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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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LIST OF ABBREVIATIONS AND ACRONYMS

3MT	3-methoxytyramine
5HIAA	5-hydroxyindoeacetic acid
5HT	5-hydroxytryptamine
AA	Acrylamide
AAMA	N-acetyl-S-(2-carbamoylethyl)-L-cysteine
AAVal	AA-Hb-terminal-valine adduct, N-(2-carbamoylethyl)valine
ABT	1-aminobenzotriazole
ADAF	age-dependent adjustment factor
afssa	Agence Francaise de Securite Sanitaire des Aliments, France
AIC	Akaike's Information Criterion
ALT	alanine aminotransferase
ANOVA	analysis of variance
AUC	area under the curve
BAG	Bundesamt für Gesundheit (Federal Office of Public Health – in Switzerland)
BB	Big Blue
BE	biomonitoring equivalent
BfR	Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment, Germany)
BMD	benchmark dose
BMDL	95% lower bound on BMD
BMDS	benchmark dose software
BMR	benchmark response
BrdU	bromodeoxyuridine
bw	body weight
CAs	chromosomal aberrations
CASRN	Chemical Abstracts Service Registry Number
C-C	control dams with control pups
CDC	Centers for Disease Control and Prevention
CERHR	[National Toxicology Program] Center for the Evaluation of Risks to Human Reproduction
CFR	Code of Federal Regulations
CI	confidence interval
CIR	The Cosmetic Ingredient Review (Expert Panel)
CNS	central nervous system
CRU	Covance Clinical Research Unit
C-T	control dams with treated pups
dAdo	2'-deoxyadenosine
dCyd	2'-deoxycytidine
dGua	2'-deoxyguanosine
DNC	degenerative nerve change
DOPAC	3,4,-dihydroxyphenylacetic acid
dThd	2'-deoxythymidine
EC	European Commission
ECG	electrocardiogram
ED	effective dose
EH	epoxide hydrolase

EIC	Environ International Corporation
E_k	elimination rate constant
ENMG	electroneuromyographic
EPA	U.S. Environmental Protection Agency
EPIC	European Prospective Investigation into Cancer and Nutrition
ER	endocrine receptor
ESI	electrospray ionization
EU	European Union
FAO	Food and Agricultural Organization
FDA	U.S. Food and Drug Administration
FFQ	food frequency questionnaire
FISH	fluorescence in situ hybridization
GA	glycidamide
GABA	gamma-aminobutyric acid
GAMA	N-(R,S)-acetyl-S-(carbamoyl-2-hydroxyethyl)-L-cysteine
GAVal	glycidamide-Hb-terminal-valine adduct, N-(2-carbamoyl-2-hydroxyethyl)valine
GC-MS	gas chromatography-mass spectrometry
GD	gestational day
GSH	glutathione
GST	GSH transferases
Hb	Hemoglobin
HBSS	Hanks' balanced salt solution
HEC	human equivalent concentration (inhalation exposure)
HED	human equivalent dose (oral exposure)
HID	highest ineffective dose/concentration
HR	hazard rate ratio
HSDB	Hazardous Substances Data Bank
HVA	homovanillic acid
i.p.	intraperitoneal or intraperitoneally
i.v.	intravenous or intravenously
IARC	International Agency for Research on Cancer
ICPEMC	International Commission for Protection Against Environmental Mutagens and Carcinogens
IRB	Institute Review Board
IRIS	Integrated Risk Information System
IRMM	Institute for Reference Materials and Measurements
IUR	inhalation unit risk
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JIFSAN	Joint Institute for Food Safety and Applied Nutrition
LC	liquid chromatography
LCL	lower confidence limit
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
LOD	limit of detection
LOH	loss of heterozygosity

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
MS	mass spectrometry
N3-GA-Ade	N3-(2-carbamoyl-2-hydroxyethyl)adenine
N7-GA-Gua	N7-(2-carbamoyl-2-hydroxyethyl)guanine
NCE	normochromatic erythrocyte
NCEA	National Center for Environmental Assessment
NCTR	National Center for Toxicological Research
NFCA	Norwegian Food Control Authority
NFCS	National Food Consumption Survey (Netherlands)
NHANES	National Health and Nutrition Examination Survey
NIOSH	National Institute of Occupational Safety and Health
NMA	N-methylolacrylamide
NMR	nuclear magnetic resonance
NO	nitric oxide
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
NTP	National Toxicology Program
OR	odds ratio
ORD	Office of Research and Development
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
PR	progesterone receptor
REF	risk extrapolation factors
REL	recommended exposure limit
RET	reticulocyte
RfC	reference concentration
RfD	reference dose
SAB	Scientific Advisory Board
SCE	sister chromatid exchange
SCF	Scientific Committee on Food of the European Commission
SD	standard deviation
SEM	standard error of the mean
SHE	Syrian hamster embryo
SMR	standardized mortality ratio
SNFA	Swedish National Food Agency
SNT	Statens næringsmiddeltilsy; (Scientific Committee of the Norwegian Food Control Authority, Mattilsynet)

T3	triiodothyronine
T4	thyroxin
T-C	treated dams with control pups
TD	toxicodynamic
TP53	tumor suppressor p53 gene
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
UDS	unscheduled DNA synthesis
UNSCEAR	United Nations scientific committee on the effects of radiation
U.S.	United States
UF	uncertainty factor
VD	volume of distribution
WHO	World Health Organization

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 (AA). It is not intended to be a comprehensive treatise on the chemical or toxicological nature of AA.

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 (AA). 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 (presumed threshold) mode of action (MOA). 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 ($\leq$ 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 a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a plausible inhalation unit risk (IUR) 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 AA has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983, 194806). U.S. Environmental Protection Agency (U.S. EPA) Guidelines and Risk Assessment Forum Technical Panel 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, 1986, 001468), *Guidelines for Mutagenicity Risk Assessment (U.S. EPA,*

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS)..

1986, 001466), Recommendations for and Documentation of Biological Values for Use in Risk Assessment (U.S. EPA, 1988, 064560), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991, 202859)*Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994, 076133), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994, 006488), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995, 005992), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996, 030019), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998, 030021), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000, 052149), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000, 052150), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000, 004421), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002, 088824), *Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005, 086237), Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005, 088823), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006, 194566), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006, 194567).

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 was reviewed through April 2009.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

2.1. CHEMICAL AND PHYSICAL INFORMATION

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

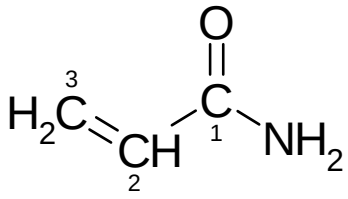


Figure 2-1. Chemical structure of AA with carbon numbers indicated.

References for the selected chemical and physical properties of AA listed in Table 2-1 or in the subsequent text include: the Hazardous Substances Data Bank (HSDB, 2005, 224272); Budavari, (2001, 224489); Verschueren, (2001, 224505); Lide (2000, 196090); Lewis (1997, 224609); Hansch et al. (1995, 224462); International Agency for Research on Cancer (IARC, 1994, 224633); and Petersen et al. (1985, 224371).

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS).

Table 2-1. Chemical and physical properties of AA

CAS number:	79-06-1 (Verschueren, 2001, 224505)
Molecular weight:	71.08 (Verschueren, 2001, 224505) 74.0 for 1,2,3-[^{13}C3]-labeled AA (Fennell et al., 2005, 224299)
Chemical Formula:	C_3H_5NO (Verschueren, 2001, 224505)
Boiling point:	192.6°C (Verschueren, 2001, 224505)
Melting point:	84.5°C (Verschueren, 2001, 224505)
Vapor pressure:	0.007 mmHg at 25°C (HSDB, 2005, 224272)
Density:	1.12 g/mL at 30°C (Budavari, 2001, 224489)
Vapor density:	2.46 (air = 1) (Verschueren, 2001, 224505)
Water solubility:	2.155 g/mL at 30°C (Verschueren, 2001, 224505)
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, 224489; Lide, 2000, 196090)
Partition coefficient (K_{ow}):	log K_{ow} = -0.67 (octanol/water) (Hansch et al., 1995, 224462)
Partition coefficient (K_{oc}):	log K_{oc} = 1 (organic carbon/water) (HSDB, 2005, 224272)
pH:	5.0–6.5 (50% aqueous solution) (HSDB, 2005, 224272)
Henry's law constant:	1.7×10^{-9} atm-m^3/mol at 25°C (HSDB, 2005, 224272)
Bioconcentration factor:	1 for fingerling trout (Petersen et al., 1985, 224371)
Stability	Stable at room temperature but may polymerize violently on melting (HSDB, 2005, 224272)
Conversion factors:	1 mg/m^3 = 0.34 ppm, 1 ppm = 2.95 mg/m^3 (Verschueren, 2001, 224505) 1 g = 14.07 mmoles

AA is a highly water-soluble α,β-unsaturated amide that reacts with nucleophilic sites in macromolecules in Michael-type additions (Calleman, 1996, 202899; Segerbäck et al., 1995, 224485). Monomeric AA readily participates in radical-initiated polymerization reactions, whose products form the basis of most of its industrial applications (Calleman, 1996, 202899).

2.2. SOURCES OF EXPOSURE, FATE AND TRANSPORT

2.2.1. AA from Industrial Sources

AA 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 AA by enzymatic hydration (HSDB, 2005, 224272; IARC, 1994, 224633). Direct uses of AA 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, 224272; IARC, 1994, 224633).

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 AA. Examples include polyacrylamide compounds used in oil well drilling operations (well drilling muds), as flocculants 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, 224268; CIR, 1991, 224274). Localized contamination may arise from the use of AA in grouting operations (HSDB, 2005, 224272). U.S. EPA (2003, 224471) requires drinking water authorities to certify that, for polyacrylamides used as coagulants or flocculants in drinking water treatment, the level of AA 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 Standard 60 for Drinking Water Treatment Chemicals - Health Effects (NSF/ANSI, 2009, 399318) provides the restrictions for the use of polyacrylamides in well drilling muds and grouts for potable water wells based on AA monomer levels.

If released to air, the vapor pressure of 0.007 mmHg at 25°C indicates that 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. AA is not expected to be susceptible to direct photolysis in sunlight since it does not absorb light with wavelengths >290 nm (HSDB, 2005, 224272).

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, 224272). AA 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, 204385). 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, 224498).

If released to water, AA is not expected to adsorb to suspended solids or sediment, based on the K_{oc} (HSDB, 2005, 224272). In a river die-away test, 90% of AA disappeared in approximately 150 hours (Croll et al., 1974, 224297). The hydrolysis half-life of AA has been reported as >38 years (HSDB, 2005, 224272). Volatilization of AA 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, 224371) suggests that bioconcentration in aquatic organisms is low (HSDB, 2005, 224272). Microbial degradation of AA can occur under light or

dark, aerobic or anaerobic conditions (Brown et al., 1980, 224478; Croll et al., 1974, 224297; Lande et al., 1979, 224498).

AA was formerly thought to only be present as an industrially manufactured chemical and not a naturally occurring contaminant (IARC, 1994, 224633). It is now known that AA is present in cigarette smoke, and can form in certain foods during cooking or processing.

2.2.2. AA in Cigarette Smoke

AA 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, 224502). 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 AA from environmental tobacco smoke. Boettcher et al. (2005, 224446) measured the AA and AA metabolites in human urine, and reported median levels in smokers (n = 13) about four times higher than in nonsmokers (n = 16) indicating that cigarette smoke is clearly an important source of AA exposure.

