates

       ( *** The model parameter(s)  -Beta(2)
            have been estimated at a boundary point, or have been specified by the user,
            and do not appear in the correlation matrix )

         Background    Beta(1)

Background        1      -0.71

  Beta(1)      -0.71        1
                     Parameter Estimates

                                      95.0% Wald Confidence Interval
     Variable      Estimate      Std. Err.    Lower Conf. Limit  Upper Conf. Limit
    Background      0.0204321      0.0851609        -0.14648        0.187344
      Beta(1)      0.0813062      0.0507808       -0.0182223        0.180835
      Beta(2)            0           NA

NA - Indicates that this parameter has hit a bound
    implied by some inequality constraint and thus
```

has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-96.2398	3			
Fitted model	-96.2474	2	0.0150352	1	0.9024
Reduced model	-108.069	1	23.6586	2	<.0001

AIC: 196.495

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0204	2.043	2	100	-0.031
1.0000	0.0969	9.693	10	100	0.104
3.0000	0.2325	23.246	23	100	-0.058

Chi^2 = 0.02 d.f. = 1 P-value = 0.9021

Benchmark Dose Computation

Specified effect = 0.1 Risk Type = Extra risk Confidence level = 0.95 BMD = 1.29585 BMDL = 0.941045	Specified effect = 1e-005 Risk Type = Extra risk Confidence level = 0.95 BMD = 0.000122993 BMDL = 8.93171e-005
Specified effect = 0.0001 Risk Type = Extra risk Confidence level = 0.95 BMD = 0.00122998 BMDL = 0.000893211	Specified effect = 1e-006 Risk Type = Extra risk Confidence level = 0.95 BMD = 1.22992e-005 BMDL = 1.21663e-005

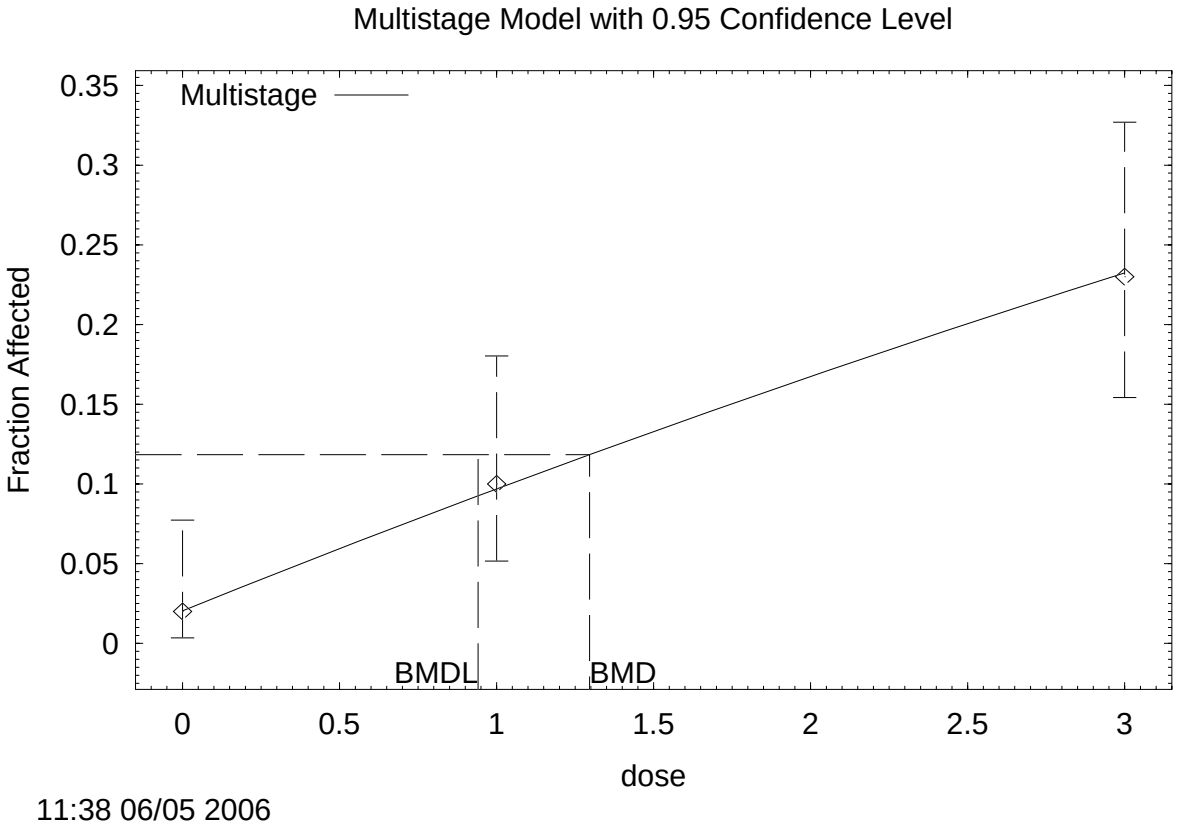

Figure D-2: Observed and predicted incidences for thyroid tumors in female rats exposed to acrylamide in drinking water for 2 years.

Source: Friedman et al. (1995).

```
 ================================================================
     Multistage Model. (Version: 2.5;  Date: 10/17/2005)
     Input Data File: G:\_BMDS\PCE\ACRYLAMIDE_FRIEDMAN_F.(d)
     Gnuplot Plotting File:  G:\_BMDS\PCE\ACRYLAMIDE_FRIEDMAN_F.plt
                                      Wed Jun 14 12:51:00 2006
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the probability function is:

  P[response] = background + (1-background)*[1-EXP(
           -beta1*dose^1-beta2*dose^2)]

  The parameter betas are restricted to be positive


  Dependent variable = com
  Independent variable = mg_kg_d

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



            Default Initial Parameter Values
              Background =    0.130609
                 Beta(1) =    0.188994
                 Beta(2) =           0


        Asymptotic Correlation Matrix of Parameter Estimates

       ( *** The model parameter(s)  -Beta(2)
            have been estimated at a boundary point, or have been specified by the user,
            and do not appear in the correlation matrix )

         Background    Beta(1)

Background        1      -0.67

  Beta(1)      -0.67        1
                      Parameter Estimates

                                        95.0% Wald Confidence Interval
     Variable      Estimate      Std. Err.    Lower Conf. Limit  Upper Conf. Limit
    Background      0.127194      0.0836998       -0.0368541        0.291243
      Beta(1)       0.192236      0.0612613        0.0721662        0.312306
      Beta(2)             0           NA