2.2.3. AA Formation in Foods during Processing

In early 2002, high concentrations of AA were reported in certain fried, baked, and deep-fried foods (SNFA, 2002, 224282). This discovery dramatically increased the interest in nonindustrial sources of AA 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., >120°C) (Tareke et al., 2000, 224368; Tareke et al., 2002, 224384). 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, 224595; Stadler et al., 2002, 224324). 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. AA has been detected in some food products that are processed at temperatures in the 98–116°C range and in high moisture conditions (e.g., canned black olives [not oil cured] and prune juice) (Roach et al., 2003, 224399), so there are other pathways of formation that do not involve temperatures over 120°C and crispiness, and these are being further evaluated (JIFSAN, 2004, 224420). 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, 224377) list AA concentrations in various foods in the United States as determined by the U.S. Food and Drug Administration (U.S. FDA, 2009, 224481) in Table 2-2, and in foods in Europe from data compiled by the Institute for Reference Materials and Measurements (IRRM, 2004, 224383) in Table 2-3.

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

Food commodity	n	Minimum	25%	Median	75%	Maximum	Standard deviation
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	2,762.0	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	1,057.0	249.1
Crackers and snack foods	32	12.0	92.5	169.0	302.3	1,243.0	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	1,925.0	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	5,399.0	1,018.8

[a]Includes cookies, pies and pastry, and 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 U.S. FDA on the Internet (2004, 399216). 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, 224377).

Table 2-3. AA levels in food (ppb) as collected by the European Union Joint Research Center

Food commodity	n	Minimum	25%	Median	75%	Maximum
French fries	741	5.0	90.0	178.0	326.0	2,228.0
Chips	569	5.0	378.0	600.0	980.0	3,770.0
Potato fritter[a]	75	15.0	215.0	492.0	797.6	2,779.0
Fine bakery ware[b]	485	5.0	67.0	160.0	366.0	3,324.0
Gingerbread	414	5.0	152.0	298.5	650.7	7,834.0
Crispbread	261	5.0	81.0	251.0	602.0	2,838.0
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	3,044.0
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	1,321.8	2,955.0

[a]Grated potatoes fried into a pancake.
[b] Products in the bread category normally have contents of sugars and fat, neither exceeding 5% on a dry weight basis. Bakery products exceeding either of these limits are termed "fine bakery ware." Savory fine bakery wares will tend to be higher in fat and sweet products in sugars. Some examples are: biscuits, cookies, dry pastry (e.g., savory biscuits, sweet biscuits and cookies); sweet breads (e.g., croissants, currant bun, dough cakes like muffins or brioche, scone, doughnut); pastry (e.g., danish pastry, baklava); tart, pie (e.g. custard tart, mince pie); cakes (e.g., fruit cake, cream cake, sponge cake).

Note: Data were calculated from the European monitoring database (updated June 2004) on AA levels in food (http://irmm.jrc.ec.europa.eu/html/activities/acrylamide/index.htm) maintained by the IRMM (2006, 399218), together with the Directorate General for Health and Consumer Affairs. This database comprises 3,442 samples of AA 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, 224377).

2.2.4. Estimates of AA exposure based on diet and AA content in foods

The FDA has estimated overall daily intake levels of AA 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, 2009, 224481). Table 2-3 is a compilation by Dybing et al. (2005, 224377) 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 (Table 2-4).

Table 2-4. Exposure estimates from 2002 to 2006

Exposure assessment (year)	Daily intake µg/kg-day		Source
	Mean (age group)	Percentile[a,b]	
WHO (2002)	0.3–0.8		http://www.who.int/foodsafety/publications/chem/en/acrylamide_full.pdf (FAO/WHO, 2002, 324865)
European Union (2002)	0.2–0.4		http://europa.eu/comm/food/fs/sc/scf/out131_en.pdf (EC, 2002, 224396)
Germany; Federal Institute for Risk Assessment (2003)	1.1 (15–18)	3.4[a]	http://www.bfr.bund.de/cm/245/assessment_of_acrylamide_intake_from_foods_containing_high_acrylamide_levels_in_germany.pdf (English) (BfR, 2003, 224437)
Switzerland; Federal Office for Public Health (2002)	0.28 (16–57)		http://www.bag.admin.ch/dokumentation/medieninformationen/01217/index.html?lang=fr&msg-id=4095 (BAG, 2002, 416785)
France; Agence Francaise de Securite Sanitaire des Aliments (2003)	0.5 (>15) 1.4 (2–14)	1.1[a] 2.9[a]	http://www.afssa.fr/Documents/RCCP2002sa0300.pdf (afssa, 2003, 416795)
U.S. FDA (2002)	0.7		http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Acrylamide/ucm053549.htm (2004, 399216)
U.S. FDA (2004)	0.43 (>2) 1.06 (2–5)	0.92[b] 2.31[b]	http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Acrylamide/ucm053549.htm (2004, 399216)
U.S. FDA (2006)	0.40 (>2) 1.07 (2–5)	0.95[a] 2.33[b]	http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Acrylamide/ucm053549.htm (2004, 399216)
Netherlands; NFCS (2003)	0.48 (1–97) 1.04 (1–6) 0.71 (7–18)	0.6[a] 1.1[a] 0.9[a]	Konings et al. (2003, 224538)
Sweden, SNFA (2002)	0.45 (18–74)	1.03	Svensson et al. (2003, 224353); (SNFA, 2002, 224282)
Norway, SNT (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, 224380) (SNT, 2002, 418763)

[a]95th percentile. [b]90th percentile.
NFCS = National Food Consumption Survey; SCF = Scientific Committee on Food of the European Commission; SNFA = Swedish National Food Agency; SNT = Statens næringsmiddeltilsy; the Norwegian Food Control Authority

Source: For all exposures estimates from 2002 to 2004, Dybing et al. (2005, 224377) except the FDA estimates; FDA exposure estimates 2002–2006 directly from the FDA website (U.S. FDA, 2009, 224481). Some links have been updated from those cited in Dybing et al. (2005, 224377).

A 2004 expert panel review of risk for human reproductive toxicity from exposure to AA compiled a table of estimates for total exposures, presented in Table 2-5 (NTP/CERHR, 2004, 224300).

Table 2-5. 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
Diet: 2- to 5-yr-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-d; human exposures are expressed as µg/kg-d. To convert figures in table to mg/kg-day, divide by 1,000.
[b]AA 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 AA 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 National Toxicology Program (NTP)/Center for the Evaluation of Risks to Human Reproduction (CERHR) Expert Panel estimated mean and upper bound workplace AA inhalation exposures at 1.4–18.6 and 43 µg/kg-day, respectively.

Source: NTP/CERHR (2004, 224300).

Alternate methods for estimating exposure to the general population are based on internal levels of biomarkers of exposure including levels of hemoglobin (Hb) adducts or urinary metabolites. Recent comparisons of biomarker studies from many different studies are being used to estimate risk (Doerge et al., 2008, 224362) or to compare estimates of exposure in the general nonsmoking population (Hartmann et al., 2008, 224480). Hartmann et al. (2008, 224480) developed exposure estimates based on levels of Hb adducts or urinary metabolites as

biomarkers in a nonsmoking population of children, adolescents, and adults from the general population in Germany (n = 91; 45 males, 46 females; aged 6–80 years; median age = 36 years). Median daily intakes were estimated at 0.43 (0.21–1.04) µg/kg-day based on Hb adducts levels; and 0.51(less than the limit of detection [LOD] -2.32) µg/kg-day based on urinary mercapturic acid levels. The internal exposure to AA and GA, measured as Hb adducts, was virtually the same for both sexes. The blood adduct levels, which can be used as long-term exposure markers, were identical in both sexes with median levels of 30 pmol/g globin for the acrylamide-hemoglobin-terminal-valine adduct, N-(2-carbamoylethyl)valine (AAVal) and 34 pmol/g globin for the glycidamide-hemoglobin-terminal-valine adduct, N-(2-carbamoyl-2-hydroxyethyl)valine (GAVal). The results, however, indicated that children take up approximately 1.3-1.5 times more AA per kg body weight (bw) than adults. The ratio of N-(R,S)-acetyl-S-(carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA)/N-acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA) was also significantly higher in the group of young children (6-10 years) with a median level of 0.5. The Hartmann et al. (2008, 224480) results are consistent with the U.S. population average intake estimates of 0.4 µg AA/kg-day, as well as intake estimates from other European countries summarized by FAO/WHO as ranging from 0.2 to 2.0 µg/kg-day, with a WHO designated representative average level for the general population of 1 µg/kg-day (FAO/WHO, 2005, 224279).

Additional information on estimates of exposure based on Hb adducts and urinary metabolites can be found in the Section 3.5 in the next chapter on the toxicokinetics of AA, and in Chapter 5 on the derivation of the human equivalent dose (HED) and reference values.

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

Much of the information in this section describes interactions of AA and its principal and toxicologically significant (epoxide) metabolite, glycidamide (GA) with various biologically significant targets such as cellular thiols (e.g., glutathione [GSH]), 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, 224360). Due to its α,β-unsaturated structure and ready capacity to undergo Michael-type additions, AA may be classified as a "soft" electrophile. Soft electrophiles like AA react readily with soft nucleophiles such as the thiol groups of proteins or GSH. GA, on the other hand, has a relatively high positive charge density, and acts as a hard electrophile, more capable of reacting with centers of high electronegativity (i.e., hard nucleophiles) such as the purine and pyrimidine bases in DNA (Dearfield et al., 1995, 224315; LoPachin and DeCaprio, 2005, 224556). 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, 224569). This information is useful in understanding the differences discussed in this section between the types of adducts formed by AA and GA (e.g., Hb and/or DNA) and the binding rates.

3.1. ABSORPTION

3.1.1. Hemoglobin Adducts as a Biomarker of Exposure/Absorption

Numerous studies, including a recent study by Fennell et al. (2005, 224299), support the use of AA Hb adducts as a biomarker of exposure. (see the Metabolism Section 3.3 for a detailed discussion of the chemistry of AA and GA Hb adducts, and GA DNA adducts). Estimates of exposure using Hb adduct levels are based on the assumption that a measured adduct level represents a steady state level from a continuous exposure to AA over the previous 120 days, which is the average life span of a red blood cell. Fennell et al. (2005, 224299) calculated AA exposure by using the results of the toxicokinetic study described above in 24 adult male volunteers. The estimated average daily background exposure to AA was 1.26 µg/kg-day based

on the subject's preexposure background AAVal levels (averaging about 80 pmol/g globin). In an occupational exposure study, Hagmar et al. (2001, 224453) reported a background range of 20–70 fmol AAVal/mg globin in the unexposed reference group. Using the Hagmar et al. (2001, 224453) 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, 224299) estimated a daily AA 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 AA. As can be seen in Table 2-4, many of the estimates of daily intakes in adults based on exposure estimates in foods are in the 0.2–1 µg/kg-day range, suggesting that adults with higher adduct levels may be exposed to AA from sources other than food (e.g., smoking, occupational, or from an as yet unknown source).

Detection of Hb 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 (Bergmark et al., 1993, 224424; Hagmar et al., 2001, 224453). Hb adduct levels were measured in 41 Chinese workers who were exposed to AA for 0.1–8 years (Bergmark et al., 1993, 224424). Adducts measured in this study were those at N-terminal valine residues in Hb. Workers were involved in the production of AA (via the hydration of acrylonitrile) and polyacrylamide. The adduct levels in exposed workers ranged from 0.3 to 34 nmol AA/g globin. Hb adducts of AA were not detected in blood samples from 10 control workers from the same city who had not been exposed to AA (or acrylonitrile). Blood samples from 5 of the 41 exposed workers were also analyzed for Hb adducts of GA (a principal metabolite of AA in both humans and animals) (Section 3.3). There was a statistically significant linear relationship between levels of Hb adducts of AA and GA in these five 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 Hb adduct levels of AA of 7.3 ± 3.4 nmol/g globin (n = 12, polymerization) and 14.7 ± 10.6 nmol/g globin (n = 14, synthesis). The study authors calculated the levels of Hb 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 globin for the polymerization workers and 0.44 (instead of 14.7) nmol/g globin for synthesis workers. Thus, Bergmark et al. (1993, 224424) 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.

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

Hagmar et al. (2001, 224453) measured Hb 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 (NMA). An important caveat in interpreting the Hb adduct data relative to AA absorption is that both AA and NMA form the same N-(2-carbamoylethyl)valine adduct in Hb and subsequent chemical measures of adduct levels cannot distinguish which parent compound formed the adduct (Fennell et al., 2003, 224295) (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 NMA; further analysis suggested that the air contained a 50:50 mixture of these compounds. Hb 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 globin. Adduct levels decreased to background levels within 120 days, consistent with the approximate 120-day life of red blood cells.

3.1.2. Human Oral/Dermal Exposure

Fennell et al. (2005, 224299) evaluated metabolism and Hb 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]-AA, 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]-AA by gavage. The [1,2,3-^{13}C]-AA 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.

AA 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, 224299) indicate much lower levels of AAVal and 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 versus 74.7 nmol/g globin/mmol AA/kg bw). 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 versus 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 versus 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, 224319) evaluated the toxicokinetics of AA in six young healthy volunteers after the consumption of a meal containing 0.94 mg of AA. Urine was collected up to 72 hours thereafter. Unchanged AA, its mercapturic acid metabolite AAMA, its epoxy derivative GA, and the respective metabolite of GA, GAMA, were quantified in the urine by liquid chromatography (LC)-mass spectrometry (MS). Toxicokinetic variables were obtained by noncompartmental methods. Overall, 60.3 ± 11.2% of the dose was recovered in the urine. Although no GA was found, unchanged AA, AAMA, and GAMA accounted for urinary excretion of (mean ± standard deviation [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 AA ingested with food is absorbed in humans.

Boettcher et al. (2006, 224451) 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 hours. Urinary AA mercapturic acid metabolites were considerably reduced after 48 hours of fasting, with levels even well below the median level in nonsmokers. These results

indicate that the AA in the diet is the main source of environmental AA exposure in humans, apart from smoking.

Bjellaas et al. (2007, 224443) 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 hour dietary recall as 21 (13–178) µg for nonsmokers and 26 (12–67) µg for smokers. The median dietary exposure to AA was estimated to be 0.47 (range 0.17–1.16) µg/kg-day. The median (range) total excretion of AA in urine during 24 hours was 16 (7–47) µg AA for nonsmokers and 74 (38–106) µg AA for smokers. In a multiple linear regression analysis, the urinary excretion of AA metabolites correlated statistically significant with intake of aspartic acid, protein, starch and coffee. Consumption of citrus fruits correlated negatively with excretion of AA metabolites.

3.1.3. Animal Oral Exposure

Studies in rats indicate that orally administered AA is rapidly and extensively absorbed by the gastrointestinal tract (Dixit et al., 1982, 061317; Doerge et al., 2005, 224348; Dow Chemical Company, 1984, 067922; Fennell et al., 2005, 224299; Kadry et al., 1999, 224596; Miller et al., 1982, 061351).

Doerge et al. (2005, 224348) compared the toxicokinetics of AA and GA in serum and tissues of male and female B6C3F_1 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 postdosing, 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, 224299) 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, 061351) and Kadry et al. (1999, 224596).

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 gavage or an i.v. dose of 10 mg/kg [2,3-^{14}C]-AA (in water vehicle) was essentially the same, indicating that 100% of the oral

dose was absorbed (Miller et al., 1982, 061351). 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]-AA 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, 061351).

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]-AA (Kadry et al., 1999, 224596). 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, 224596).

3.1.4. Animal Inhalation Exposure

Animal studies indicate that inhaled AA is readily absorbed (Sumner et al., 2003, 224347). Male F344 rats and B6C3F$_1$ mice were exposed to approximately 3 ppm of a mixture of [^{13}C]-labeled AA and [^{14}C]-labeled AA 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; <3% was in exhaled air; and 56% remained in the body. In mice, 51% was recovered in urine, feces, and nose-tube and cage washes; <3% was in exhaled air; and 46% remained in the body. Fractional absorption could not be determined from the presented data because ventilation rates were apparently not measured.[1]

[1] 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 AA inhaled in the 6-hour exposure period are calculated to be 6.5 and 2 µmol AA/exposure period for rats and mice, respectively. Given that the measured amounts of recovered AA 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: 3 ppm × 71.08/24.45 = 8.7 mg/m^3; (8.7mg/m^3) × (0.7cm^3/min-g) × (60 min/hour)×(6 hours/exposure) × (211 g/rat)×(m^3/10^6 cm^3) × (mmol/71.08 mg) × (10^{3}µmol/mmol) = 6.5 µmol/rat-exposure period.

3.1.5. 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 (Dow Chemical Company, 1984, 067922; Frantz et al., 1995, 224304; Sumner et al., 2003, 224347).

In male F344 rats, 14–30% (mean 22%) of an occluded dermal dose of [2,3-^{14}C]-labeled AA (162 mg/kg in distilled water) was absorbed during a 24-hour exposure period (Sumner et al., 2003, 224347). By 24 hours postapplication, 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, 224304) applied a 0.5% aqueous solution of [^{14}C]-labeled AA 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 Company, 1984, 067922). Aqueous solutions (1%) of [1,3-^{14}C]-labeled AA 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 AA. The fraction of dermally applied compound that was absorbed was not reported.

Results of several in vitro studies describe dermal absorption of AA. Frantz et al. (1995, 224304) applied a 0.5% [^{14}C]-labeled AA 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, 224331) applied a 0.5% [^{14}C]-labeled AA 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, 224582) applied [^{14}C]-labeled AA (in an aqueous gel of 2% polyacrylamide) to biopsied human abdominal skin for 24 hours at AA 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 AA 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 AA 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, 224414; Crofton et al., 1996, 145493; Dow Chemical Company, 1984, 067922; Edwards, 1975, 224382; Hashimoto and Aldridge, 1970, 063972; Ikeda et al., 1985, 224352; Kadry et al., 1999, 224596; Marlowe et al., 1986, 224576; Miller et al., 1982, 061351) and late-staged spermatids (Sega et al., 1989, 224477).

3.2.1. Animal Oral Exposure

Following 13 daily oral doses of [1,3-^{14}C]-labeled AA (at levels of 0.05 or 30 mg/kg), tissue concentrations of AA 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 Hb adducts of AA or GA (Dow Chemical Company, 1984, 067922). In rats exposed to 30 mg/kg, mean concentrations (µg equivalents [^{14}C]-AA per g 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]-AA 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]-AA equivalents in the other tissues (Dow Chemical Company, 1984, 067922).

In Sprague-Dawley rats given single oral doses of 50 mg/kg [1-^{14}C]-labeled AA, 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, 224596). 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 five 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.

3.2.2. Animal Dermal Exposure

Following 24-hour dermal exposure of male F344 rats to [^{14}C]-labeled AA (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, 224347).

3.2.3. Animal Inhalation Exposure

Immediately following a 6-hour inhalation exposure of male F344 rats to 3 ppm [^{14}C]/[^{13}C]-labeled AA 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, 224347). Immediately following a 6-hour inhalation exposure to the same concentration, male B6C3F$_1$ 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.

3.2.4. 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, 063972). 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, 063972).

Doerge et al. (2005, 224344) measured DNA adducts following a single intraperitoneal i.p. administration of AA and GA to adult B6C3F$_1$ 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, 061351). 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 Hb. 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, 061351).

Following i.p. injection of [^{14}C]-labeled AA (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, 224477). 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, 224477). In another study using whole-body autoradiography of Swiss-Webster mice orally exposed to [^{14}C]-labeled AA, (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, 224576).

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 from 10 to 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, 145493). 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 (Ikeda et al., 1983, 224332; Ikeda et al., 1985, 224352; Marlowe et al., 1986, 224576). 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, 224352). 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, 224352). 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, 224576). The autoradiographic technique used, however, provided only qualitative information.

3.3. METABOLISM

3.3.1. Human Metabolism

In the Fennell et al. (2005, 224299) study on 24 adult male volunteers previously discussed in the absorption section, approximately 86% of the urinary metabolites were derived from GSH conjugation and excreted as N-acetyl-S-(3-amino-3-oxopropyl)cysteine and its S-oxide. 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 Hb. 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 GSH 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 GSH 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, 224347])) and in mice (59% at 50 mg/kg ([Sumner et al., 1992, 224339])).

Boettcher et al. (2005, 224446) 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 AA 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 versus 29 µg/L for AAMA and 19 µg/L versus 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 Hb 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 Hb compared to GSH in humans.

Boettcher et al. (2006, 224449) 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 AA (d(3)-AA), representing 13 µg/kg bw, in drinking water. Urine samples before dosing and within 46 hours after the dose were analyzed for d(3)-AAMA and d(3)-GAMA by LC-electrospray ionization (ESI)-MS/MS. Total recovery in urine after 24 hours was about 51% as the sum of AAMA and GAMA and was similar to recoveries in rats (53–66%) given a gavage dose of 0.1 mg/kg bw (Doerge et al., 2007, 224359). 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., 2005, 224344). 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 Hb adducts.

Fuhr et al. (2006, 224319) 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 AA. Urine was collected up to 72 hours thereafter. No GA was found. Unchanged AA, 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 GSH exceeded the formation of the reactive metabolite GA. The data suggests an at least two- and fourfold lower relative internal exposure for GA from dietary AA in humans compared with rats or mice, respectively.