NA - Indicates that this parameter has hit a bound
    implied by some inequality constraint and thus
```

has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-158.381	3			
Fitted model	-158.4	2	0.0370709	1	0.8473
Reduced model	-175.349	1	33.9343	2	<.0001

AIC: 320.8

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.1272	12.211	12	96	-0.065
1.0000	0.2798	26.305	27	94	0.160
3.0000	0.5097	48.422	48	95	-0.087

Chi^2 = 0.04 d.f. = 1 P-value = 0.8471

Benchmark Dose Computation

Specified effect = 0.2

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.16078

BMDL = 0.88194

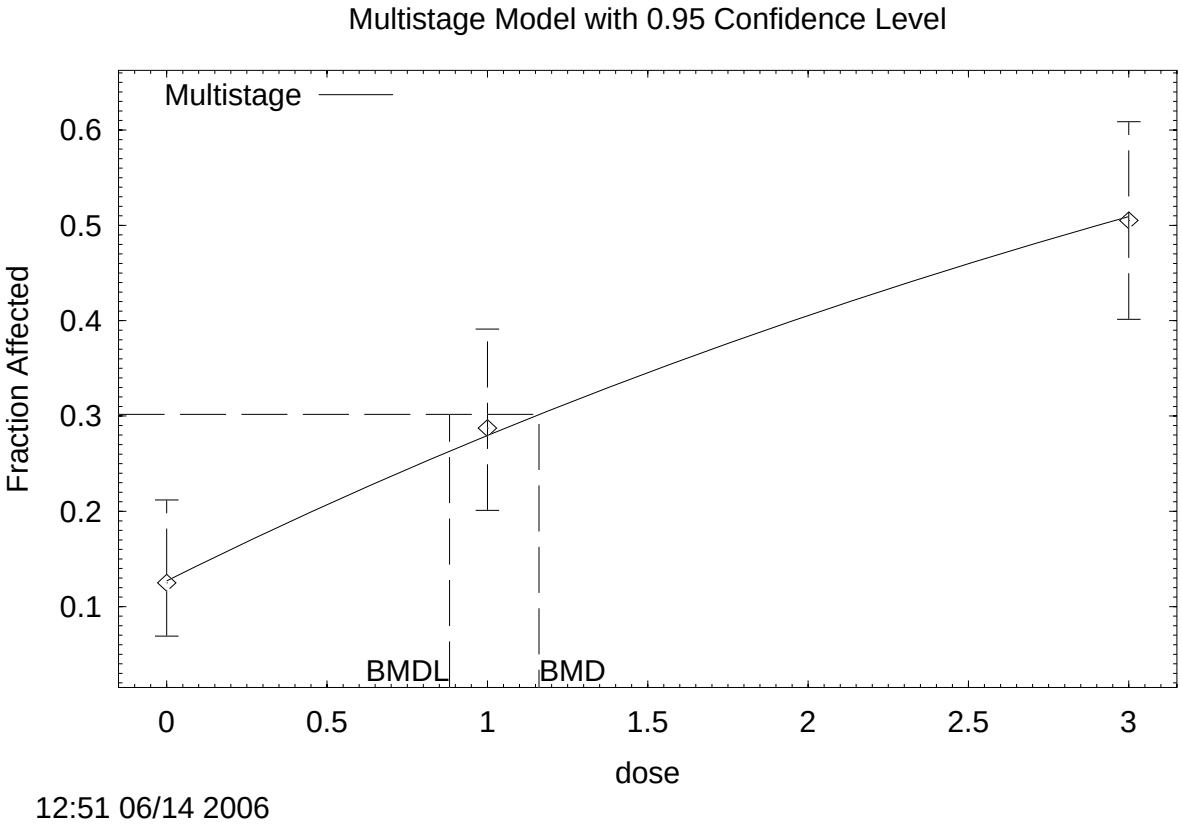

Figure D-3: Observed and predicted incidences for mammary or thyroid tumors in female rats exposed to acrylamide in drinking water for 2 years.

Source: Friedman et al. (1995).

MALE RAT, TUNICA VAGINALIS MESOTHELIOMA, ACRYLAMIDE WITH INDUCTION TIME ESTIMATED (TIME UNIT = WEEKS)

DATA SOURCE: Tegeris Laboratories, 1989

DATE: 06-07-03 TIME: 18:11:17

MULTI-WEIB (MAR 1985)

K.S. CRUMP & COMPANY, INC.
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

THE 36 OBSERVATIONS AT LEVEL 1 WITH A DOSE OF .000000

TIME	#	TUMOR INDICATOR	TIME	#	TUMOR INDICATOR
41.0	1	1	58.0	1	1
69.0	1	1	73.0	2	1
74.0	1	1	76.0	2	1
77.0	2	1	78.0	2	1
79.0	1	1	82.0	2	1
85.0	1	1	87.0	2	1
88.0	1	1	89.0	3	1
90.0	5	1	91.0	5	1
93.0	3	1	94.0	1	1
95.0	3	1	96.0	1	2
96.0	2	1	97.0	1	1
98.0	1	1	99.0	1	2
99.0	9	1	100.0	3	1
101.0	3	1	102.0	3	1
103.0	12	1	104.0	9	1
105.0	4	1	106.0	17	1
107.0	4	2	107.0	57	1
108.0	2	2	108.0	36	1

THE 46 OBSERVATIONS AT LEVEL 2 WITH A DOSE OF .100000

TIME	#	TUMOR INDICATOR	TIME	#	TUMOR INDICATOR
46.0	1	1	61.0	1	1
63.0	1	1	65.0	1	1
67.0	1	1	68.0	1	1
72.0	1	1	76.0	1	2
78.0	2	1	79.0	1	1
80.0	1	1	81.0	1	1
82.0	3	1	83.0	2	1
84.0	1	1	85.0	2	1
86.0	2	1	87.0	3	1
89.0	1	1	90.0	2	1
91.0	1	1	92.0	1	1
93.0	1	2	93.0	1	1
94.0	4	1	95.0	1	1
96.0	3	1	97.0	1	2
97.0	2	1	98.0	1	2
98.0	5	1	99.0	2	1

100.0	1	2	100.0	3	1
101.0	2	1	102.0	1	2
102.0	5	1	103.0	11	1
104.0	10	1	105.0	6	1
106.0	1	2	106.0	15	1
107.0	1	2	107.0	57	1
108.0	1	2	108.0	38	1

THE 26 OBSERVATIONS AT LEVEL 3 WITH A DOSE OF .500000

TIME	#	TUMOR INDICATOR	TIME	#	TUMOR INDICATOR
2.0	1	1	32.0	1	1
49.0	1	1	72.0	1	1
77.0	1	1	78.0	1	2
78.0	1	1	79.0	2	1
86.0	2	1	88.0	1	1
90.0	3	1	91.0	1	1
95.0	1	1	97.0	2	1
98.0	3	1	99.0	3	1
101.0	1	1	103.0	2	2
103.0	7	1	104.0	4	1
105.0	3	1	106.0	3	2
106.0	6	1	107.0	2	2
107.0	30	1	108.0	19	1

THE 39 OBSERVATIONS AT LEVEL 4 WITH A DOSE OF 2.00000

TIME	#	TUMOR INDICATOR	TIME	#	TUMOR INDICATOR
51.0	1	1	55.0	1	1
58.0	1	1	61.0	1	1
67.0	1	2	67.0	1	1
72.0	1	1	74.0	1	1
76.0	1	1	77.0	1	1
78.0	1	2	79.0	2	1
80.0	1	2	81.0	1	1
82.0	1	2	83.0	2	1
86.0	1	2	88.0	4	1
92.0	1	1	93.0	1	2
93.0	1	1	95.0	1	2
95.0	1	1	97.0	1	1
98.0	1	2	98.0	1	1
100.0	3	1	101.0	2	1
102.0	1	1	103.0	1	2
103.0	5	1	104.0	1	2
104.0	5	1	105.0	1	2
105.0	1	1	106.0	6	1
107.0	2	2	107.0	14	1
108.0	2	1			

FORM OF PROBABILITY FUNCTION:

P(DOSE) = 1 - exp((-Q0 - Q1 * D - Q2 * D^2 - Q3 * D^3) * (T - T0)^J)

THE MAXIMUM LIKELIHOOD ESTIMATION OF:

PROBABILITY FUNCTION COEFFICIENTS

Q(0)= .384153255996E-03
Q(1)= .812864704009E-03
Q(2)= .000000000000
Q(3)= .000000000000

TIME FUNCTION COEFFICIENTS

T0 = .000000000000
J = 1.00000000000

THE MAXIMUM LIKELIHOOD IS -133.655450741

MAXIMUM LIKELIHOOD ESTIMATES OF EXTRA RISK
****

WEIBULL LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK
****

RISK	MLE DOSE	ON DOSE	LOWER BOUND ON RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT INTERVAL	TIME	
.100000	1.20015	.747920	.155548		95.0%	108.000	
1.000000E-03	1.139660E-02	7.102226E-03		1.604166E-03		95.0%	108.000
1.000000E-06	1.139090E-05	7.116445E-06		1.600645E-06		95.0%	108.000

WEIBULL UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE
****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT INTERVAL		TIME
.500000	4.294526E-02	6.801233E-02	95.0%	108.000	
2.00000	.161029	.252245		95.0%	108.000

NORMAL COMPLETION!

MALE RAT, FOLLICULAR CELL ADENOMA AND CARCINOMA, ACRYLAMIDE WITH NO INDUCTION TIME ESTIMATED

DATA SOURCE: Tegeris Laboratories, 1989

[NOTE FOR THE RECORD: When SRC examined the individual male rat pathology reports provided in the Tegeris Laboratories 1989 Report (provided on CD by Marvin Friedman), 2 rats with follicular cell adenomas (#138 and #175), and one rat with a follicular cell carcinoma (#182) were found in Control Group 1. These numbers agree with the numbers reported in Table 4 of the Friedman et al. (1995) report. Among the individual animal pathology reports for male rats in Control Group 2, however, SRC found two male rats with follicular cell carcinomas (#'s 335 and 345), but no male rats with follicular cell adenomas. This does not agree with Table 4 in Friedman et al. (1995), which reported that Control Group 2 had 2 male rats with follicular cell carcinomas and one male rat with a follicular cell adenoma. The dose-response analysis described in here in Appendix D for the male rat follicular cell adenomas plus carcinomas used the Tegeris Laboratories 1989 report numbers. In addition, based on SRC's examination of the individual animal pathology reports, the total number of male rats assessed for thyroid histopathology in the two control groups was 202 (rather than the 204 male rats included in these control groups); 2 male rats in Control Group 1 did not have thyroid histopathology.]

DATE: 06-09-03 TIME: 19:38:24
MULTI-WEIB (MAR 1985)
(C) COPYRIGHT CLEMENT ASSOCIATES, INC. 1983-198
K.S. CRUMP & COMPANY, INC.
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

THE 35 OBSERVATIONS AT LEVEL 1 WITH A DOSE OF .000000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
41.0	1	1	58.0	1	1
69.0	1	1	73.0	2	1
74.0	1	1	76.0	2	1
77.0	2	1	78.0	2	1
79.0	1	1	82.0	2	1
85.0	1	1	87.0	2	1
88.0	1	1	89.0	3	1
90.0	5	1	91.0	5	1
93.0	3	1	94.0	1	1
95.0	3	1	96.0	3	1
97.0	1	1	98.0	1	1
99.0	10	1	100.0	3	1
101.0	3	1	102.0	3	1
103.0	12	1	104.0	1	2
104.0	8	1	105.0	4	1
106.0	16	1	107.0	2	2

107.0	58	1	108.0	2	2
108.0	36	1			

THE 44 OBSERVATIONS AT LEVEL 2 WITH A DOSE OF .100000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
46.0	1	1	61.0	1	1
63.0	1	1	65.0	1	1
67.0	1	1	68.0	1	1
72.0	1	1	76.0	1	1
78.0	2	1	79.0	1	1
80.0	1	1	81.0	1	1
82.0	3	1	83.0	2	1
84.0	1	1	85.0	2	1
86.0	2	1	87.0	3	1
89.0	1	1	90.0	2	1
91.0	1	1	92.0	1	1
93.0	2	1	94.0	4	1
95.0	1	1	96.0	3	1
97.0	3	1	98.0	6	1
99.0	2	1	100.0	1	2
100.0	3	1	101.0	2	1
102.0	6	1	103.0	1	2
103.0	10	1	104.0	1	2
104.0	9	1	105.0	6	1
106.0	1	2	106.0	15	1
107.0	4	2	107.0	54	1
108.0	4	2	108.0	35	1

THE 26 OBSERVATIONS AT LEVEL 3 WITH A DOSE OF .500000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
2.0	1	1	32.0	1	1
49.0	1	1	72.0	1	1
77.0	1	1	78.0	1	2
78.0	1	1	79.0	2	1
86.0	1	2	86.0	1	1
88.0	1	1	90.0	3	1
91.0	1	1	95.0	1	1
97.0	2	1	98.0	3	1
99.0	3	1	101.0	1	1
103.0	9	1	104.0	4	1
105.0	3	1	106.0	9	1
107.0	2	2	107.0	30	1
108.0	1	2	108.0	18	1

THE 38 OBSERVATIONS AT LEVEL 4 WITH A DOSE OF 2.00000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
51.0	1	1	55.0	1	1
58.0	1	1	61.0	1	1
67.0	2	1	72.0	1	1

74.0	1	2	76.0	1	1
77.0	1	1	78.0	1	1
79.0	1	2	79.0	1	1
80.0	1	1	81.0	1	1
82.0	1	1	83.0	2	1
86.0	1	1	88.0	4	1
92.0	1	1	93.0	1	2
93.0	1	1	95.0	2	1
97.0	1	1	98.0	1	2
98.0	1	1	100.0	3	1
101.0	2	1	102.0	1	2
103.0	2	2	103.0	4	1
104.0	1	2	104.0	5	1
105.0	2	1	106.0	2	2
106.0	4	1	107.0	7	2
107.0	9	1	108.0	2	1

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp((-Q0 - Q1 * D) * (T - T0)^J)

THE MAXIMUM LIKELIHOOD ESTIMATION OF:
PROBABILITY FUNCTION COEFFICIENTS
Q(0)= .107582747873E-08
Q(1)= .420830494317E-08
TIME FUNCTION COEFFICIENTS
T0 = .000000000000
J = 3.71285084690

THE MAXIMUM LIKELIHOOD IS -127.749366108

MAXIMUM LIKELIHOOD ESTIMATES OF EXTRA RISK
****

WEIBULL LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK
****

RISK	MLE DOSE	ON DOSE	LOWER BOUND ON RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT INTERVAL	TIME
.100000	.705946	.451674	.151830		95.0%	108.000
1.000000E-03	6.703644E-03	4.289084E-03		1.562515E-03	95.0%	108.000
1.000000E-06	6.700295E-06	4.308122E-06		1.555270E-06	95.0%	108.000

WEIBULL UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE
****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT INTERVAL	TIME
.500000	7.190726E-02	.110089	95.0%	108.000
2.00000	.258066	.372827	95.0%	108.000

NORMAL COMPLETION!

Time-to-Tumor Model Results for the Combined Incidence of Thyroid Tumors or TVM in Male Rats Exposed to Acrylamide in the Drinking Water

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RUSTON, LA 71270
(318) 255-4800

THE 28 OBSERVATIONS AT LEVEL 1 WITH A DOSE OF 0.000000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
42.0	1	1	73.0	1	1
74.0	1	1	76.0	2	1
77.0	1	1	78.0	2	1
82.0	2	1	87.0	1	1
89.0	3	1	90.0	4	1
91.0	2	1	93.0	1	1
94.0	1	1	95.0	3	1
96.0	2	1	96.0	1	2
97.0	1	1	99.0	5	1
101.0	3	1	102.0	3	1
103.0	5	1	104.0	2	1
105.0	1	1	106.0	10	1
107.0	25	1	107.0	5	2
108.0	13	1	108.0	1	2

THE 25 OBSERVATIONS AT LEVEL 2 WITH A DOSE OF .000000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
58.0	1	1	69.0	1	1
73.0	1	1	77.0	1	1
79.0	1	1	85.0	1	1
87.0	1	1	88.0	1	1
90.0	1	1	91.0	3	1
93.0	1	1	94.0	1	1
98.0	1	1	99.0	4	1
99.0	1	2	100.0	3	1
103.0	7	1	104.0	6	1
104.0	1	2	105.0	3	1
106.0	7	1	107.0	32	1
107.0	1	2	108.0	22	1
108.0	2	2			

THE 48 OBSERVATIONS AT LEVEL 3 WITH A DOSE OF .100000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
46.0	1	1	61.0	1	1
63.0	1	1	65.0	1	1
67.0	1	1	68.0	1	1
72.0	1	1	76.0	1	2
78.0	2	1	79.0	1	1
80.0	1	1	81.0	1	1
82.0	3	1	83.0	2	1
84.0	1	1	85.0	2	1
86.0	2	1	87.0	3	1
89.0	1	1	90.0	2	1