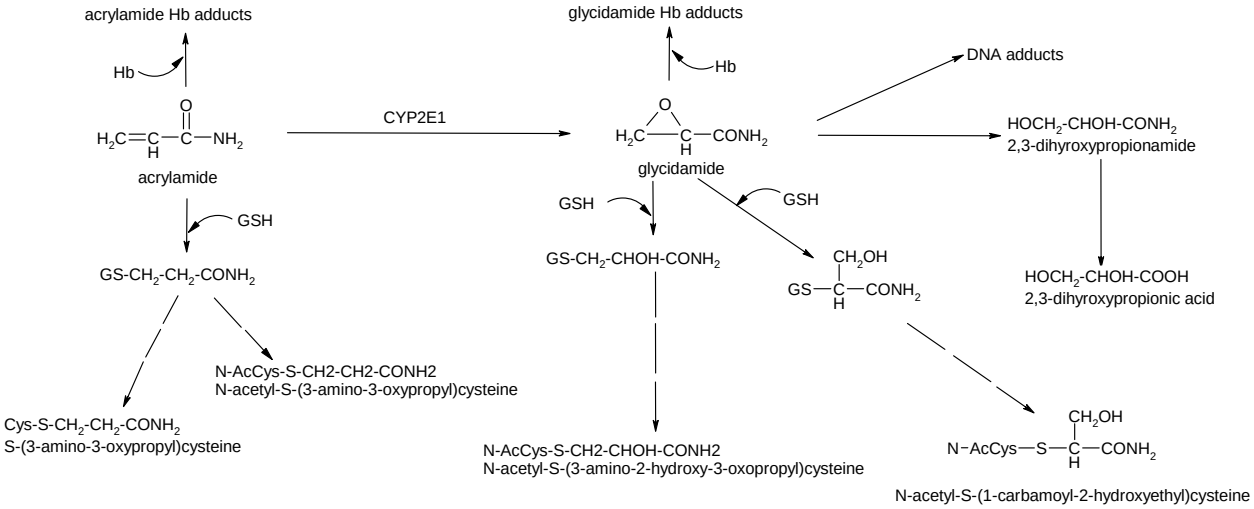
Paulsson et al. (2005, 224349) evaluated variability in human metabolism of acrylamide and glycidamide due to genetic polymorphic enzymes in the detoxification of AA and its metabolite glycidamide. Enzymes that enhance conjugation with GSH, the GSH transferases (GSTs), may influence the detoxification of both AA and glycidamide, whereas the enzyme epoxide hydrolase (EH) should only catalyse the hydrolysis of glycidamide. Paulsson et al. (2005, 224349) estimated the internal doses of AA or GA measured as specific adducts to Hb in blood samples after in vitro incubation with these compounds. Blood samples from individuals with different genotypes for GSTT1 and GSTM1 were studied. No significant differences in adduct levels depending on genotype were noted. In a parallel experiment, incubation with ethylene oxide was used as positive control. In this experiment individuals carrying GSTT1 showed lower adduct level increments from ethylene oxide than individuals lacking GSTT1. Furthermore, addition of ethacrynic acid or laurylamine, compounds which inhibit GST and EH, respectively, did not affect the adduct levels. Based on their results, the authors suggest that neither GSTs nor EH has any significant effect on the blood dose, measured as Hb-adducts over time, after exposure to AA or GA.

3.3.2. Animal Studies

Results from rat and mouse studies also indicate that AA is rapidly metabolized and excreted predominantly in the urine as metabolites (Dixit et al., 1982, 061317;

Dow Chemical Company, 1984, 067922; Edwards, 1975, 224382; Miller et al., 1982, 061351; Sumner et al., 1992, 224339; Sumner et al., 1999, 224342; Sumner et al., 2003, 224347; Twaddle et al., 2004, 224447). Formation of AA and GA Hb adducts in rats was initially reported by Bergmark et al. (1991, 224423) and second rate constants have been subsequently derived by in vitro and in vivo studies (Bergmark et al., 1993, 224424; Fennell et al., 2005, 224299; Tareke et al., 2006, 224387; Törnqvist et al., 2008, 224428). Bergmark et al. (1991, 224423) reported that the Hb binding index of AA to cysteine was found to be 6,400 pmol/g globin/µmol AA/kg, higher than for any other substance studied so far in the rat, and the Hb binding index of GA to cysteine was 1,820 pmol/g globin/µmol GA/kg. 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). The more recent studies have focused on the AA and GA binding to the N-terminal valine residue (Bergmark et al., 1993, 224424; Fennell et al., 2005, 224299; Tareke et al., 2006, 224387; Törnqvist et al., 2008, 224428).

A metabolic scheme for AA, based on results from these and other studies, is illustrated in Figure 3-1. AA reacts readily with GSH to form a GSH 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 AA in male F344 rats exposed to oral doses of 1–100 mg/kg [2,3-^{14}C]-labeled AA (Miller et al., 1982, 061351) and in male F344 rats and B6C3F$_1$ mice exposed to oral doses of 50 mg/kg [1,2,3-^{13}C]-labeled AA (Sumner et al., 1992, 224339).



Sources: Adapted from (Calleman, 1996, 202899; IARC, 1994, 224633; Sumner et al., 1999, 224342).

Figure 3-1. Metabolic scheme for AA. Note: Processes involving several steps are represented with broken arrows. Abbreviations: N-AcCys = N-acetylcysteine

Table 3-1 lists the relative amounts of AA metabolites determined by [^{13}C]- nuclear magnetic resonance (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 AA, 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, 224342).

Table 3-1. Urinary metabolites collected for 24 hours following oral administration of [1,2,3-^{13}C]-labeled AA (50 mg/kg) to male F344 rats or male B6C3F$_1$ mice

Metabolite[a]	Percent of total metabolites excreted in urine in 24 hrs (mean ± SD, n = 3)	
	Rat	Mouse[b]
From AA precursor		
N-acetyl-S-(3-amino-3-oxopropyl)cysteine	67.4 ± 3.6	41.2 ± 2.2
GA	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 AA was detected in urine but was not quantified. In other studies with F344 rats exposed to [2,3-^{14}C]-labeled AA, <2% of administered radiolabel was excreted in urine and bile as unchanged AA (Miller et al., 1982, 061351).
[b] In mice, an epoxide degradation product accounted for 4% of the total metabolites excreted.

Source: (Sumner et al., 1992, 224339).

Another initial step, catalyzed by CYP2E1, involves oxidation of AA to the epoxide derivative, GA. GA (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. GA may also undergo hydrolysis, perhaps catalyzed by epoxide hydrolases (1992, 224339; Sumner et al., 1999, 224342), leading to the formation of 2,3-dihydroxypropionamide and 2,3-dihydroxypropionic acid. GA and metabolites (or degradation products) derived from it accounted for about 33 and 59% of the total metabolites excreted in rat and mouse urine, respectively, within 24 hours (Table 3-2), indicating that, under these test conditions, the rate of transformation from AA to GA 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 AA (Sumner et al., 2003, 224347). GA and its metabolites derived from it accounted for 36 ± 2.4 and 73 ± 3.7% of total metabolites excreted in rat and mouse urine, respectively, within 24 hours (Sumner et al., 2003, 224347).

Table 3-2. Comparison of molar percentage of dose excreted in urine of rodents and humans after oral administration of AA

Species dose	AA	AAMA	AAMA-sulfoxide	GA	GAMA	Glyceramide	∑GA[a]/ ∑AA[b]	Total of dose[c]
Mouse 50 mg/kg[d]	NQ	21.0 ± 1.10	ND	8.6 ± 1.1	17 ± 0.60	2.70 ± 0.60	1.3	50.4
Mouse 0.1 mg/kg[e]	0.6–0.7	5–9	ND	16–18	9–22	ND	4.2	33–48
Rat/ 0 mg/kg[d]	NQ	34.0 ± 1.80	ND	2.8 ± 0.50	12 ± 0.60	1.20 ± 0.40	0.47	50.7
Rat 50 mg/kg[f]	NQ	38	ND	3.9	10.5	0.6	0.39	53
Rat 3 mg/kg[g]	NQ	29.0 ± 4.50	ND	ND	21 ± 2.42	ND	0.72	50.0 ± 8.60
Rat 0.1 mg/kg[e]	2	31	ND	6	27–29	ND	1–1.1	64–66
Rat 20 µg/kg[h]	ND	29.7 ± 5.13	ND	ND	25.4 ± 6.20	ND	0.86	55.1 ± 11.8
Rat 0.1 mg/kg[g]	ND	34.9 ± 7.40	ND	ND	26.7 ± 4.64	ND	0.77	61.7 ± 10.5
Hamster 3.0 mg/kg[g]	NQ	22.0 ± 5.30	4.20 ± 1.10	0.79 ± 0.24	ND	3.30 ± 1.10	0.16	34.0 ± 5.70
Hamster 13 µg/kg[i]	ND	45.1	ND	ND	2.8	ND	0.06	47.7
Hamster 0.5 mg/kg[j]	4.67 ± 1.34	31.2 ± 6.55	8.26 ± 2.39	0.43 ± 0.20	0.82 ± 0.16	ND	0.03	45.6 ± 8.50
Hamster 1.0 mg/kg[j]	5.02 ± 1.65	34.4 ± 5.21	8.68 ± 1.21	0.63 ± 0.33	0.82 ± 0.11	ND	0.03	49.9 ± 6.30
Hamster 3.0 mg/kg[j]	3.23 ± 0.49	27.8 ± 7.99	7.25 ± 2.40	0.65 ± 0.21	0.70 ± 0.22	ND	0.03	39.9 ± 9.90
Hamster 0.5 µg/kg[k]	ND	41.4 ± 3.47	7.19 ± 1.40	ND	3.83 ± 0.78	ND	0.08	52.4 ± 3.59
Hamster 20 µg/kg[k]	ND	37.4 ± 2.92	6.33 ± 1.77	ND	3.23 ± 0.69	ND	0.07	46.9 ± 3.70

Note: All information given is referenced to collection periods of 24 hrs after administration.

[a]This sum represents GA + GAMA + glyceramide.
[b]This sum represents AA + AAMA + AAMA-sulfoxide.
[c]Total amount excreted within 24 hrs after exposure calculated as percentage of dose.
[d](Sumner et al., 1992, 224339); gavage male rats; gavage male mice.
[e](Doerge et al., 2007, 224359); gavage male mice; gavage male rats.
[f](Sumner et al., 2003, 224347); gavage me rats.
[g](Fennell et al., 2005, 224299); gavage male rats; oral administration male humans.
[h](Hartmann et al., 2008, 224480); gavage male rats.
[i](Boettcher et al., 2006, 224451); oral administration male human; excretion within 22 hrs following exposure.
[j](Fennell et al., 2005, 224299); oral administration male humans.
[k](Hartmann et al., 2008, 224480); oral administration male and female humans; excretion within 22 hrs following exposure.
ND = not determined; NQ = not quantified

Source: Hartmann et al. (2008, 224480).

Doroshyenko et al. (2009, 224430) investigated AA toxicokinetics in 16 healthy volunteers in a four-period change-over trial and evaluated the respective role of cytochrome P450 2E1 (CYP2E1) and GSTs. Participants ingested potato chips containing AA (1 mg) without co-medication, after CYP2E1 inhibition (500 mg disulfiram, single dose) or induction (48 g-day ethanol for 1 week), and were phenotyped for CYP2E1 with chlorzoxazone (250 mg, single dose). AA-containing potato chips were prepared by frying 150g batches of self-prepared potato chips (‘‘Princess’’ potatoes) at 190ºC for 5 minutes. Unchanged AA and the mercapturic acids N-acetyl- S-(2-carbamoylethyl)-cysteine (AAMA) and N-acetyl- S-(2-hydroxy-2-carbamoylethyl)-cysteine (GAMA) accounted for urinary excretion [geometric mean (percent coefficient of variation)] of 2.9% (42), 65% (23), and 1.7% (65) of the AA dose in the reference period. Hb adducts clearly increased following the AA test-meal. The increases in cumulative amounts of AA, AAMA, and GAMA excreted, and in AA adducts were significant during CYP2E1 blockade (point estimate [90% confidence interval or CI]) to the 1.34-fold (1.14-1.58), 1.18-fold (1.02-1.36), 0.44-fold (0.31-0.61), and 1.08-fold (1.02-1.15) of the reference period, respectively, but were not changed significantly during moderate CYP2E1 induction. Individual baseline CYP2E1 activity, CYP2E1*6, GSTP1 313A>G and 341T>C single nucleotide polymorphisms, and GSTM1- and GSTT1-null genotypes had no major effect on AA disposition. The changes in AA toxicokinetics upon CYP2E1 blockade provide evidence that CYP2E1 is a major but not the only enzyme mediating AA epoxidation in vivo to glycidamide in humans. The authors reported that no obvious genetic risks or protective factors in xenobiotic-metabolizing enzymes could be determined for exposed subjects.

Age related increases in human CYP2E1 expression have been reported. Johnsrud et al. (2003, 224616) 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 (GDs 93–186 ; 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 31–90 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, posthoc). 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 such as AA (i.e., decreased levels of GA) compared with older infants, children, and adults. However, actual differences in the total amount metabolized and parent compound cleared (and the resulting spectrum of adverse effects) would depend upon the delivery rate and substrate concentration relative to the value of the Michaelis-Menten constant (Km) for CYP2E1 (Lipscomb, 2004, 224551; Lipscomb et al., 2003, 192847).

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.

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 AA, 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, 224342). In contrast, urine collected from wild-type mice contained considerable amounts of metabolites derived from GA (Sumner et al., 1999, 224342). With wild-type mice in this study, 22% of excreted metabolites were accounted for by metabolites derived from GSH conjugation with GA (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 GA 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.

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 AA 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, 063972), but following administration of [2,3-^{14}C]-labeled AA to male F344 rats, no radioactivity was detected in exhaled breath (Miller et al., 1982, 061351). Sumner et al. (1992, 224339) 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 AA. In other experiments, no exhaled $^{14}CO_2$ was detected following oral administration of 50 mg/kg [1-^{14}C]-labeled AA to male Sprague-Dawley rats (Kadry et al., 1999, 224596), whereas 3–4% of i.v. injected [1,3-^{14}C]-AA (2 or 100 mg/kg) was detected as $^{14}CO_2$ in exhaled breath in male F344 rats (Dow Chemical Company, 1984, 067922). During a 24-hour period following a 24-hour dermal exposure of male F344 rats to 162 mg/kg [2,3-^{14}C]-labeled AA, $^{14}CO_2$ in exhaled breath accounted for 1.8 ± 0.2% of radioactivity recovered in exhaled air, urine, feces, and tissues (Sumner et al., 2003, 224347). 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 B6C3F$_1$

mice and F344 rats, respectively, following nose-only inhalation exposure to 3 ppm of a mixture of [1,2,3-^{13}C]-AA and [2,3-^{14}C]-AA (Sumner et al., 2003, 224347).

3.3.3. Route-to-Route Differences

Results from a rat kinetic study by Sumner et al. (2003, 224347) indicate that an i.p. or gavage route of exposure had a small effect on the percentage of AA conjugated to GSH versus the percentage of AA converted to GA. Following i.p. or gavage administration of 50 mg/kg [1,2,3-^{13}C]-AA 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 GSH. Similarly, in the only available animal inhalation kinetic study (i.e., no human inhalation kinetic studies are available), the metabolites associated with direct conjugation of AA with GSH following a 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]-AA accounted for 64 ± 2.4% of metabolites in urine collected for 24 hours. 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.

In this same study, Sumner et al. (2003, 224347) reported statistically significantly larger percentages of urinary metabolites associated with GA formation following an inhalation exposure compared with an i.p. and gavage exposure. GAVal levels were also higher and AAVal levels were lower (as indicators of serum AUCs), following the single 6 hr inhalation exposures versus the single gavage dose in rats, however, statistical significance was not reported for the adduct level differences, and the numbers were within twofold of each other. Doerge et al. (2005, 224348; 2005, 224355) reported an increased percentage of GA formation observed in mice and F344 rats from a gavage or dietary exposure compared to an i.v. exposure that, in conjunction with the Sumner et al. (2003, 224347) results, indicate first pass metabolism in the lungs following an inhalation exposure similar to the first pass metabolism in the liver from an oral exposure, but apparently the lungs may have a larger percent of oxidative metabolism of AA to GA.

Lehning et al. (1998, 224454) reported 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) induced axonal degeneration, but shorter-term (11 days) exposure to higher i.p. doses (50 mg/kg-day) did not. Barber et al. (2001, 224414) 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 AA by gavage (24 hours after the last dose of a drinking water solution of 20 mg/kg-day nonlabeled AA 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, 224414) also measured the activities of CYP2E1 and epoxide hydrolase in liver microsomes, as well as concentrations of AA-Hb and GA-Hb 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 ($t_{1/2}$) 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 versus time curves (AUCs) indicated that a larger proportion of plasma AA was converted to GA following a single oral dose of 20 mg/kg (22%) than following a single i.p. dose of 50 mg/kg (10%). A larger proportion of plasma AA was also converted to GA following the 34 days of repeated oral dosing (30%) compared with 11-days of i.p. dosing (8%). No correlation was found to the different enzyme activities involved in GA formation (CYP2E1) or metabolism (epoxide hydrolase). Concordant with the serum data, concentrations of AA-Hb adducts were about 36% lower in the oral dosing regimen (8 µmol adduct/g globin at 15 days) compared with the i.p. regimen (12.5 µmol adduct/g globin at 11 days), and concentrations of GA-Hb adducts were about twofold higher. Barber et al. (2001, 224414) 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 AA.

Doerge et al. (2005, 224348) compared the toxicokinetics of AA and GA in serum and tissues of male and female B6C3F_1 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, due either to a first-pass effect or some other factors that affect the kinetic disposition from an i.v. dose. Similar results were observed by Doerge et al. (2005, 224355) in a comparable study with F344 rats.

In comparing the results of the Doerge et al. (2005, 224348) mouse study with previous studies from that laboratory at a 500-fold higher concentration (Twaddle et al., 2004, 224447), an increase in relative internal GA levels was observed, suggesting that as the dose rate decreases, the conversion of AA to GA in mice is more efficient.

3.3.4. Differences in Mouse and Rat Metabolism

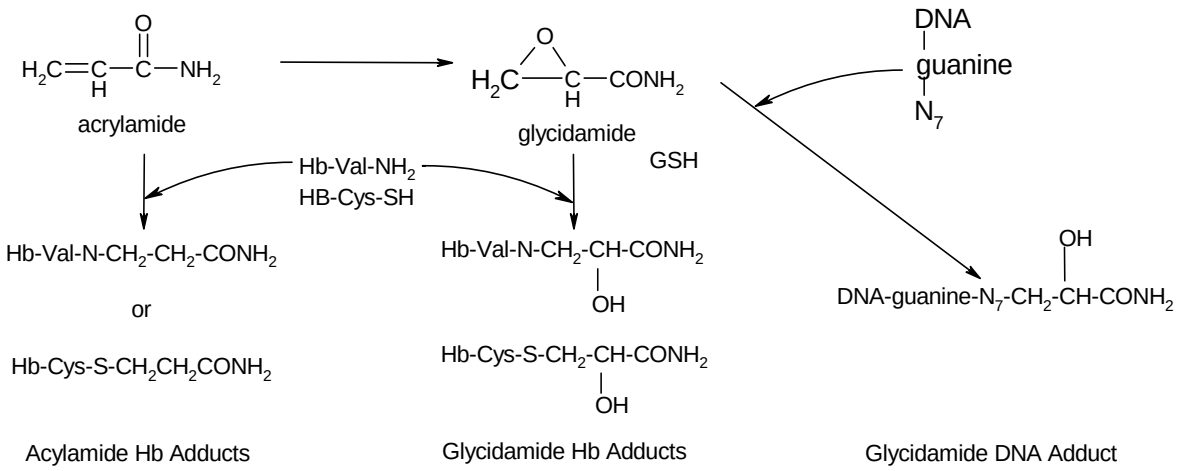
Twaddle et al. (2004, 224447) administered AA at approximately 50 mg/kg via gavage to adult male and female B6C3F$_1$ mice. Serum concentrations of AA and GA were taken at 0.5, 1, 2, 4, and 8 hours postdosing. Livers were removed from control and AA-treated mice at all exposure times, and analyzed for 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, 224447) estimated an AA half-life of elimination from plasma at 0.73 hours in these B6C3F$_1$ 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, 224414). Miller et al. (1982, 061351) 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, 224447) 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, 224414) in rats. Barber et al. (2001, 224414) also reported a GA/AA-AUC ratio of 0.18 for Sprague-Dawley rats treated with 20 mg/kg AA by gavage. This is in contrast to the Twaddle et al. (2004, 224447) observation of equal AUCs for AA and GA in B6C3F$_1$ mice. Since rats and mice had a comparable GA elimination half-life, this approximately fivefold difference in internal exposure to GA for mice compared with rats (i.e., a GA/AA-AUC ratio of 1 in mice versus 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.

3.3.5. Formation of DNA Adducts

Doerge et al. (2005, 224344) measured DNA adducts following a single i.p. administration of AA to adult B6C3F$_1$ mice and F344 rats at 50 mg AA/kg, or an equimolar dose of GA (61 mg/kg). They reported that 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. (2005, 224344) also measured DNA adduct formation following oral administration of a single dose of AA (50 mg/kg), and accumulation from repeated 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.

AA and GA react with nucleophilic sites in macromolecules (including Hb and DNA [Figure 3-2]) in Michael-type additions (Bergmark et al., 1991, 224423; Bergmark et al., 1993, 224424; Segerbäck et al., 1995, 224485; Solomon et al., 1985, 224306). Solomon et al. (1985) (1985, 224306) conducted in vitro studies for the reaction of AA 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 AA concentrations (Solomon et al., 1985, 224306). Based on the second order rate constant derived by Solomon et al. (1985, 224306), Segerbäck et al. (1995, 224485) 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 GA, 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, 224485). Two additional GA-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 da Costa et al., 2003, 194572). Using LC with tandem MS and isotope dilution, Gamboa da Costa et al. (2003, 194572) 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 2,000 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.



Sources: Dearfield et al. (1995, 224315); Bergmark et al. (1991, 224423; 1993, 224424).

Figure 3-2. Hb and DNA adducts of AA and glycidamide.

3.3.6. Potential Confounders for the Hb Adduct Biomarker of AA exposure

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

Studies that use AA Hb 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 AA N-(2-carbamoylethyl)valine adduct with gas chromatography (GC)-MS analysis after derivatization with pentafluorophenyl isothiocyanate (Bergmark et al., 1993, 224424). NMA, however, forms the same adduct as AA, 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, 224295). There are also differing results on the relative rate of formation of the N-(2-carbamoylethyl) valine adduct from AA or NMA (Fennell et al., 2003, 224295; Paulsson et al., 2002, 224334).

Paulsson et al. (2002, 224334) measured Hb adducts (and micronucleus [MN] frequencies) in mice and rats after AA 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 Hb adduct measurement. One group of rats was sacrificed after 24 hours and one group after 48 hours for the Hb 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 Hb 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 Hb adduct level and MN frequency in peripheral erythrocytes. Per unit of administered dose, NMA showed only half the potency for inducing MN 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, 224295) also measured levels of N-(2-carbamoylethyl)valine adducts following gavage exposure of male F344 rats (4/group) to equimolar levels of AA 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, 224334), Fennell et al. (2003, 224295) reported that AA exposure resulted in the formation of 21 ± 1.7 nmol/g globin (mean ± SD), less than the equimolar dose of NMA that resulted in 41 ± 4.9 nmol/g globin. 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 AA exposure based on N-terminal valine levels if there is also a potential for concurrent exposure to NMA.

3.4. ELIMINATION

3.4.1. Human Data

Boettcher et al. (2005, 224446) measured the mercapturic acid of AA and its epoxide GA, i.e., AAMA and GAMA in human urine. 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 versus 29 µg/L for AAMA and µg/L versus µg/L for GAMA indicating that cigarette smoke is clearly an important source of AA exposure. The level of AAMA in the occupationally nonexposed collective (n = 29) ranged from 3 to 338 µg/L, and the level of GAMA from <LOD to 45 µg/L.