91.0	1	1	92.0	1	1
93.0	1	1	93.0	1	2
94.0	4	1	95.0	1	1
96.0	3	1	97.0	3	1
97.0	1	2	98.0	4	1
98.0	1	2	99.0	2	1
100.0	2	1	100.0	2	2
101.0	2	1	102.0	5	1
102.0	1	2	103.0	10	1
103.0	1	2	104.0	9	1
104.0	1	2	105.0	6	1
106.0	14	1	106.0	2	2
107.0	53	1	107.0	5	2
108.0	33	1	108.0	6	2

THE 28 OBSERVATIONS AT LEVEL 4 WITH A DOSE OF .500000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
2.0	1	1	32.0	1	1
49.0	1	1	72.0	1	1
77.0	1	1	78.0	1	1
78.0	1	3	79.0	2	1
86.0	1	1	86.0	1	3
88.0	1	1	90.0	3	1
92.0	1	1	95.0	1	1
97.0	2	1	98.0	3	1
99.0	3	1	101.0	1	1
103.0	6	1	103.0	3	3
104.0	4	1	105.0	3	1
106.0	6	1	106.0	3	3
107.0	29	1	107.0	3	3
108.0	18	1	108.0	1	3

THE 40 OBSERVATIONS AT LEVEL 5 WITH A DOSE OF 2.00000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR

51.0	1	1	55.0	1	1
58.0	1	1	61.0	1	1
67.0	1	1	67.0	1	2
72.0	1	1	74.0	1	2
76.0	1	1	77.0	1	1
78.0	1	2	79.0	1	1
79.0	1	2	80.0	1	2
81.0	1	1	82.0	1	2
83.0	2	1	86.0	1	2
88.0	4	1	92.0	1	1
93.0	1	1	93.0	1	2
95.0	1	1	95.0	1	2
97.0	1	1	98.0	2	2
100.0	3	1	101.0	2	1
102.0	1	2	103.0	4	1
103.0	2	2	104.0	4	1
104.0	2	2	105.0	1	1
105.0	1	2	106.0	4	1
106.0	2	2	107.0	8	1
107.0	8	2	108.0	2	1

FORM OF PROBABILITY FUNCTION:

P(DOSE) = 1 - exp((-Q0 - Q1 * D - Q2 * D^2 - Q3 * D^3) * (T - T0)^J)

THE MAXIMUM LIKELIHOOD ESTIMATION OF:

PROBABILITY FUNCTION COEFFICIENTS

Q(0)= .106410244171E-11
Q(1)= .135503864185E-11
Q(2)= .000000000000
Q(3)= .499366216636E-12

TIME FUNCTION COEFFICIENTS

T0 = .000000000000
J = 5.39821051674

THE MAXIMUM LIKELIHOOD IS -185.712125973

MAXIMUM LIKELIHOOD ESTIMATES OF EXTRA RISK

****

WEIBULL LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

****

CONFIDENCE

LOWER BOUND UPPER BOUND LIMIT

RISK	MLE DOSE	ON DOSE	ON RISK	INTERVAL	TIME
.100000	.695915	.304814	.213802	95.0%	108.000
5.000000E-02	.379173	.148395	.122838	95.0%	108.000
1.000000E-02	7.805583E-02	2.907622E-02	2.661966E-02	95.0%	108.000
1.000000E-03	7.787649E-03	2.894507E-03	2.688219E-03	95.0%	108.000
1.000000E-06	7.783932E-06	3.575852E-06	2.176804E-06	95.0%	108.000

WEIBULL UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE

****

		CONFIDENCE UPPER BOUND LIMIT			
DOSE	MLE RISK	ON RISK	INTERVAL		TIME
.100000	1.281155E-02	3.397492E-02	95.0%		108.000
.500000	6.774880E-02	.158717	95.0%		108.000
2.00000	.470433	.600987	95.0%		108.000

NORMAL COMPLETION!

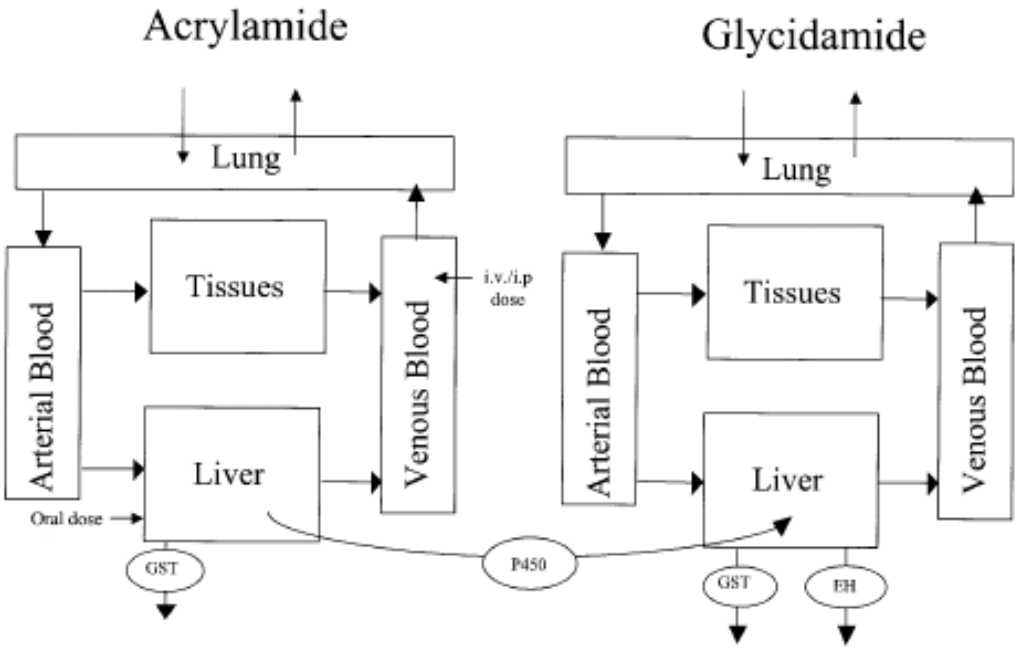
APPENDIX E. KIRMAN ET AL. (2003) PBTK MODEL SUPPORTING DOCUMENTATION

This appendix contains supporting documentation for the orginal Kirman et al. (2003) PBTK model for acrylamide and metabolites, and for the EPA recalibrated model using more recent data for rats and humans. The model schematic and original model parameter values presented below were extracted directly from the original article ─ Kirman, CR; Gargas, ML; Deskin, R; et al. (2003) A physiologically based pharmacokinetic model for acrylamide and its metabolite, glycidamide, in the rat. J Toxicol Environ Health A 66(3):253–74

The reader is referred to the orginal text for further details about the original Kirman et al. (2003) model development and simulation results including the mass-balance equations. The original Kirman et al (2003) ACSL code and command files, and the updated code in acslXtreme are available from--*[Note: website address or email address will be included in the final draft]*.

The diagram below of the Kirman et al. (2003) model illustrate the distribution of AA and GA within five compartments—arterial blood, venous blood, liver, lung, and all other tissues lumped together. The arterial and venous blood compartments are further divided into serum and blood cell subcompartments to model specific data sets. Different routes of exposure to AA are represented in the Kirman et al. model including intravenous (i.v.), intraperitoneal (i.p.), oral gavage, oral drinking water, and inhalation. Metabolism of AA and GA are represented only in the liver.

Diagram of the Kirman et al. (2003) PBTK Model for Acrylamide and Glycidamide



The model parameter values and sources in the original Kirman model are presented in Table E-1. The sources include measured or calculated values for rat physiological parameters from the literature (tissue volumes, blood flows), estimates for the tissue partition coefficients for AA based on a published algorithm or specific chemical properties (e.g., solubility in water and octanol, vapor pressure), estimates for GA tissue partition coefficients from values for AA using a proportionality constant of 3.2 derived from the ratio of structural analogs (acrylonitrile and its epoxide metabolite, cyanoethylene oxide), and estimates of metabolism and tissue binding rates optimized to fit tissue levels of administered [14C]-radiolabeled AA (Ramsey et al., 1984; Miller et al., 1982), or to urinary metabolite levels (Raymer et al. 1993 Sumner et al., 1992; Miller et al., 1982). The urinary metabolite data of Summer et al. (1992) was largely used to calibrate the balance between different routes of metabolism, while the overall rate of metabolism was calibrated to AA concentrations in blood and nervous tissue (Raymer et al., 1993) and total radioactivity levels (Ramsey et al., 1984; Miller et al., 1982). Kirmal et al. developed a single set of model parameter values that best fit these kinetic data. Optimized values of Vmax = 1.6 mg/hour-kg and Km =10 mg/L were determined for the metabolism of AA via cytochrome P-450. The second-order rate constant for metabolism of AA via reaction with GSH was best fit for 0.55 L/hour-mmol GSH. Similarly, the metabolism parameters for GA via epoxide hydrolase were a Vmax of 1.9 mg/hour-kg, a Km of 100 mg/L, and a second-order rate constant for GA-GSH conjugation of 0.8 L/hour-mmol GSH.

Table E-1: Original Model Parameter Values for Rats in the Kirman et al. (2003) PBTK Model. Source: Kirman et al. (2003)

Parameter group	Parameter	Symbol (units)	Value	Reference/source
Rat physiology	Body weight	BW (kg)	Study specific	Study specific
	Cardiac output	QCC (L/h-kg)	14	Kedderis et al. (1996)
	Alveolar ventilation	QPC (L/h-kg)	14	
	Liver blood flow	QLC (fraction QCC)	0.25	
	Fraction arterial	FABC (fraction VB)	0.35	
	Fraction venous	FVBC (fraction VB)	0.65	
	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 (ip dose)	KA (/h)	5	Model simulations fit to Raymer et al. (1993), Miller et al. (1982), Ramsey et al. (1984)
	Infusion time (iv dose)	TINF (h)	0.003	
Partition coefficients	Blood:air, AMD	PB1 (unitless)	31,000,000	Estimated (Poulin & Krishnan, 1995, 1996a, 1996b)
	Liver:blood, AMD	PL1 (unitless)	0.83	
	Tissue:blood, AMD	PT1 (unitless)	0.95	
	Blood:air, GLY	PB2 (unitless)	98,000,000	
	Liver:blood, GLY	PL2 (unitless)	2.7	
	Tissue:blood, GLY	PT2 (unitless)	3.0	
Metabolism	Cytochrome P-450 oxidation rate, AMD	VMAXC1 (mg/h-kg)	1.6	Model simulations fit to Raymer et al. (1993), Miller et al. (1982), Sumner et al. (1992)
	Cytochrome P-450, Michaelis-Menten constant, AMD	KMC1 (mg/L)	10	
	Epoxide hydrolase hydrolysis rate, GLY	VMAXC2 (mg/h-kg)	1.9	
	Epoxide hydrolase, Michaelis-Menten constant, GLY	KMC2 (mg/L)	100	
	Reaction with glutathione, AMD	KGSTC1 (L/mmol GSH-hr)	0.55	
	Reaction with glutathione, GLY	KGSTC2 (L/mmol GSH-h)	0.8	
Tissue binding	Binding to hemoglobin, AMD	KHGB1 (L/g HGB-h)	0.5	Model simulations fit to Miller et al. (1982), Ramsey et al. (1984)
	Binding to hemoglobin, GLY	KHGB2 (L/g HGB-h)	0.25	
	Binding to liver macromolecules, acylamide	KFEEL1 (/h)	0.2	

Table E-1 (continued):

Parameter group	Parameter	Symbol (units)	Value	Reference/source
	Binding to liver macromolecules, GLY	KFEEL2 (/h)	0.1	
	Binding to tissue macromolecules, AMD	KFEET1 (/h)	0.08	
	Binding to tissue macromolecules, GLY	KFEET2 (/h)	0.04	
	Binding to blood macromolecules, AMD	KFEEB1 (/h)	0.01	
	Binding to blood macromolecules, GLY	KFEEB2 (/h)	0.005	
	Protein turnover	KPT (/h)	0.008	
Glutathione	GSH production rate	KGSHP (mmol/h)	0.025	D'Souza et al. (1988)
	GSH loss rate	KGSHL (/h)	0.35	
	Initial GSH concentration in liver	GSHL0 (mmol/L)	7.0	

References cited by Kirman et al (2003) in the above Table of model parameters

Brown, R. P., Delp, M. D., Lindstedt, S. L., Rhomberg, L. R., and Beliles, R. P. 1997. Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol. Ind. Health* 13:407–484.

D'Souza, R. W., Francis, W. R., and Andersen, M. E. 1988. Physiological model for tissue glutathione depletion and increased resynthesis after ethylene dichloride exposure. *J. Pharmacol. Exp. Ther.* 245:563–568.

Kedderis, G. L., Teo, S. K., Batra, R., Held, S. D., and Gargas, M. L. 1996. Refinement and verification of the physiologically based dosimetry description for acrylonitrile in rats. *Toxicol. Appl. Pharmacol.* 140:422–435.

Miller, M., Carter, D., and Sipes, I. 1982. Pharmacokinetics of acrylamide in Fischer 344 rats. *Toxicol. Appl. Pharmacol.* 63:36–44.

Poulin, P., and Krishnan, K. 1995. A biologically-based algorithm for predicting human tissue:blood partition coefficients of organic chemicals. *Hum. Exp. Toxicol.* 14:273–280.

Poulin, P., and Krishnan, K. 1996a. A mechanistic algorithm for predicting blood:air partition coefficients of organic chemicals with the consideration of reversible binding in hemoglobin. *Toxicol. Appl. Pharmacol.* 136:131–137.

Poulin, P., and Krishnan, K. 1996b. A tissue composition-based algorithm for predicting tissue:air partition coefficients of organic chemicals. *Toxicol. Appl. Pharmacol.* 136:126–130.

Ramsey, J., Young, J., and Gorzinski, S. 1984. Acrylamide: Toxicodynamics in rats. Midland, MI: Dow Chemical USA.

Raymer, J. H., Sparacino, C. M., Velez, G. R., Padilla, S., MacPhail, R. C., and Crofton, K. M. 1993. Determination of acrylamide in rat serum and sciatic nerve by gas chromatography–electron–capture detection. *J. Chromatogr.* 619:223–234.

Sumner, S., MacNeela, J., and Fennell, T. 1992. Characterization and quantitation of urinary metabolites of [1,2,3-13C]acrylamide in rats and mice using 13C nuclear magnetic resonance spectroscopy. *Chem. Res. Toxicol.* 5:81–89.

Recalibrated Rat PBTK Model

The data used to calibrate the original Kirman et al. (2003) model were limited to urinary metabolite data, tissue levels of AA radiolabel, and some information on binding to hemoglobin and other tissue macromolecules. Additional kinetic and hemoglobin binding data in rats and mice have subsequently been published (Sumner et al., 2003, Fennell et al. 2005; Doerge et al., 2005a,b,c). The US EPA contracted Dale Hattis and Katherine Walker (George Perkins Marsh Institute, Clark University, Worcester, MA) to assist in the recalibration and testing of the Kirman et al. (2003) rat model.

Table E-2 summarizes the rat hemoglobin adduct data used to estimate area under the time-concentration curves (AUC) for acrylamide and glycidamide. The basic adduct measurements were translated into units of AUC in μmoles/liter-hour in blood with the aid of second-order rate constants derived from in vitro measurement of the reaction of hemoglobin with acrylamide and glycidamide.

Hemoglobin adducts on the terminal valine, as measured by Fennell et al. (2005):

	Reaction rate constants L/g-hr	
	AAVal	GAVal
Rat	3.82E-06	4.96E-06
Human	4.27E-06	6.72E-06

Adducts on the free cysteine of rat hemoglobin as measured by Bergmark et al. (1991):

	Reaction rate constants L/g-hr	
	AACys	GACys
Rat	1.8E-03	0.92E-03

For short-term measurements post exposure (e.g., at 24 hours following a single exposure), adduct concentrations expressed in femptomoles/mg Hb are simply converted into AUC units:

$$\frac{(\text{adducts in fmoles/mg Hb})(1\text{e-}9\ \mu\text{moles/fmole})*1000\ \text{mg/g}}{\text{rate constant in L/g-hr}} = \mu\text{mole-hr/L}$$

When measuring adduct levels after repeated exposures over many days, one must take into account the normal loss of red cells (e.g., lifespan of about 61 days in rats; Derelanko [1987][12]) and effective dilution of the adducted red cells via growth of the animal (and

[12] Derelanko MJ. 1987. Determination of erythrocyte life span in F-344, wistar, and Sprague-Dawley rats using a modification of the [3H]diisopropylfluorophosphate ([3H]DFP) method. Fundam. Appl Toxicol. 9: 271-276.

consequent growth of the blood). Calleman et al. (1996)[13] utilize the following equation for repeated treatments over time:

$$\text{Accumulated adducts} = \sum_{t=1 \text{ to } 61} a(1 - t/61) * b_t$$

where "a" is the accumulation on each day based on 1-day experiments; 61 is the lifetime of erythrocytes; and b_t is a coefficient that corrects for the growth in blood volume from the start of exposure to day t.

It can be seen in Table E-2 that the adduct-derived AUC observations fall into two distinct groups: 1) recent measurements of Hb-terminal valine adducts in Fischer-344 rats and humans (Fennell et al., 2003; Fennell et al, 2005; Sumner et al. 2003); and 2) older measurements of Hb-cysteine adducts by Bergmark et al. (1991) in Sprague-Dawley rats. The Fennell and Sumner measurements indicate several fold higher internal glycidamide AUC exposures per unit of acrylamide external dose than derived from Bergmark et al. (1991) data. The AUCs based on the Fennel and Sumner data, however, were given more weight for calibrating the acrylamide PBTK model parameters because they used the same strain of rats (F344 rats) as was used in the two co-principal rats bioassays (Johnson et al., 1986; Friedman et al., 1995), the oral dose in Fennell et al. (2005) of 3 mg/kg bw corresponds roughly to the highest daily dose in the chronic bioassays, and the measurement of the terminal valine adduct are preferred to the cysteine adducts measured by Bergmark et al. (1991) because valine adducts were also measured in the more recent human studies (Fennell et al., 2005) and are used in the calibration of the human version of the PBTK model.

A caveat for the choice of the Fennell and Sumner data and AUCs as the primary target for the model parameter calibration is that the measurements are for single exposures. These data therefore will not reflect possible effects of enzyme induction that may arise during achronic exposure, phenomena that may be reflected in the Bergmark et al. (1991) data from 10- and 33-day repeated exposures.

[13] Calleman CJ. 1996. The metabolism and pharmacokinetics of acrylamide: Implications for mechanisms of toxicity and human risk estimation. Drug Metabolism Reviews 28:527-590.

Table E-2: Data used to recalibrate the Kirman et al. (2003) model parameters

Reference Source, Route and Chemical Administered	Rat Strain	Type of Adducts Measured	Acrylamide or Glycidamide Dose mg/kg	Acrylamide AUC µM-hr	Acrylamide AUC Std Error	Glycidamide AUC µM-hr	Glycidamide AUC Std Error	Observed Acrylamide AUC/dose µM-hr/(mg/kg AA)	Observed Glycidamide AUC/dose µM-hr/(mg/kg AA)
Fennell (2005a) oral	Male Fischer-344	terminal valine	3	237	Not Available	156	Not Available	79	52
Sumner (2003) Inhalation	Male Fischer-344	terminal valine	6.5 (retained at 6 hr)	363	17	322	21	56	50
Sumner (2003) IP	Male Fischer-344	terminal valine	46.5	3395	85	1861	47	73	40
Fennell (2003) Observations (oral)	Male Fischer-344	terminal valine	59.5	5457	219	1588	76	92	27
Bergmark (1991) IP acrylamide 30 d	Sprague-Dawley	cysteine	3.3	109	7	45	2	33	14
Bergmark (1991) IP acrylamide 10 d	Sprague-Dawley	cysteine	10	366	32	124	11	37	12
Bergmark (1991) IP acrylamide	Sprague-Dawley	cysteine	10	Not Available	Not Available	137	17		
Bergmark (1991) IP acrylamide	Sprague-Dawley	cysteine	50	2617	167	400	35	52	8
Bergmark (1991) IP acrylamide	Sprague-Dawley	cysteine	100	4789	164	407	28	48	4
Bergmark (1991) IP glycidamide	Sprague-Dawley	cysteine	10			267	Not Available		
Bergmark (1991) IP glycidamide	Sprague-Dawley	cysteine	50			1185	73		
Bergmark (1991) IP glycidamide	Sprague-Dawley	cysteine	100			2543	54		

The original Kirman et al. (2003) model parameter values were used to evalute the model predictions against the AUCs derived from hemoglobin adduct data. The results of the observed versus the predicted values are presented in Table E-3, and reflect a much closer fit to the AUCs based on the Bergmark et al (1991) data, with two fold or higher model predicted levels than estimated AUCs based on the Fennell and Sumner data. Since the AUCs based on Fennell and Sumner adduct data were considered more relevant to the derivation of toxicity values for this assessment, a recalibration of the model parameters was conducted to improve the fit to these data. The following model parameters were chosen as good candidates for the recalibration because their values were less certain and the more recent data was informative:

1. the tissue/blood partition coefficient multiplier of 3.2 for glycidamide;
2. the balance between P450 vs. GSH and other non-P450 metabolic routes for acrylamide, on the basis that the urinary metabolite profile observed at 24 hours (considered to not fully represent the complete metabolic fate of acrylamide, i.e., the fraction that does not appear in urine but is irreversibly bound to tissues, or completely metabolized to building blocks that are incorporated into tissue constituents or exhaled;
3. the tissue/blood partition coefficients for acrylamide, which were based on statistical model projections (based on Poulin and Krishnan 1995; 1996a; 1996b) rather than direct measurements.

A number of model runs were conducted against the hemoglobin adduct data and some of the previous blood level data (Raymer et al, 1993) to evaluate model results for different estimates of the tissue/blood partition coefficients including use of the original values, re-estimation using a different algorithm based on octanol/water partition coefficients; and ultimately calibration based on the volume of distributions derived directly from measurement of serum AA and GA levels in mice and rats following i.v. low dose exposures (Doerge et al., 2005 b,c).

Table E-4 lists the recalibrated parameter values, and Table E-5 presents the fits of the final recalibrated rat PBTK model predictions versus the data based estimates of the AUCs, as limited by the Fennel et al. (2005) urinary metabolite data. Urinary output data provided a lower bound on the amount of a particular metabolite that the model needs to simulate, but not necessarily a precise estimate of the amount actually produced. This is because there is no guarantee that all of the metabolite of a particular pathway produced in animals (or people) will actually show up in the urine—in general, an unknown fraction of the metabolite that is actually produced can undergo further metabolism to undetected or unquantified metabolites (including, for example, exhaled carbon dioxide) and an unknown fraction may remain in the body at the end of the urine collection period. By contrast, the model parameters were adjusted to fit the