Fennell et al. (2005, 224299) reported 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. (2006, 224449) 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

AA (d(3)-AA), representing 13 µg/kg bw, in drinking water. Urine samples before dosing and within 46 hours after the dose were analyzed 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 hours with a broad plateau between 8 and 18 hours for AAMA, and 22 hours for GAMA. Elimination half-lives of both AAMA and GAMA were estimated to be approximately 3.5 hours for the first phase and more than 10 hours up to few days for the second phase. Total recovery in urine after 24 hours was about 51% as the sum of AAMA and GAMA and was similar to recoveries in rats (53–66%) given a gavage dose of 0.1 mg/kg bw (Doerge et al., 2007, 224359). 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, 224319) 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 AA. Overall, 60.3 ± 11.2% of the dose was recovered in the urine. Although no GA was found, unchanged AA, 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 AA, AAMA, and GAMA of 2.4 ± 0.4, 17.4 ± 3.9, and 25.1 ± 6.4 hours, respectively.

Boettcher et al. (2006, 224451) evaluated urinary mercapturic acid metabolites derived from AA in three healthy volunteers who fasted for 48 hours. Urinary AA mercapturic acid metabolites were considerably reduced after 48 hours of fasting, with levels well below the median level in nonsmokers. These results indicate that, for nonsmokers, AA in the diet is the main source of environmental AA exposure in humans.

Hartmann et al. (2008, 224480) determined the relationship between the oxidative and reductive metabolic pathways of AA in the nonsmoking general population, measuring both blood protein adducts and the urinary metabolites of AA and GA in an especially designed study group with even distribution of age and gender. The Hb adducts AAVal and GAVal were detected by GC-MS/MS in all blood samples with median levels of 30 and 34 pmol/g globin, respectively. Concentrations ranged from 15 to 71 pmol/g globin for AAVal and from 14 to 66 pmol/g globin for GAVal. The ratio GAVal/AAVal was 0.4-2.7 (median = 1.1). The urinary metabolites were determined by LC-MS/MS. Of all urine samples examined 99% of AAMA levels and 73% of GAMA levels were above the LOD (1.5 µg/L). Concentrations ranged from <LOD to 229 µg/L (median = 29 µg/L) for AAMA and from <LOD to 85 µg/L (median = 7 µg/L) for GAMA. The ratio of GAMA/AAMA varied from 0.004 to 1.4 (median = 0.3).

Hartmann et al. (2008, 224480) summarized the data from human and rat urinary metabolite studies in a table that indicated reasonable concordance of results (Table 3-2).

3.4.2. Animal Data

Results from animal studies indicate that urinary excretion of metabolites is the principal route of elimination of absorbed AA, with minor amounts of metabolites being excreted via bile in the feces, and as CO_2 in exhaled breath (Barber et al., 2001, 224414; Dow Chemical Company, 1984, 067922; Hashimoto and Aldridge, 1970, 063972; Kadry et al., 1999, 224596; Miller et al., 1982, 061351; Sumner et al., 1992, 224339; Sumner et al., 1999, 224342).

Fennell et al. (2005, 224299) 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, 224347). 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, 224596) and Miller et al. (1982, 061351).

In male F344 rats given i.v. (10 mg/kg) or oral (1, 10, or 100 mg/kg) doses of [2,3-^{14}C]-AA, about 60 and 70% of the administered radioactivity was excreted in urine collected within 24 hours and 7 days, respectively (Miller et al., 1982, 061351). 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, 202899) 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, 061351). Bile-duct-cannulated rats given single i.v. doses of 10 mg/kg [2,3-^{14}C]-labeled AA 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 AA were placed in metabolism cages designed to trap expired air (Miller et al., 1982, 061351). 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 AA (Hashimoto and Aldridge, 1970, 063972) and about 4% of an injected dose of 2 mg/kg [1,3-^{14}C]-labeled AA (Dow Chemical Company, 1984, 067922) 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 AA to male Sprague-Dawley rats (Kadry et al., 1999, 224596).

In studies with male F344 rats given single i.v. doses of [1,3-^{14}C]-labeled AA, 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 Company, 1984, 067922). 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 AA 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 AA, whereas the second phase was due to clearance of radiolabeled metabolites from the plasma.

Tong et al. (2004, 224421) estimated the second order rate constants for reaction of AA with human serum albumin and GSH 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, 224359) 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 i.v., gavage, and dietary routes of administration. The results were compared with serum/tissue toxicokinetic and adduct data (DNA and Hb) from previous studies in the same laboratory using the identical dosing protocols (Doerge et al., 2005, 224348; 2005, 224355; 2005, 224344). 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 percentages of the total i.v. delivered dose that was recovered as free AA and metabolites in a 24 hour urine collection were 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 versus AAMA, $r^2 = 0.78$, $p < 0.001$; GA versus GAMA, $r^2 = 0.81$, $p < 0.001$) and mice (AA versus AAMA, $r^2 = 0.86$, $p < 0.001$; GA versus 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 versus AA, $r^2 = 0.74$, $p < 0.001$; AUC-AA versus AAMA, $r^2 = 0.83$, $p < 0.001$) and mice (AUC-AA versus AA, $r^2 = 0.41$, $p < 0.011$; AUC-AA versus 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 versus GA, $r^2 = 0.53$, $p < 0.001$; AUC-GA versus GAMA, $r^2 = 0.32$, $p < 0.02$) and mice (AUC-GA versus GA, $r^2 = 0.34$, $p < 0.022$; AUC-GA versus 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. HB ADDUCTS AND URINARY METABOLITES AS BIOMARKERS OF EXPOSURE

Hemoglobin adducts are increasingly being used as biomarkers of exposure (Ogawa et al., 2006, 597196), and the literature in this area is increasing. Hb adducts were first proposed as biomarkers of exposure to AA by WHO (1985, 224533), and the initial analytical techniques were developed by Bailey et al. (1986, 224410). Early studies in people who were occupationally exposed to or who smoked tobacco evaluated the relationship between AA and GA Hb adducts and exposure (Bergmark, 1997, 224422; Bergmark et al., 1993, 224424; Calleman et al., 1994, 202900). AA was reported to form the N-(2-carbamoylethyl) valine and GA to form the N-(2-carbamoyl-2-hydroxyethyl)valine and the N-(1-carbamoyl-2-hydroxyethyl)-valine (Bergmark, 1997, 224422; Bergmark et al., 1993, 224424; Calleman et al., 1994, 202900). The detection of GA adducts of Hb in AA-exposed workers demonstrated the transformation of AA to GA in humans (Bergmark et al., 1993, 224424). Other related compounds like acrylonitrile and NMA also form Hb adducts, so these potential confounders should always be considered in studies that use AA Hb adducts as the basis for estimating exposure to AA.

Hays and Aylward (2008, 224510) and Aylward (2008, 224507) reported the results of a workshop that developed methods to generate a biomonitoring equivalent (BE) for AA among other case studies. BEs are estimates of the concentration of a chemical or metabolite in a biological medium that is consistent with an existing exposure guidance value such as a tolerable daily intake or an RfD. BEs address the need for a context to interpret data that are increasingly

becoming available on trace concentrations of chemicals in human biological media. Case studies for four chemicals (toluene, 2,4-dichlorophenoxyacetic acid, cadmium and AA) were published to demonstrate the derivation of BEs for various kinds of data. The case study for AA (Hays and Aylward, 2008, 224510) clearly shows the utility of AA Hb adduct and urinary metabolite concentrations as biomarkers of exposure, as well as the methods to estimate daily intake levels based upon those concentrations. Hays and Aylward (2008, 224507; 2008, 224510) summarized the advantages and disadvantages of the various biomarkers of exposure for AA (Table 3-3). This current toxicological review of AA utilizes the direct relationship between AA and GA Hb adducts and serum levels of AA and GA to estimate the dose-response relationship in humans based on the observed dose-response relationship from animal studies.

Table 3-3. The advantages and disadvantages of available biomarkers of exposure for AA

Analyte	Medium	Advantages	Disadvantages
AA	Serum	Relevant to effect(s) of interest.	Short half-life; invasive (requires blood sample)
GA	Serum	Relevant to effect(s) of interest.	Short half-life; invasive (requires blood sample)
AA–as hemoglobin adduct	Blood	Longer half-life; relevant to effect(s) of interest.	Invasive (requires blood sample)
GA–as hemoglobin adduct	Blood	Longer half-life; relevant to effect(s) of interest.	Invasive (requires blood sample)
AAMA (mercapturic acid metabolite of AA)	Urine	Noninvasive; slower half-life leads to more stable profile in urine under chronic exposure conditions.	Not directly related to critical target tissue dose(s); measure of metabolic deactivation.
GAMA (mercapturic acid conjugate of GA) and other urinary metabolites	Urine	Noninvasive; slower half-life leads to more stable profile in urine under chronic exposure conditions.	Not directly related to critical target tissue dose(s); lower proportion of administered dose than AAMA.

Source: Hays and Aylward (2008, 224510).

3.5.1. Use of Measured Hb Adducts to Estimate Administered Dose or Serum AUC

The equations used to estimate the AUC of AA or GA in serum based upon measured Hb adduct levels are straightforward. For a single dose over a short time frame (i.e., no need to adjust for accumulation or steady state levels of adducts from multiple doses) the serum AUC is calculated as:

$$\text{Serum AUC} = \frac{\text{Hb adduct concentration/second order rate constant for adduct formation}}{} \qquad \text{Equation-1}$$

If the adduct levels are normalized to the administered dose an estimate of the AUC per dose can be derived, as exemplified in the following data and discussion from Fennell et al. 2005.

Fennell et al. (2005, 224299) measured the amount of Hb adducts from AA and GA following administration of a single 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 bw) 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.

Table 3-4 shows the data used by Fennell et al. (2005, 224299) to estimate the internal serum concentrations of AA based on adduct levels and second order rate constants that they measured in vitro by adding AA or GA to extracted human Hb.

Table 3-4. Estimated human serum AA-AUC normalized to administered dose based on measured Hb adduct levels and in vitro derived second-order rate constants

		Hb adducts/actual administered dose	Human AA-AUC per administered dose[b]
Human nominal dose (route) (mg/kg)	**Actual dose (μM AA/kg bw)**	**Concentration of [$^{13}C_3$]-AAVal normalized to actual dose (nmole/g globin/mmole AA/kg bw)**	**([$^{13}C_3$] mM-hr AA/mmole AA/kg bw)**
Human Dose			
0.5 (oral)	5.9 ± 0.2	86.4 ± 7.5[a]	20.2
1.0 (oral)	12.5 ± 0.2	73.4 ± 9.8[a]	17.2
3 (oral)	38.7 ± 0.5	64.2 ± 17.7[a]	15.0
Combined (oral)		74.7 ± 14.9[a]	17.5

[a]Values are normalized by actual dose (mmol/kg bw).
[b]Hb adduct levels divided by second-order formation rate constant of 4.27×10^{-6} L/g globin/hr.

Sources: Fennell et al. (2005, 224299).

The above AA-AUC of 17.5 mM-hr AA/mmoles of AA/kg bw was derived by dividing the [$^{13}C_3$]-AAVal adduct concentration normalized to administered dose of 74.7 nmoles/g globin/mmole AM/kg bw by a second order rate constant for the formation of AAVal of 4.2×10^{-6} L/g globin/hour that was measured in in vitro studies.