acrylamide and glycidamide hemoglobin adduct-derived AUCs at the approximately 3 mg/kg bw dose. Departures of the model predictions from the observations at higher acute doses were tolerated because as dose increases it is increasingly likely that phenomena such as GST enzyme induction or induction of increased glutathione production levels following an initial depletion will change the results via mechanisms that are not in the model and would not be expected to substantially influence the toxicokinetics at the lower doses in the two principal chronic bioassays of interest in this assessment. In particular, reducing the Km for the CYP2E1 metabolism of acrylamide to glycidamide (thus introducing this saturating nonlinearity at lower doses) tends to worsen the fit of the model to the acrylamide AUC data at high doses, holding the fit at the lowest dose level constant. The nonlinearity that was indicated by the observed acrylamide AUC derivations at the higher doses is therefore in the opposite direction than would be produced by a lower Km for CYP2E1.

Departures of the model predictions from even the low dose observations of Bergmark et al (1991) were also tolerated because the Bergmark group used Sprague-Dawley rats, i.p doses, measured cysteine (rather than valine) hemoglobin adducts, and administered much higher doses than used in the chronic bioassays.

One possible model adaptation that would bring the model predictions closer to the observations at higher doses is to assume, contrary to the current model formulation, that the glutathione depletion at higher doses does not appreciably slow down the rates of the GST-mediated reactions. There is some indication in the prior literature that the Km for binding of glutathione to the active site of some GSTs can be very low (Sun and Morgenstern, 1997[14]) — .018 mM compared to a basal liver concentration in this version of our model of 7 mM — indicating that modest changes in glutathione levels would not be reflected in proportionately decreased rates of the GSH-acrylamide and GSH-glycidamide reactions . This was evaluated in a series of model runs where liver glutathione was held constant at 7 mM rather than appreciably depleted at high doses (approximately to 4.3 mM at minimum for the 59.5 mg/kg dose). The results of this modification did indicate closer agreement to the AUC data at high does, while causing only modest changes to the model fit of doses in the region where the bioassay was conducted.

The current model reflects a considerable reduction in the estimates of the rates of reaction between acrylamide and miscellaneous proteins in the liver, tissue, and blood, but

[14] Sun TH, Morgenstern R. 1997. Binding of glutathione and an inhibitor to microsomal glutathione transferase. Biochem J. 326 (Pt 1):193-196.

relatively modest changes in other parameters to accommodate the revised tissue/blood partition coefficients and the revised adduct-based AUC estimates.

Table E-3: AUC Predictions from the Original Kirman Model versus AUCs Derived from Hemoglobin Adduct Data.

	Acrylamide AUC Comparisons					Glycidamide AUC Comparisons		
Data Source and Route of Admin	**mg/kg Acrylamide dose**	**Obs AA AUC µM-hr**	**Model pred. AA AUC µM-hr**	**Ratio--Model Predicted/Obs.**		**Obs GA AUC µM-hr**	**Model pred. GA AUC µM-hr**	**Ratio—Model Predicted/Obs**
Fennell (2005a) Oral	3	237	84	0.354		156	14	0.087
Sumner (2003) Inhalation	6.5	363	192	0.529		322	28	0.088
Sumner (2003) IP	46.5	3395	1919	0.565		1861	168	0.090
Fennell (2003) Observations (oral)	59.5	5457	2631	0.482		1588	210	0.133
Bergmark (1991) IP acrylamide 30 d	3.3	109	93	0.852		45	15	0.328
Bergmark (1991) IP acrylamide 10 d	10	366	309	0.844		124	42	0.339
Bergmark (1991) IP acrylamide	10					267	42	0.157
Bergmark (1991) IP acrylamide	50	2617	2104	0.804		1185	180	0.152
Bergmark (1991) IP acrylamide	100	4789	5212	1.088		2543	335	0.132

Table E-4:Recalibrated PBTK Model Parameter Values for the Rat

Parameter group	Parameter	Symbol (units)	Value
Basic physiology	Body weight	BW (kg)	0.25
	Alveolar ventilation	QCC (L/h-kg$^{0.74}$)	14
	Cardiac output (total body blood flow)	QPC (L/h-kg$^{0.74}$)	14
	Liver blood flow	QLC (fraction QCC)	0.25
	Tissue blood flow	QTC (fraction QCC)	0.75
Compartment Volumes	Volume blood	VBC (fraction BW)	0.06
	Fraction arterial/total blood	FABC (fraction VB)	0.35
	Fraction venous/total blood	FVBC (fraction VB)	0.65
	Liver volume	VLC (fraction BW)	0.04
	Tissue volume	VTC (fraction BW)	0.87
	Fraction blood cells/blood	FBC (fraction VB)	0.44
	Fraction blood serum/blood	FBS (fraction VB)	0.56
Absorption	Absorption rate from gastrointestinal tract (oral dose) or intraperitoneal cavity (ip dose)	KA(/h)	5
Partition coefficients	Blood:air, AA	PB1 (unitless)	3.1E7
(equilibrium concentration ratios)	Liver: blood, AA	PL1 (unitless)	0.797
	Tissue: blood, AA	PT1 (unitless)	0.619
	Blood:air, GA	PB2 (unitless)	9.8E7
	Liver: blood, GA	PL2 (unitless)	0.923
	Tissue: blood, GA	PT2 (unitless)	0.716

Table E-4 continued:

Parameter group	Parameter	Symbol (units)	Value
Metabolism	Cytochrome P-450 oxidation rate, AA	VMAXC1 (mg/h-kg$^{0.7}$)	4.00
	Cytochrome P-450 Michaelis-Menten constant, AA	KMC1 (mg/L)	31
	Epoxide hydrolase hydrolysis rate, GA	VMAXC2 (mg/h-kg$^{0.7}$)	0.988
	Epoxide hydrolase Michaelis-Menten constant, GA	KMC2 (mg/L)	100
	Reaction with glutathione, AA	KGSTC1 [L/(mmolGSH-kg$^{0.3}$ -h)]	0.225
	Reaction with glutathione, GA	KGSTC2 [L/(mmolGSH-kg$^{0.3}$ -h)]	0.630
Tissue binding	Binding to hemoglobin, AA	KHGB1 (L/gHGB-h)	0.00135
	Binding to hemoglobin, GA	KHGB2 (l/gHGB-h)	0.1300
	Binding to liver macromolecules, AA	KFEEL1 (/h)	0.00054
	Binding to liver macromolecules, GA	KFEEL2 (/h)	0.0520
	Binding to tissue macromolecules, AA	KFEET1 (/h)	0.000216
	Binding to tissue macromolecules, GA	KFEET2 (/h)	0.0208
	Binding to blood macromolecules other than hemoglobin, AA	KFEEB1 (/h)	0.000027
	Binding to blood macromolecules other than hemoglobin, GA	KFEEB2 (/h)	0.0026)
	Protein turnover	KPT (/h)	0.008
Glutathione	GSH production rate (Based on a 0.25 Kg rat)	KGSHP (mmol/h)	0.025
	GSH loss rate	KGSHL (/h)	0.35
	Initial GSH concentration in liver	GHSL0 (mmol/L)	7.0

Table E-5: Results of the Recalibratedl Kirman et al (2003) Model versus Urinary Metabolite Data and AUCs Derived from Hemoglobin Adduct Data

		Acrylamide AUC Comparisons			Glycidamide AUC Comparisons			Urinary Metabolite Comparisons		
Data Source and Route of Admin	mg/kg Acrylamide dose	Obs AA AUC µM-hr	Model pred. AA AUC µM-hr	Ratio--Model Predicted/Obs.	Obs GA AUC µM-hr	Model pred. GA AUC µM-hr	Ratio—Model Predicted/Obs	Obs AA-GSH excreted (µmoles)	Pred AA-GSH produced (µmoles)	Model/ Minimum Obs
Fennell (2005) oral	2.86	237	210	0.886	156	152	0.973	2.6	2.62	1.01
								Obs GA-GSH excreted (µmoles)	Pred GA-GSH produced (µmoles)	Model/ Minimum Obs
Fennell (2005) oral	2.86							1.8	6.17	3.43
Sumner (2003) Inhalation	6.5	363	479	1.322	322	332	1.032			
Sumner (2003) IP	46.5	3395	5224	1.544	1861	2655	1.427			
Fennell (2003) Observations (oral)	59.5	5457	7432	1.362	1588	3499	2.204			
Bergmark (1991) IP acrylamide 30d	3.3	109	232	2.134	45	167	3.685			
Bergmark (1991) IP acrylamide 10d	10	366	773	2.113	124	516	4.164			
Bergmark (1991) IP acrylamide	10				267	516	1.931			
Bergmark (1991) IP acrylamide	50	2617	5803	2.218	1185	2879	2.430			
Bergmark (1991) IP acrylamide	100	4789	16200	3.383	2543	6240	2.454			

Use of the PBTK Model to Estimate the Internal Dose Metric from the Chronic Bioassay Data

To model the drinking water exposures to rats in the chronic bioasssays, values for hourly percent of total water consumed were modeled based on data reported by Johnson and Johnson (1990)[15] for diurnal drinking patterns in rats. Johnson and Johnson (1990) observed that drinking behavior in rats follows a nocturnal pattern, with the greatest intensity in the hours just after the lights go out (by convention, labeled 6:00 PM), and just before the lights are due to go back on again at 6:00 AM. About 94% of the total water consumption appears to take place during the 12 hours of darkness.

Table E-6 contains the recalibrated rat model-predictions of the relationship between delivered AUC doses of acrylamide and glycidamide, and mg/kg daily doses administered according to the diurnal pattern of drinking behavior described by Johnson and Johnson (1990). To derive these AUCs, the model was run for a 36 hour period, 24 hours with rat pattern of drinking water consumption, followed by 12 hours with no further dosing. Infinite-time AUCs for acrylamide and glycidamide were projected using the following formulae:

$AUC_{infinite} = AUC_{0\text{-}36\ hr} + C_{36}/k$

where C_{36} = the acrylamide or acrylamide blood concentration;

k = the rate constant for exponential decline in acrylamide or glycidamide blood concentration between 35 and 36 hours.

It can be seen from Tables E-6 that the effect of this projection beyond 36 hours is minimal, showing up sometimes as a change in only the third decimal place of the AUC results, and sometimes with no additional effect. Overall, the current model predicts very little nonlinearity in AA and GA AUCs between the limits of low dosage and the highest dose rates used in the Friedman et al. (1995) bioassays. Linear interpolation formulae were therefore derived between the closely spaced doses in Tables E-6 with the AUCs as the dependent variables and mg/kg-day in all cases the independent variable yielding intercepts (b's) and slopes (m's) of the interpolation lines. The intercepts and slopes were used to derive lifetime average daily internal AUCs for benchmark dose levels derived for the noncancer and cancer dose-response data. These internal AUC levels for blood AA or GA were used as the basis for deriving the human equivalent concentration in lieu of using the default uncertainty factor for interspecies toxicokinetic differences.

[15] Johnson RF, Johnson AK. 1990. Light-dark cycle modulates drinking to homeostatic challenges. Am J Physiol. 1990 Nov;259(5 Pt 2):R1035-42.

Table E-6: Estimated Internal AUC Acrylamide and Glycidamide Doses Produced by Various Drinking Water Intakes

mg/kg DW	Cum 36 hr µM-hr AA in blood	AABlood Conc at 35 hr (µM)	AA Blood Conc at 36 hr (µM)	35-36 hr Half Life for AA decline (hr)	Infinite AUC for AA µM-hr	AA AUC µM-hr/mg/kg AA Dose	Cum 36 h µM-hr GA in blood	GA Blood Conc at 35 hr--µM	GA Blood Conc at 36 hr--µM	35-36 hr Half Life for GA decline (hr)	Infinite AUC for GA µM-hr	GA AUC µM-hr/mg/kg AA Dose
0.001	6.68E-02	1.76E-05	1.28E-05	2.205	6.69E-02	66.88	5.00E-02	8.00E-05	6.32E-05	2.948	5.03E-02	50.29
0.01	6.68E-01	1.76E-04	1.28E-04	2.205	6.69E-01	66.89	5.00E-01	8.00E-04	6.32E-04	2.948	5.03E-01	50.29
0.1	6.69E+00	1.76E-03	1.28E-03	2.205	6.69E+00	66.92	5.00E+00	8.01E-03	6.33E-03	2.948	5.03E+00	50.31
0.5	3.35E+01	8.81E-03	6.43E-03	2.205	3.35E+01	67.09	2.51E+01	4.02E-02	3.18E-02	2.947	2.52E+01	50.38
1	6.73E+01	1.77E-02	1.29E-02	2.205	6.73E+01	67.31	5.02E+01	8.08E-02	6.39E-02	2.947	5.05E+01	50.48
1.5	1.01E+02	2.66E-02	1.94E-02	2.205	1.01E+02	67.52	7.54E+01	1.22E-01	9.64E-02	2.946	7.59E+01	50.57
2	1.35E+02	3.56E-02	2.60E-02	2.205	1.35E+02	67.74	1.01E+02	1.63E-01	1.29E-01	2.945	1.01E+02	50.66
2.5	1.70E+02	4.47E-02	3.26E-02	2.205	1.70E+02	67.96	1.26E+02	2.05E-01	1.62E-01	2.945	1.27E+02	50.75
3	2.04E+02	5.38E-02	3.93E-02	2.205	2.05E+02	68.17	1.52E+02	2.48E-01	1.96E-01	2.944	1.53E+02	50.85
3.5	2.39E+02	6.30E-02	4.60E-02	2.205	2.39E+02	68.39	1.77E+02	2.91E-01	2.30E-01	2.944	1.78E+02	50.94
4	2.74E+02	7.22E-02	5.27E-02	2.206	2.74E+02	68.61	2.03E+02	3.34E-01	2.64E-01	2.943	2.04E+02	51.03

Table E-7: Available Data for Calibration of the Human PBTK model

Nominal dose (mg/kg)	Measured dose mg/kg	µmole/kg administered	AAVal AUC/dose µMol-hr/(mg/kg AA)	GAVal AUC/dose µMol-hr/(mg/kg AA)	µmole excreted as AA-GSH metabolite 24 hr	µmole excreted as GA 24 hr	µmole excreted as GA-Epoxide hydrolase product 24 hr	Ratio of GA-GSH to AA-GSH metabolite excretion at low doses
0.5	0.437	5.9	275.7	63.4				0.206
1	0.925	12.5	231.4	55.3				
3	2.864	38.7	202.7	55.9	823	25.4	103.8	
		Average	236.6	58.2				
		Std Dev	36.8	4.5				
		Std Error	21.2	2.6				
		Lower 95% CL	215.4	55.6				
		Upper 95% CL	257.8	60.8				

The data are all from Fennell et al. (2005), with the exception of those in the last column, which are derived from the observations of general population urinary excretion of glutathione metabolites by Boettcher et al. (2005).

Parameter Values for the Human PBTK Model

To estimate the human external concentration that would result in the same internal AUC of AA or GA in the blood as that prodiced from the BMDL, the Kirman et al. PBTK model parameters values were adjusted to simulate an adult human male. Table E-7 lists the data used for calibrating the human model including hemoglobin and urinary metabolite data primarily from Fennell et al. (2005), with some observations of general population urinary excretion of glutathione metabolites reported by Boettcher et al. (2005).

The Fennell et al. (2005) urinary data have inherent uncertainties in reflecting AA and GA kinetics because of the possible incomplete excretion of both acrylamide and glycidamide by the end of the 24 hour observation time, i.e., the model only predicts the amount of each metabolite made at a particular observation time, not the amount that is actually excreted in the urine, in the absence of renal clearance data. The Boettcher (2005) data were generated from long term exposures and are not as sensitive to the timing issue, but still have inherent uncertainties for total recovery since at least two potential glycidamide metabolites other than GAMA remained unmeasured: 1) N-acetyl-S-(1-carbamoyl-2-hydroxyethyl)cysteine, and 2) a hydrolysis product that is quantified by Fennell et al. (2005). Therefore, the molar ratio of total glycidamide to acrylamide metabolites may well be understated by the urinary GAMA/AAMA ratios reported by Boettcher et al (2005). For model calibration purposes, the urinary data can only be considered to establish a lower limit on the amount of a particular metabolite that must be produced. Any model output above the lower limit is acceptable. By contrast, the AUC estimates, derived from the hemoglobin adduct data, should be considered central estimates of the AUC that should be relatively precisely targeted in the model calibration.

The human model was parameterized for males because the study subjects in the Fennell et al. (2005) study were males with an average body weight of 81.65 Kg. Central values for body weight, tissue volumes and blood flows for the human PBTK model were developed from the National Health and Nutrition Examination Survey (NHANES III) database using the P3M[16] software program (Price et al., 2003). Initial values of metabolism parameters were estimated by scaling overall body metabolic capacities (e.g. Vmax values for the liver as a whole) in proportion to overall metabolic rates, which approximately scale across species with the three

[16] The P3M model was created to use the anthropometric measurements in the NHANES III database (age, gender, race, height, weight, circumferences, etc.) to predict physiological parameters for each individual in the database using empirical equations describing these relationships in the published literature. The objective behind the development of this model was to assist efforts to incorporate interindividual variability into PBTK modeling. The databases generated using this program provide a set of internally consistent estimates of the physiological parameters needed for PBTK modeling for each individual in the database. Estimates of interindividual variability based on sampling from independent distributions representing each physiological parameter have to specify the correlation structure for all parameters. These correlations are inherently preserved in the individual records generated by P3M.

quarters power of body weight (Rees and Hattis, 1994[17]). Because the liver makes up only about half the proportion of body weight in people as it does in rats, metabolism rates per unit of liver tissue were further adjusted upward by a factor of (0.04 / 0.195) related to this proportion. Parameters that were expressed in terms of a rate per unit time were adjusted downward in proportion to body weight$^{0.25}$. The liver glutathione parameters were adapted to establish steady state at the same liver glutathione concentration (7 mmol/liter) as was used by Kirman et al. (2003) for rats. For example, both the liver volume and the relative rate adjustments contributed to the estimated baseline loss rate for glutathione in the liver of 0.164/hour, compared to the comparable rat value of 0.35/hour. In general, only relatively modest adjustments in parameters were needed to accommodate the new data. An extra pathway for renal removal of glycidamide was added to reflect the newly reported observation of glycidamide in the urine of the studied subjects.

Various model parameters were then adjusted in an iterative process that evaluated various physiologically feasible modeling options to achieve the best fit to the data. The final model parameter values are shown in Table E-8, and the model fits to the data are presented in Table E-9.

The final iteration of the human model had nearly half of the acrylamide being converted to glycidamide, (compared with only a little over a quarter for earlier versions) and an apparent half-life for glycidamide of about 6 hours (the actual half life might be less than this because the glycidamide levels present at 23-24 h levels might be limited by the conversion from the declining level of acrylamide). In comparing different versions of the model, it was also noted that the model parameters were underdetermined, that is, there is just not enough basic pharmacokinetic data to derive a unique set of optimal parameter values, given the number of "adjustable" parameters in the current model. The last two iterations of the model calibrations closely fit the hemoglobin-adduct-derived AUC values, and do not yield appreciably different estimates of mean "human equivalent doses" even though they reflect different assumptions about the fraction of acrylamide that is processed by CYP2E1 and glutathione-S-transferase dependent pathways. If additonal data were available to further resolve the model parameters for these pathways, the human PBTK model could also be used to predict population distributions and interindividual variability in internal AUCs for acrylamide and glycidamide based on interindividual differences in metabolic rates. Until those data are available, however, the model is only suitable for use in deriving a human equivalent concentration based on the rat bioassay data in lieu of the interspecies toxicokinetic uncertainty factor, and not suitable to replace the uncertainty factor for variability within the human population (i.e., to identify the most senstivie subpopulation) resulting from intrahuman differences in AA's or GA's toxicokinetics.