The AA-AUC units of mM-hr AA/mmoles of AA/kg bw can be converted to units of μM-hr AA/mg AA/kg bw by dividing by the molecular weight of AA (71.08), and multiplying by 1,000 to convert mmoles to μmoles (Fennell et al., (2005, 224299):

$$\frac{17.5\,mM-hr\,AA}{mmoles\,AA/kg\,bw}\,x\,\frac{1\,mmole\,AA}{71.08\,mg\,AA}=\frac{0.227\,mM-hr\,AA}{mg\,AA/kg\,bw}\,x\,\frac{1000\,\mu M}{1mM}$$

$$=\frac{246\,\mu M-hr\,AA}{mg\,AA/kg\,bw}$$ Equation-2

Fennell et al. (2005, 224299) 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 pmol AAVal/g globin/day) for the life span of the erythrocyte (120 days) was estimated to result in the accumulation of adducts to 63 pmol/g globin. Daily dermal exposure to 1 μg/kg AA (0.18 pmol AAVal/g globin/day) for the life span of the erythrocyte (120 days) would result in the accumulation of adducts to 10.8 pmol AAVal/g globin. With workplace exposure of 5 days/week, this would decrease to approximately 7.8 pmol AAVal/g globin.

Other researchers have developed equations to estimate daily increment of adducts or steady state levels depending on the available data.

Based on measurements of total AAVal levels in F344 rat blood following exposure to AA in drinking water for one week, Törnqvist et al. (2008, 224428) derived a parameter value of 1.07 to be used in the following equation developed by Granath et al. (1992, 224411) to adjust the total adduct concentration after 7 days to an estimate of the daily increase in adduct level:

$$AAVal_{DAILY} = AAVal_{TOTAL} \frac{1.07}{7\ (Days\ of\ Treatment)} \qquad \text{Equation-3}$$

Bergmark et al. (1991, 224423) used the following equations to estimate steady state level of adducts or to estimate the AUC based on an assumed steady state adduct level:

$$AAVal = AA_{AUC} \times k_{AA} \times \frac{t_{RBC}}{2} \qquad \text{Equation-4}$$

$$GAVal = GA_{AUC} \times k_{GA} \times \frac{t_{RBC}}{2} \qquad \text{Equation-5}$$

where AAVal is the steady state level of AA-Hb adducts (µmol/g globin), AA_{AUC} is the daily serum area under the curve for AA (µM hr-day), k_{AA} is the rate constant for the reaction of AA with the N-terminal valine residue of Hb, and t_{RBC} is the life span of a human red blood cell with often used values ranging from 120 days (Osterman-Golkar et al., 1976, 224312) to 126 days in humans (Hartmann et al., 2008, 224480).

The above equations are important since, in practice, dosimetry by means of hemoglobin adducts in humans often are based on steady-state adduct levels of chemically stable adducts. An important caveat, however, is that the assumption of steady-state level is valid, and that rate of elimination is determined only by erythrocyte turnover.

Hartmann et al. (2008, 224480) used more elaborate equations (Calleman, 1996, 202899; Schettgen et al., 2003, 224452) and the parameter values below to estimate a daily intake of 0.43 (0.21-1.04) µg AA/kg-day in the nonsmoking general population based on measured Hb adduct levels. These equations adjust an "assumed" steady state adduct level to estimate the internal serum AUC, and then apply values for an elimination rate constant (E_k) and the volume of distribution (VD) to convert an AUC to an estimated daily intake.

$$AA[\mu g / kg\text{ - day}] = \frac{AAVal\ [pmol\ /\ g\ globin]}{k \times erthrocyte\ \ lifespan \times 1/2} \times E_k \times MW\ \ acrylamide\ \times VD \qquad \text{Equation-6}$$

where: *k* is the human Hb adduct formation rate constant for AAVal (4.4×10^{-6} L/g of globin/hr (Bergmark et al., 1993, 224424); the value for the middle erythrocyte lifespan is 63 days, the

elimination rate constant E_k in humans is 0.15 h^{-1} (Calleman, 1996, 202899) and the VD of 0.38 L/kg is an estimate from Fennell et al. (2005, 224299).

An additional caveat about estimating an administered dose or AUC based on measured adduct levels is the sensitivity of the estimate to the value of the second order rate constant. Currently, there are no available second order adduct formation rate constants that have been measured "in vivo" in humans, which would require human studies where data were collected for all three of the critical variables needed to derive an in vivo human adduct formation rate, namely: (1) the administered dose; (2) the time course serum levels; and (3) the time course adduct levels (including sufficient post dosing sample times to determine elimination rates). Kopp and Dekant (2009, 224532) only measured human serum data and administered dose, and Fennell et al. (2005, 224299) only measured Hb adduct levels and administered dose. Thus, current derivations of human serum AUCs or daily intakes that have been reported in the published literature are based on Hb adduct levels and second order rate constants that were derived from in vitro studies where AA or GA are added to extracted human Hb (Bergmark et al., 1993, 224424; Fennell et al., 2005, 224299; Tareke et al., 2006, 224387). Second order rate constants have also been estimated from in vitro studies using rat Hb (Bergmark et al., 1993, 224424; Fennell et al., 2005, 224299; Tareke et al., 2006, 224387; Törnqvist et al., 2008, 224428). Recently, however, rat and mice in vivo data have been published by Doerge et al. (2005, 224344; 2005, 224348; 2005, 224355) and Tareke et al. (2006, 224387) sufficient to allow the generation of in vivo animal Hb adduct formation rates. EPA used these data, which include in vivo time course serum data from single doses of AA with different routes of administration (Doerge et al., 2005, 224355) and the corresponding Hb adduct levels (Tareke et al., 2006, 224387) to derive second order rate constants for AA and GA Hb adducts in rats. Table 3-5 is a compilation of these various rat and human adduct formation rate constants. The in vivo rat adduct formation rates were then used to estimate rat internal AUCs for AA and GA based upon the time course Hb adduct levels reported by Tareke et al. (2006, 224387) for rats given AA in drinking water for 42 days (Doerge et al., 2005, 224344; Tareke et al., 2006, 224387). Table 3-6 and Table 3-7 present these estimated serum AA and glycidamide AUCs normalized to the administered dose of AA. The values of the AUC per administered dose in Tables 3-6 and 3-7 depend on the route of exposure (see text in the first column) as well as, perhaps, the vehicle/mode of administration due to potential differences in bioavailability. Estimates of AUC per administered dose based on the adduct data following drinking water exposure for 7 weeks from Doerge et al. (2005, 224355) are therefore the most appropriate for use in estimating internal doses in the Friedman et al. (1995, 224307) and Johnson et al. (1984, 067926; 1986, 061340) studies, which administered AA in drinking water.

The values in these tables were used to derive the reference values in this assessment, and are discussed in greater detail in Chapter 5.

Table 3-5. Second-order rate constants for reaction of AA or GA with the N-terminal valine residue of Hb

Source	Second-order rate constant for formation of Hb adducts (L/g globin/hr)×10^{-6}					
	Male rat	Female rat	Pooled male and female rat data	Gender not specified rat	Pooled rat and mouse	Human
AAVal in vivo adduct formation rate[a]						
Based on all rat and mice Tareke et al. (2006, 224387) adduct data and measured serum AUCs in Doerge et al. (2005, 224348; 2005, 224355) single-dose studies					7.5	
Based on gender-specific rat Tareke et al. (2006, 224387) adduct data and measured serum AUCs in Doerge et al. (2005, 224355) single dose studies	8.9	5.9				
Based on all rat Tareke et al. (2006, 224387) adduct data and measured serum AUCs in Doerge et al. (2005, 224355) single dose studies			7.5			
AAVal in vitro rate adduct formation rate						
As reported by Fennell et al. (2005, 224299)	3.82					4.27
As reported by Bergmark et al. (1993, 224424)						4.4
As reported by Tareke et al. (2006, 224387)				2.9		7.4
As reported by Törnqvist et al. (2008, 224428)				4.6		
GAVal in vivo adduct formation rate[a]						
Based on all rat and mice Tareke et al. (2006, 224387) adduct data and measured serum AUCs in Doerge et al. (2005, 224348; 2005, 224355) single-dose studies					32.5	
Based on gender-specific rat Tareke et al. (2006, 224387) adduct data and measured serum AUCs in Doerge et al. (2005, 224355) single-dose studies	38.4	30.7				
Based on all rat Tareke et al. (2006, 224387) adduct data and measured serum AUCs in Doerge et al. (2005, 224355) single-dose studies			34.0			
GAVal in vitro rate adduct formation rate						
As reported by Fennell et al. (2005, 224299)	4.96					6.72
As reported by Bergmark et al. (1993, 224424)				12.0[b]		11.0
As reported by Tareke et al. (2006, 224387)				9.5		59.0
As reported by Törnqvist et al. (2008, 224428)				13.6		

[a]See Appendix E for a description of the derivation of the in vivo adduct formation rates.
[b]Bergmark derived the rat GAVal residue such that kval = (GAVal *kcys)/GA cys; the human GAVal adduct was measured directly.

Table 3-6. Measured and estimated AA-AUCs normalized to dose in humans and F344 rats

	AA-AUC in µM-hr per mg AA/kg bw			
	Male rat	Female rat	Gender not specified rat	Human
AA in humans				
Measured				
Kopp and Dekant (2009, 224532); human serum data AA (single dose of 20 µg/kg, n = 3F,3M)				2.83
Estimated using human adduct data and test animal in vivo rate constants				
Fennell et al. (2005, 224299); human adduct data (single oral exposure) and in vivo rate constants derived from Tareke et al. (2006, 224387) adduct data for all rat and mice in Doerge et al. (2005, 224348; 2005, 224355) single-dose AUCs				140.1
Estimated using human adduct data and human in vitro rate constants				
Fennell et al. (2005, 224299); human adduct data (single oral exposure) and in vitro rate constants				246.0
Fennell et al. (2005, 224299); human adduct data (single oral exposure) and Bergmark et al. (1993, 224424) in vitro rate constants				238.8
Estimated using human adduct data and rat in vitro rate constants				
Fennell et al. (2005, 224299); human adduct data (single oral exposure) and Törnqvist et al. (2008, 224428) in vitro rate constants				228.5
AA in F344 rats				
Measured				
Doerge et al. (2005, 224355); time course data from a single dietary exposure	18.0	15.0		
Doerge et al. (2005, 224355); time course data from a single gavage exposure	24.0	45.0		
Estimated using rat adduct data and rat in vivo rate constants				
Tareke et al. (2006, 224387) adduct data for the Doerge et al. (2005, 224344) 49-day drinking water study, and gender-specific in vivo derived rate constants from Tareke et al. (2006, 224387) and Doerge et al. (2005, 224355)	23	37.7		
Tareke et al. (2006, 224387) (2006) adduct data for the Doerge et al. (2005, 224344) 49-day drinking water study, and nongender in vivo derived rate constants from Tareke et al. (2006, 224387) (2006) and Doerge et al. (2005, 224355)	27.4	29.7		
Estimated using rat adduct data and rat in vitro rate constants				
Törnqvist et al. (2008, 224428); adduct data from a 7-day drinking water study and in vitro rate constants	34.0	48.0		
Fennell et al. (2005, 224299); adduct data (single dose gavage) and in vitro rate constant			80.2	

Table 3-7. Measured and estimated GA-AUCs normalized to dose in humans and F344 rats