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[17] Rees DC, Hattis D 1994. "Developing Quantitative Strategies for Animal to Human Extrapolation" Chapter 8 in Principles and Methods of Toxicology, 3rd Edition, A. W. Hayes, ed., Raven Press, New York, 1994, pp. 275- 315.

Use of the Human PBTK Model to Derive the Human Equivalent Concentration for a Drining Water Exposure to the Rat Neurotoxicity or Carcinogenicity Benchmark Doses

To derive human equivalent mg/kg-day doses and drinking water levels for the acrylamide AUCs estimated in rats at the BMDL, oral exposures via drinking water were modeled with the following schedule:

8:00 AM (Start of simulation; Time 0 hours)—25% of daily dose
10:30 AM—12.5% of daily dose
1:00 PM—25% of daily dose
3:30 PM—12.5% of daily dose
6:00 PM—25% of daily dose

Simulations were then continued until 48 hours after the start of dosing and infinite-time AUCs for both acrylamide and glycidamide were projected using the equations described earlier. The results are presented in Table E- 10.

Based on these results, interpolations of the the mg/kg-day dose corresponding to the animal acrylamide AUCs estimated above were conducted similar to as those derived from the rat modeling results. The resulting human equivalent drinking water exposure levels are before application of uncertainty factors for human interindividual variability and/or any other circumstances that are deemed necessary (except interspecies projection).

Human Equivalent Concnetrations from an Inhalation Exposure to the Rat Neurotoxicity or Carcinogenicity Benchmark Doses

The inhalation exposure was modeled as a 24 hour continuous inhalation exposure without allowances for diurnal changes in inhalation rates and tissue blood flows. In contrast to the drinking water models, acrylamide is supplied directly to the arterial circulation, rather than to an "unabsorbed" compartment that feeds in to the liver. Table E-11 has the AUC predictions as a function of dose from the human model simulatation of an inhalation exposure. Overall the differences between the inhalation and drinking water versions of the models are modest. Because of the absence of first pass metabolism in the liver from an inhalation exposure, the cumulative AUCs for acrylamide following inhalation are slightly higher and cumulative AUCs for glycidamide are slightly lower than the corresponding drinking water AUCs.

Table E-8. Parameters for the Human (male) Acrylamide PBTK Model

Parameter group	Parameter	Symbol (units)	Value
Basic physiology	Body weight	BW (kg)	81.65
	Total Alveolar ventilation	QC (L/hour-kg)	8.33
	Cardiac output (total body blood flow)	QP (L/hour-kg)	5.28
	Liver blood flow	QLC (fraction QC)	0.183
	Tissue blood flow	QTC (fraction QC)	0.8842
Compartment volumes	Volume blood	VBC (fraction BW)	0.0675
	Fraction arterial/total blood	FABC (fraction VB)	0.35
	Fraction venous/total blood	FVBC (fraction VB)	0.65
	Liver volume	VLC (fraction BW)	0.183
	Tissue volume	VTC (fraction BW)	0.8842
Absorption	Absorption rate from gastrointestinal tract (oral dose) or intraperitoneal cavity (i.p. dose)	KA (/hour)	5
Partition coefficients (Equilibrium concentration ratios)	Blood:air, AA	PB1 (unitless)	3.1 H 10^7
	Liver:blood, AA	PL1 (unitless)	0.88
	Tissue:blood, AA	PT1 (unitless)	0.40
	Blood:air, GA	PB2 (unitless)	3.1 H 10^7
	Liver:blood, GA	PL2 (unitless)	0.88
	Tissue:blood, GA	PT2 (unitless)	0.40
Metabolism	Cytochrome P-450 oxidation rate, AA	$V_{max}C1$ (mg/hour-$kg^{0.7}$)	1.12
	Cytochrome P-450 Michaelis-Menten constant, AA	KmC1 (mg/L)	7
	Epoxide hydrolase hydrolysis rate, GA	$V_{max}C2$ (mg/hour-$kg^{0.7}$)	3.27
	Epoxide hydrolase Michaelis-Menten constant, GA	KmC2 (mg/L)	100
	Reaction with glutathione, AA	$K_{GST}C1$ [L/(mmolGSH-$kg^{0.3}$-hour)]	0.1769
	Reaction with glutathione, GA	$K_{GST}C2$ [L/(mmolGSH-$kg^{0.3}$–h)]	0.5029
Tissue binding	Binding to hemoglobin, AA (previously eliminated from human model because of the lack of the free cysteine present in rat hemoglobin)	$K_{HGB}1$ (L/gHGB-h)	0
	Binding to hemoglobin, GA (previously eliminated from human model because of the lack of the free cysteine present in rat hemoglobin)	$K_{HGB}2$ (1/gHGB-h)	0
	Binding to liver macromolecules, AA	$K_{FEE}L1$ (/hour)	0.055
	Binding to liver macromolecules, GA	$K_{FEE}L2$ (/hour)	0.215
	Binding to tissue macromolecules, AA	$K_{FEE}T1$ (/hour)	0.022
	Binding to tissue macromolecules, GA	$K_{FEE}T2$ (/hour)	0.086
	Binding to blood macromolecules other than hemoglobin, AA	$K_{FEE}B1$ (/hour)	0.0028
	Binding to blood macromolecules other than hemoglobin, GA	$K_{FEE}B2$ (/hour)	0.01075
Renal Excretion (new pathway)	Direct renal elimination from arterial blood compartment	L/hour	0.082
Glutathione	GSH production rate	$K_{GSH}P$ (µmol/h)/kg	21.6687
	GSH loss rate	$K_{GSH}L$ (/hour)	0.16953
	Initial GSH concentration in liver	GHSL0 (mmol/L)	7.0

Table E-9: Human PBTK Model Predictions versus AUCs and Urinary Metabolites
(Bolded numbers are the parameters used for comparison of model outputs with calibrating data)

mg/kg dose	% AA converted to GA	% AA directly reacted with GSH	% GA eliminated via GSH reaction	% GA eliminated via epoxide hydrolase	% GA eliminated via direct renal excretion	AA AUC/dose µM-hr/mg/kg AA	GA AUC/dose µM-hr/mg/kg AA	Absolute µmoles AA Metabolized to GSH Conjugate	Cum µmoles GA metabolized to epoxide hydrolase metabolite by 24 hours	Cum µmoles GA excreted in urine by 24 hours	Ratio of GA-GSH metabolite to AA-GSH metabolite production
0.437	49.67	34.91	56.84	7.76	1.88	230.3	60.11	164	17.1	4.15	**0.733**
0.925	48.95	35.34	56.67	7.78	1.89	234.2	59.23	352	35.7	8.67	0.710
2.864	46.42	36.81	56.00	7.88	1.92	248.1	56.12	**1126**	**104.3**	**25.4**	0.633
					Average model prediction	**237.5**	**58.49**				
					Observed	**236.6**	**58.22**	**≥ 823**	**≥ 103.8**	**≥ 25.4**	**≥ 0.206**

Apparent acrylamide half life for decline, 1-2 hours after dosing with the lowest dose: 5.8 hours
Apparent glycidamide half life for decline, 23-24 hours after dosing with the lowest dose: 6.1 hours (this value may be limited by the rate of decline of acrylamide concentrations; the true elimination half life could be less if glycidamide were administered separately; rather than being made from acrylamide)

Table E-10: Estimated AUCs in Humans for Acrylamide and Glycidamide from a Drinking Water Exposure

mg/kg dose	Cum 48 hr µM-hr AA in blood	47-48 hr AA T1/2	48 hr AA blood conc (µM)	Infinite AUC µM-hr AA	Cum 48 h µM-hr GA in blood	47-48 hr GA T1/2	48 hr GA blood conc (µM)	Infinite AUC µM-hr GA
0.001	2.372E-01	5.49	1.50E-04	2.384E-01	6.713E-02	5.58	9.21E-05	6.787E-02
0.01	2.373E+00	5.49	1.50E-03	2.384E+00	6.712E-01	5.58	9.21E-04	6.786E-01
0.1	2.378E+01	5.49	1.51E-02	2.390E+01	6.706E+00	5.59	9.26E-03	6.781E+00
0.3	7.168E+01	5.49	4.58E-02	7.20E+01	2.01E+01	5.59	2.81E-02	2.030E+01
0.5	1.200E+02	5.49	7.731E-02	1.21E+02	3.339E+01	5.59	4.73E-02	3.377E+01
1	2.429E+02	5.49	1.592E-01	2.44E+02	6.644E+01	5.60	9.73E-02	6.723E+01
1.5	3.686E+02	5.50	2.459E-01	3.71E+02	9.916E+01	5.61	1.50E-01	1.004E+02
2	4.971E+02	5.50	3.375E-01	5.00E+02	1.316E+02	5.63	2.06E-01	1.332E+02
2.5	6.284E+02	5.50	4.341E-01	6.32E+02	1.636E+02	5.64	2.64E-01	1.658E+02
3	7.623E+02	5.50	5.359E-01	7.67E+02	1.954E+02	5.65	3.25E-01	1.980E+02

Table E-11: Estimated AUCs in Humans for Acrylamide and Glycidamide from An Inhalation Exposure

mg/kg dose	Cum 48 hr µM-hr AA in blood	47-48 hr AA T1/2	48 hr AA blood conc (µM)	Infinite AUC µM-hr AA	Cum 48 h µM-hr GA in blood	47-48 hr GA T1/2	48 hr GA blood conc (µM)	Infinite AUC µM-hr GA
0.001	2.453E-01	5.49	4.76E-04	2.491E-01	6.503E-02	5.79	2.80E-04	6.737E-02
0.01	2.454E+00	5.49	4.76E-03	2.492E+00	6.502E-01	5.79	2.80E-03	6.736E-01
0.1	2.457E+01	5.49	4.78E-02	2.495E+01	6.498E+00	5.80	2.81E-02	6.733E+00
0.3	7.394E+01	5.49	1.45E-01	7.51E+01	1.95E+01	5.80	8.48E-02	2.018E+01
0.5	1.236E+02	5.50	2.430E-01	1.26E+02	3.239E+01	5.81	1.42E-01	3.359E+01
1	2.491E+02	5.50	4.962E-01	2.53E+02	6.455E+01	5.84	2.90E-01	6.698E+01
1.5	3.765E+02	5.51	7.600E-01	3.82E+02	9.646E+01	5.86	4.42E-01	1.002E+02
2	5.057E+02	5.51	1.035E+00	5.14E+02	1.281E+02	5.88	5.99E-01	1.332E+02
2.5	6.368E+02	5.52	1.321E+00	6.47E+02	1.595E+02	5.91	7.62E-01	1.660E+02
3	7.697E+02	5.52	1.618E+00	7.83E+02	1.907E+02	5.93	9.30E-01	1.987E+02

APPENDIX F. YOUNG ET AL (2007) PBTK/TD MODEL SUPPORTING DOCUMENTATION

The following tables and figures provide supporting documentation for the Young et al. (2007) PBTK/TD model for acrylamide and glycidamide. All of the information presented in Appendix F has been extracted directly from the original article ─ Young, JF; Luecke, RH; Doerge, DR. (2007) Physiologically based pharmacokinetic/pharmacodynamic model for acrylamide and its metabolites in mice, rats, and humans. Chem Res Toxicol. 20(3):388-99. The reader is referred to the orginal text for further details about the Young et al. (2007) model development and simulation results.

Schematic of the Young et al. (2007) PBTK/TD Model For Acrylamide and Glycidamide

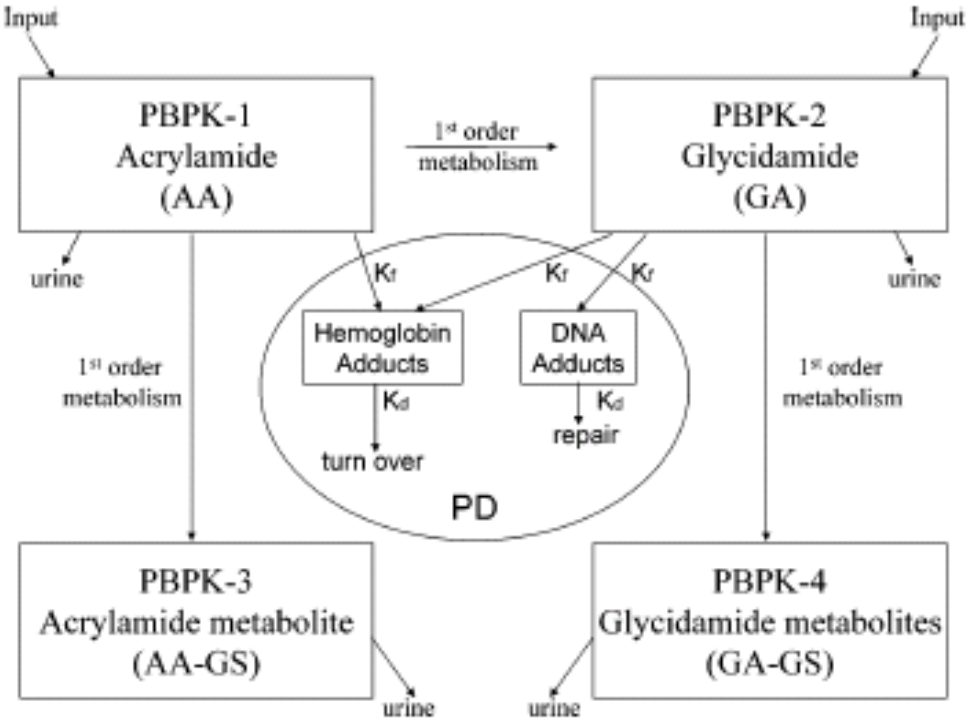

Figure 2. Block diagram for the metabolism of AA to GA and further metabolism of both to their glutathione conjugates. All pharmacokinetic and pharmacodynamic (PD) processes are first order.

Table F-1: Data Generated at NCTR on AA and GA in rats and mice.

species	gender	dose (mg/kg)	compd dosed	route	single or multidose	serum analysis		adduct data
						AA	GA	
mouse	M and F	0.12	GA	iv	single		yes	GA Hb adducts at 6 h
	M and F	0.12	GA	gavage	single		yes	GA liver and GA Hb adducts at 8 h
	M and F	0.1	AA	iv	single	yes	yes	AA and GA Hb adducts at 6 h
	M and F	0.1	AA	gavage	single	yes	yes	GA liver and AA and GA Hb adducts at 8 h
	M and F	~0.1	AA	diet	single/30 min	yes	yes	AA and GA Hb adducts at 12 h
	M and F	50	AA	gavage	single	yes	yes	GA liver adduct values out to 8 h
	M and F	~1	AA	drinking water	42 days	yes	yes	GA liver and AA and GA Hb adduct values out to 42 days and then decay
rat	M and F	0.12	GA	IV	single		yes	GA Hb adducts at 8 h
	M and F	0.12	GA	gavage	single		yes	GA liver and GA Hb adducts at 10 h
	M and F	0.1	AA	IV	single	yes	yes	AA and GA Hb adducts at 8 h
	M and F	0.1	AA	gavage	single	yes	yes	GA liver and AA and GA Hb adducts at 10 h
	M and F	~0.1	AA	diet	single/30 min	yes	yes	AA and GA Hb adducts at 12 h
	M and F	~1	AA	drinking water	42 days	yes	yes	GA liver and AA and GA Hb adduct values out to 42 days and then decay

Table F-2: Pharmacokinetic and Pharmacodynamic Parameters from AA and GA Administration to Rats [Mean ± Standard Deviation (Range)]

		male rat								female rat					
chemical	units	GA	GA	AA	AA	AA	AA	literature AA[d]	literature AA[e]	literature AA[f]	GA	GA	AA	AA	AA	AA
dose (mg/kg)		0.12	0.12	0.1	0.1	0.094	0.96	20	50	75	0.12	0.12	0.1	0.1	0.121	1.07
route		iv	gavage	iv	gavage	diet	drinking water	gavage	IP	IP	iv	gavage	iv	gavage	diet	drinking water
n		1[c]	6	1[c]	7	7	1[c]	1[c]	1[c]	1[c]	1[c]	7	1[c]	6	5	1[c]
pharmacokinetic rate parameters[a]																
stomach absorption half-life	min		87 ± 44 a (39−152)		189 ± 52 b (142−272)	344 ± 65 c (281−450)	infusion	108	40[g]	17[g]		68 ± 24 a (53−122)		56 ± 7 a (48−65)	524 ± 160 d (382−775)	infusion
fraction absorbed from stomach			1		1	1		1	1	1		1		1	1	
first-order rate constants																
AA to GA	min^{-1}			5.3	19.1 ± 4.1 a (13−27)	27.1 ± 3.4 b (23−33)	same as	10.1	5.2	5.9			4.09	6.16 ± 0.61 c (5.5−7.3)	25.5 ± 2.2 b (22.5−27.5)	same as
AA to AA-GS	min^{-1}			13.7	17.2 ± 3.7 a (12−24)	16.2 ± 2.0 a (13−20)	low dose gavage	21.2	11.8	12.3			7.35	6.77 ± 0.67 b (6.1−8.0)	7.66 ± 0.66 b (6.7−8.3)	low dose gavage
GA to GA-GS	min^{-1}	17.4	18.0 ± 3.3 a (13−22)	14.6	22.4 ± 4.5 a (18−31)	19.2 ± 3.7 a (13−23)		36.5	37.3	37	12.5	11.2 ± 1.7 b (9−15)	19.8	7.35 ± 0.73 c (6.6−8.6)	22.2 ± 3.1 a (20.3−27.8)	
AA to urine	min^{-1}			1.5	1.6 ± 0.3 a (1.2−2.3)	1.3 ± 0.2 b (1.0−1.6)							0.9	0.59 ± 0.06 c (0.5−0.7)	0.41 ± 0.06 d (0.3−0.5)	
GA to urine	min^{-1}	10.0	8.8 ± 1.8 a (6−11)	9.2	11.4 ± 2.4 a (9−16)	4.4 ± 0.9 b (2−6)		31	35.4	25.7	6.89	4.83 ± 0.71 c (4.0−6.2)	11.8	3.45 ± 0.30 d (3.0−3.9)	6.40 ± 1.11 e (5.2−7.8)	
AA-GS to urine	min^{-1}			12.0	13.3 ± 1.3 a (11−16)	3.6 ± 0.2 b (3−4)		16.2	10.2	11.3			6.90	7.23 ± 0.44 c (6.6−7.9)	2.08 ± 0.35 d (1.7−2.6)	
GA-GS to urine	min^{-1}	10.0	11.3 ± 0.8 a (10−13)	12.4	14.0 ± 0.9 b (12−16)	3.9 ± 0.2 c (3−5)		14.9	8.8	10.2	5.67	6.51 ± 0.21 d (6.2−6.9)	6.39	9.15 ± 0.77 e (8.5−10.4)	1.59 ± 0.29 f (1.3−2.0)	
elimination pattern (% of excretion)																
AA				4.3	2.4	4.5	same						4.5	2.6	2.2	
AA-GS				69.4	46.0	34.0	as		69.6				60.9	50.9	21.0	
GA		20.8	16.9	5.5	8.9	13.4	low dose		6.7		26.8	20.6	9.2	9.9	32.3	
GA-GS		79.2	83.1	20.8	42.6	48.1	gavage		23.7		73.2	79.4	25.4	36.6	44.5	
pharmacodynamic rate constants[a]																
formation of Hb AA adducts	min^{-1}			11.4	5.5 ± 1.1 a (4.2−7.1)	13.9 ± 2.0 b (11−17)	11.0		5.4				10.7	5.96 ± 1.03 a (4.5−7.4)	16.7 ± 2.8 b (14−22)	5.73
formation of Hb GA adducts	min^{-1}	48.2	47.4 ± 12.5 a,d (38−69)	45.7	83.8 ± 9.6 b (67−95)	70.9 ± 12.6 b (62−96)	50.1		44.7		48.2	41.3 ± 9.8 a (25−56)	69.3	53.7 ± 6.6 c,d (47−63)	73.9 ± 17.0 b (45−89)	30.1
formation of liver GA adducts	min^{-1}		4.9 ± 0.4 a (4.4−5.4)		6.3 ± 1.2 b (4.6−7.7)		6.0		8.7			4.21 ± 0.52 c (3.6−5.0)		7.73 ± 1.50 b (6.0−10.0)		3.0
decay of Hb AA adducts[b]	min^{-1}			0.0024	0.0024	0.0024	0.0024		0.0024				0.0024	0.0024	0.0024	0.0024
decay of Hb GA adducts[b]	min^{-1}	0.0023	0.0023	0.0023	0.0023	0.0023	0.0023		0.0023		0.0025	0.0025	0.0025	0.0025	0.0025	0.0025
decay of liver GA adducts[b]	min^{-1}		0.0114		0.0114		0.0114		0.0114			0.0118		0.0118		0.0118
hemoblobin AA adducts	pmol/g globin			48	11.0 ± 1.8 a (9−14)	21.0 ± 4.9 b (15−29)	3820		12970				50.4	23.2 ± 3.9 b (19−29)	24.5 ± 2.8 b (21−29)	
hemoblobin GA adducts	pmol/g globin	143	129 ± 19 a,c (109−151)	37	95 ± 14 b (80−119)	98.5 ± 19.8 b (76−123)	8870		9232		177	165 ± 48 a (104−227)	49.8	132 ± 17 a (118−153)	103 ± 18 b,c (71−116)	
liver GA adducts	in 10^8 nucleotides		5.5 ± 0.8 a (4.5−6.6)		2.9 ± 0.8 b (2−4)		105		651			6.9 ± 1.1 c (4.9−8.4)		8.1 ± 1.8 c (6.5−11.4)		

[a] Statistical comparisons were made for each parameter; a different letter designates a statistical difference at the $2p < 0.05$ level for each row. Comparisons that were made were male vs female, GA gavage vs AA gavage, GA gavage vs AA diet, and AA gavage vs AA diet. [b] Single value that was not allowed to vary within species. [c] Data from a group of rats were combined for a single PBPK/PD analysis. [d] Refs *12* (AA and GA plasma concentration data) and *14* (urinary excretion data). [e] Refs 12 (AA and GA plasma concentration data), *14* and *18* (urinary excretion and Hb adduct data), and *17* (liver GA−DNA adduct data). [f] Refs *11* (AA plasma concentration data) and *14* (urinary excretion data). [g] IP absorption half-life.

Table F-3: Pharmacokinetic and Pharmacodynamic Parameters from AA and GA Administration to Mice[a]

		male mouse							female mouse						
chemical	units	GA	GA	AA	AA	AA	AA[b]	AA	GA	GA	AA	AA	AA	AA[b]	AA
dose (mg/kg)		0.12	0.12	0.1	0.1	0.16	50	2.41	0.12	0.12	0.1	0.1	0.28	50	2.96
route		iv	gavage	iv	gavage	diet	gavage	drinking water	iv	gavage	iv	gavage	diet	gavage	drinking water
n		mean	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean
pharmacokinetic rate parameters															
stomach absorption half-life	min		13		10	80	24	infusion		10		1	82	28	infusion
fraction absorbed from stomach			1		0.73	0.62	1			1		0.74	0.33	1	
intestinal absorption half-life	min				275	321						333	224		
fraction absorbed from intestines					0.27	0.38						0.26	0.67		
first-order rate constants															
AA to GA	min^{-1}			9.0	20.1	30.0	11.5	same as low dose gavage			12.3	23.3	13.7	10.6	same as low dose gavage
AA to AA-GS	min^{-1}			12.6	4.02	13.8	8.04				3.69	4.66	4.80	7.42	
GA to GA-GS	min^{-1}	13.6	16.7	7.0	10.0	10.8	8.5		14.1	12.2	19.1	6.90	7.04	6.25	
AA to urine	min^{-1}			0.8	0.2	0					0.22	0.45	0		
GA to urine	min^{-1}	4.22	2.35	3.1	2.2	7.5	3.8		3.02	2.60	7.19	4.34	4.47	3.41	
AA-GS to urine	min^{-1}			1.6	1.0	3.5	10				1.26	0.87	1.51	6	
GA-GS to urine	min^{-1}	0.95	0.50	1.2	0.7	2.2	11		0.66	0.30	0.65	0.57	0.66	12	
elimination pattern (% of excretion)															
AA				5.7	1.6	0					2.4	2.4	0		
AA-GS				54.5	16.3	32.0	41.3				23.8	13.8	25.7	41.4	
GA		60.3	52.1	23.7	42.8	42.3	25.9		56.2	70.2	41.9	59.6	56.0	25.7	
GA-GS		39.7	47.9	16.1	39.2	25.7	32.8		43.8	29.8	32.0	24.2	18.3	33.0	
pharmacodynamic rate constants															
formation of Hb AA adducts	min^{-1}			13.8	13.3	18.8		3.2			14.6	20.3	10.4		11.0
formation of Hb GA adducts	min^{-1}	51.5	50.1	61.7	66.2	84.5		20.8	58.2	48.8	88.6	84.3	55.4		45.0
formation of liver GA adducts	min^{-1}		6.10		4.34		2.73	1.49		5.04		4.05		1.81	2.02
decay of Hb AA adducts	min^{-1}			0.0033	0.0033	0.0033		0.0033			0.0038	0.0038	0.0038		0.0038
decay of Hb GA adducts	min^{-1}	0.0033	0.0033	0.0033	0.0033	0.0033		0.0033	0.0034	0.0034	0.0034	0.0034	0.0034		0.0034
decay of liver GA adducts	min^{-1}		0.0110		0.0110		0.0110	0.0110		0.0110		0.0110		0.0110	0.0110
biomarkers: number of adducts															
hemoblobin AA adducts	pmol/g globin			19.5	11.9	13.4		1400			23.1	12.0	18.5		2570
hemoblobin GA adducts	pmol/g globin	143	146	83	136	124		19350	158	149	75.3	131	135		24730
liver GA adducts	in 10^8 nucleotides		6.7		4.6		1271	259		6.7		4.6		1153	238

[a] Each route yielded only one set of data with three mice sacrificed at each time point, and all mean data combined for a single simulation and set of pharmacokinetic and pharmacodynamic parameters. [b] This pilot study data set only had one mouse per gender for each time point and utilized the urinary excretion data from ref *14*.

Table F-5:. Pharmacokinetic Parameters from AA Administration to Human Volunteers

	units				
literature ref		Fuhr[a]		Boettcher[b]	
dose (mg/kg)		0.0124		0.00023	
route		diet, single		oral, daily	
gender (*n*)		male (3)	female (3)	male (3)	female (3)
		pharmacokinetic rate parameters			
stomach absorption half-life	min	30	30		
fraction absorbed from stomach		0.78 ± 0.03	0.64 ± 0.15		
		first-order rate constants		same as each individual from Fuhr	same as each individual from Fuhr
AA to GA	min^{-1}	2.55 ± 0.13	2.72 ± 0.26		
AA to AA-GS	min^{-1}	10.2	10.2		
GA to GA-GS	min^{-1}	16.3	16.3		
AA to urine	min^{-1}	1399 ± 278	1505 ± 361		
GA to urine	min^{-1}	22296 ± 4660	16673 ± 10742		
AA-GS to urine	min^{-1}	3140 ± 839	2984 ± 1638		
GA-GS to urine	min^{-1}	1390 ± 218	972 ± 315		
		elimination pattern (% of excretion)			
AA		5.9 ± 1.7	6.7 ± 1.8		
AA-GS		76.3 ± 1.5	75.7 ± 2.2		
GA		10.9 ± 0.3	9.9 ± 0.9		
GA-GS		6.9 ± 1.0	7.8 ± 1.6		
		pharmacodynamic rate constants			
formation of Hb AA adducts	min^{-1}			584 ± 19	861 ± 196
formation of Hb GA adducts	min^{-1}			3612 ± 705	4541 ± 2731
formation of liver GA adducts	min^{-1}			25.6 ± 4.3	32.6 ± 16.9
decay of Hb AA adducts	min^{-1}			0.003	0.003
decay of Hb GA adducts	min^{-1}			0.003	0.003
decay of liver GA adducts	min^{-1}			0.0113	0.0113

[a] Refs 19 and *20*. [b] Ref *21*.

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