	GA-AUC in µM-hr per mg AA/kg bw			
	Male rat	Female rat	Gender not specified rat	Human
GA in humans				
Estimated using human adduct data and test animal in vivo rate constants				
Fennell et al. (2005, 224299); human adduct data (single oral exposure) and in vivo rate constants derived from Tareke et al. (2006, 224387) adduct data for all rat and mice in Doerge et al. (2005, 224348; 2005, 224355) single dose AUCs				12.5
Estimated using human adduct data and human in vitro rate constants				
Fennell et al. (2005, 224299); human adduct data (single oral exposure) and in vitro rate constants				60.4
Fennell et al. (2005, 224299); human adduct data (single oral exposure) and Bergmark et al. (1993, 224424) in vitro rate constants				37.0
Estimated using human adduct data and rat in vitro rate constants				
Fennell et al. (2005, 224299); human adduct data (single oral exposure) and Törnqvist et al. (2008, 224428) in vitro rate constants				29.9
GA in F344 rats				
Measured				
Doerge et al. (2005, 224355); time course data from a single dietary exposure	19.0	15.0		
Doerge et al. (2005, 224355); time course data from a single gavage exposure	13.0	44.0		
Estimated using rat adduct data and rat in vivo rate constants				
Tareke et al. (2006, 224387) adduct data for the Doerge et al. (2005, 224344) 49-day drinking water study, and gender-specific in vivo derived rate constants from Tareke et al. (2006, 224387) and Doerge et al. (2005, 224355)	14.4	32.3		
Tareke et al. (2006, 224387) adduct data for the Doerge et al. (2005, 224344) 49-day drinking water study, and nongender in vivo derived rate constants from Tareke et al. (2006, 224387) and Doerge et al. (2005, 224355)	16.2	29.2		
Estimated using rat adduct data and rat in vitro rate constants				
Törnqvist et al. (2008, 224428); adduct data from a 7-day drinking water study and in vitro rate constants	18.0	34.0		
Fennell et al. (2005, 224299); adduct data (single dose gavage) and in vitro rate constant			52.1	

3.5.2. Urinary metabolites as Biomarkers of Exposure

Urinary metabolites (along with Hb adducts) have been measured in a number of studies to estimate daily intake levels in the general population. Doerge et al. (2008, 224362) compiled a summary of selected studies reproduced below as Table 3-8.

Table 3-8. Selected published measurements of AA-derived Hb adducts and urinary metabolites in groups of nonsmokers

Study	Group size (n)	AAMA[a] (µg/L)	GAMA[a] (µg/L)	AAVal[a] (pmol/g globin)	GAVal[a] (pmol/g globin)
Paulsson et al. (2003, 224345)	5	–	–	27	26
Boettcher et al. (2005, 224446)	16	29	5	19	17
Bjellaas et al. (2007, 224444)	65	39	31	–	–
Bjellaas et al. (2005, 224440)	44	–	–	38	20
Urban et al. (2006, 224476)	60	73	16	28	3
Vesper et al. (2007, 224511)	6	–	–	43	26
Fennell et al. (2005, 224299)	24	ND	ND	76	29
Kellert et al. (2006, 224571)	13	26	3	–	–
Chevolleau et al. (2007, 224269)	52	–	–	27	22
Vesper et al. (2005, 224508)	61	–	–	51	34

[a]Urinary concentrations of AAMA and GAMA in urine and AAVal and GAVal are reported from the respective studies of nonsmoking humans.
– = not measured; ND = not detected

Source: Doerge et al. (2008, 224362).

Hays and Aylward (2008, 224510) discuss approaches for interpreting measured urinary concentration data following known exposures to AA. As an example for the GSH metabolite of AA, AAMA, under steady-state exposure conditions consistent with chronic exposure, the daily elimination of AAMA on a molar basis should be equal to approximately 50% of the daily intake (Boettcher et al., 2006, 224449; Fuhr et al., 2006, 224319). The daily mass of AAMA excreted in urine as a function of the daily intake of AA can be estimated as follows:

$$AAMA_{urine} = AA \times \left[\frac{MW_{AAMA}}{MW_{AA}} \right] \times 0.5 \qquad \text{Equation-7}$$

where: $AAMA_{urine}$ is the mass of AAMA excreted in urine per day (mg); AA is the total daily dose of AA (mg); MW_{AAMA} and MW_{AA} are the molecular weights of AAMA and AA (234.1 and 71.08), respectively.

These estimates of daily intake based upon urinary concentrations require some additional assumptions (with the accompanying uncertainties). The best estimates would be based on a 24-hour urine specimen, and the subject's age, gender, lean body mass (a function of height and weight), dietary patterns, and other factors including kidney function status would all be known. Hays and Aylward (2008, 224510) note that, in practice, collection of 24-hour samples is difficult and impractical for large biomonitoring studies such as the NHANES/CDC effort. As a result, urinary concentrations are generally reported based on spot urine sample collection. The absolute concentration of compounds in such samples can vary substantially due simply to differences in hydration rates and to other factors. Thus, in addition to reporting absolute urinary concentrations of such chemicals (for example, in units of µg/L), CDC and other researchers generally also report levels adjusted to creatinine levels (e.g., µg chemical/g creatinine). While hydration status introduces variability into interpretation of urinary concentrations on a volume basis, creatinine adjustment also introduces variability into the analysis. Because the total intake is also a function of weight (these values are generally specified in terms of mg of intake per kg bodyweight per day), estimates of the creatinine-adjusted concentration in urine associated with any daily intake can also vary substantially among individuals. The reader is referred to the Hays and Aylward (2008, 224510) AA case study for additional discussion of these uncertainties and approaches for interpreting urinary metabolite levels that adjust for creatinine (µg chemical/g creatinine) and for urinary volume (µg chemical/L of urine) in the context of reference values.

As an example of a daily intake estimate, Bjellaas et al. (2007, 224443) reported urinary mercapturic acid derivatives of AA in a clinical study of 53 subjects. Urinary metabolite levels were determined using solid-phase extraction and LC with positive electrospray MS/MS detection. The median (range) total excretion of AA in urine during 24 hours was 16 (7–47) µg AA for nonsmokers and 74 (38–106) µg AA for smokers. Median intakes (range) of AA were estimated based on 24 hour dietary recalls as 21 (13–178) µg for nonsmokers and 26 (12–67) µg for smokers. The median dietary exposure to AA was estimated to be 0.47 (range 0.17–1.16) µg/kg-day. In a multiple linear regression analysis, the urinary excretion of AA metabolites correlated statistically significant with intake of aspartic acid, protein, starch and coffee. Consumption of citrus fruits correlated negatively with excretion of AA metabolites.

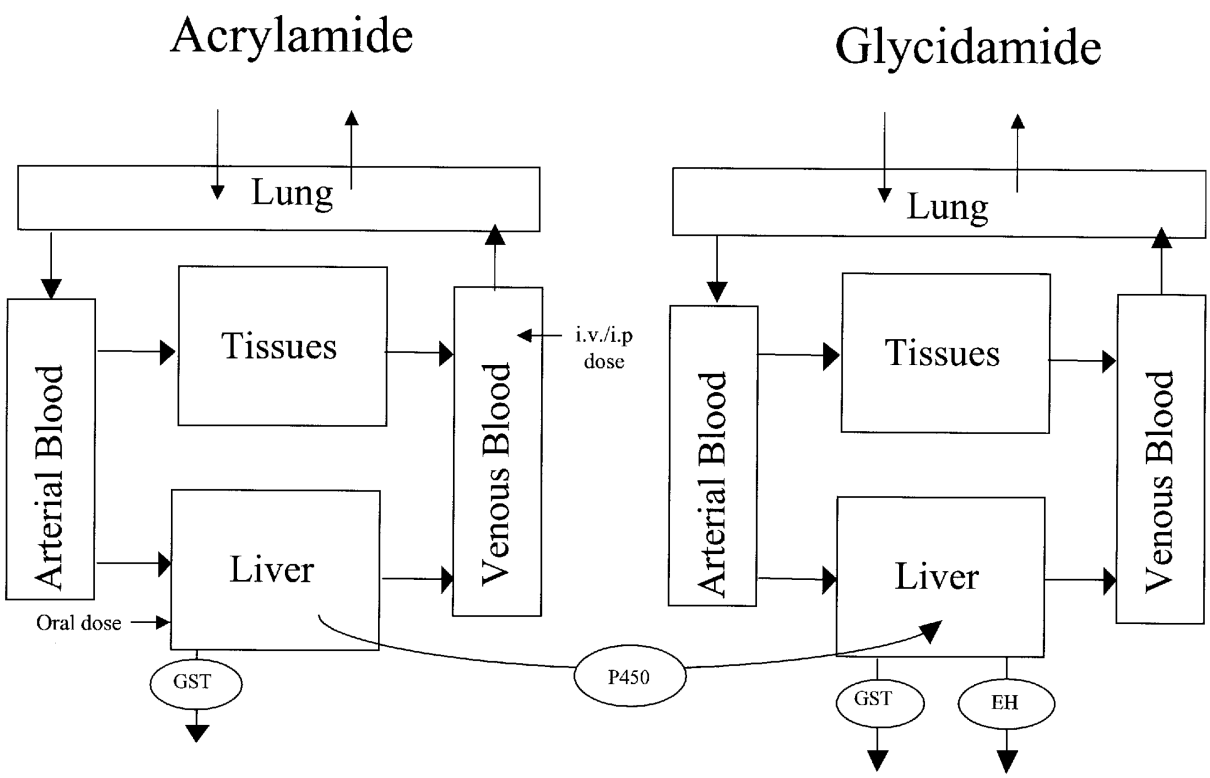
In the Hartmann et al. (2008, 224480) analysis of AA exposure in the nonsmoking general population, Hb adduct levels in blood and mercapturic acid excretion in urine were used to calculate daily AA intake, and gave practically identical values. The estimated median daily intakes were 0.43 (0.21-1.04) µg/kg-day using Hb adducts and 0.51 (<LOD-2.32) µg/kg-day using mercapturic acids for calculations. Children were reported to have intakes of up to

approximately 1.3-1.5 times more AA per kg bw than adults. The ratio GAMA/AAMA was significantly higher in the group of young children (6-10 years) with a median level of 0.5. A gender-related difference in internal exposure and metabolism was not observed.

Similarly, Heudorf et al. (2009, 224517) measured AAMA and GAMA in urine specimens of 110 children, and provided evidence for a background exposure by nutrition. Median (95th percentile) uptake of AA in children was 0.54 (1.91) mg/kg-day, exceeding exposure in adults by 50%. The authors suggest that children may have a higher AA intake than adults, and that children more effectively oxidize AA.

3.6. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

Three physiologically based toxicokinetic (PBTK) models for AA are available from the peer reviewed published literature (Kirman et al., 2003, 087778; Walker et al., 2007, 224527; Young et al., 2007, 224545). Kirman et al. (2003, 087778) developed a PBTK model (Figure 3-3) that simulated the disposition of AA and its epoxide metabolite, GA, in the rat based upon the available kinetic data from the 1980s and early 1990s, including limited measurements of AA blood and nervous tissue concentrations (Raymer et al., 1993, 224375), measurements of total radioactivity (Miller et al., 1982, 061351; Ramsey et al., 1984, 067937) and urinary metabolite data to set the metabolic parameters (Sumner et al., 1992, 224339). Walker et al. (2007, 224527) recalibrated the Kirman et al. (2003, 087778) PBTK model based upon rat and human Hb adduct and urinary metabolite data (Bergmark et al., 1991, 224423; Fennell et al., 2005, 224299; Sumner et al., 2003, 224347), and second order adduct formation rate constants that were developed from in vitro addition of AA or GA and human or rat red blood cell Hb (Fennell et al., 2005, 224299). EPA used the Walker et al. (2007, 224527) model in the derivation of reference values in a previous draft of the EPA AA assessment (U.S. EPA, 2007, 418811) specifically to estimate the human internal dose equivalent to the point of departure (POD) from rat bioassay dose-response data.

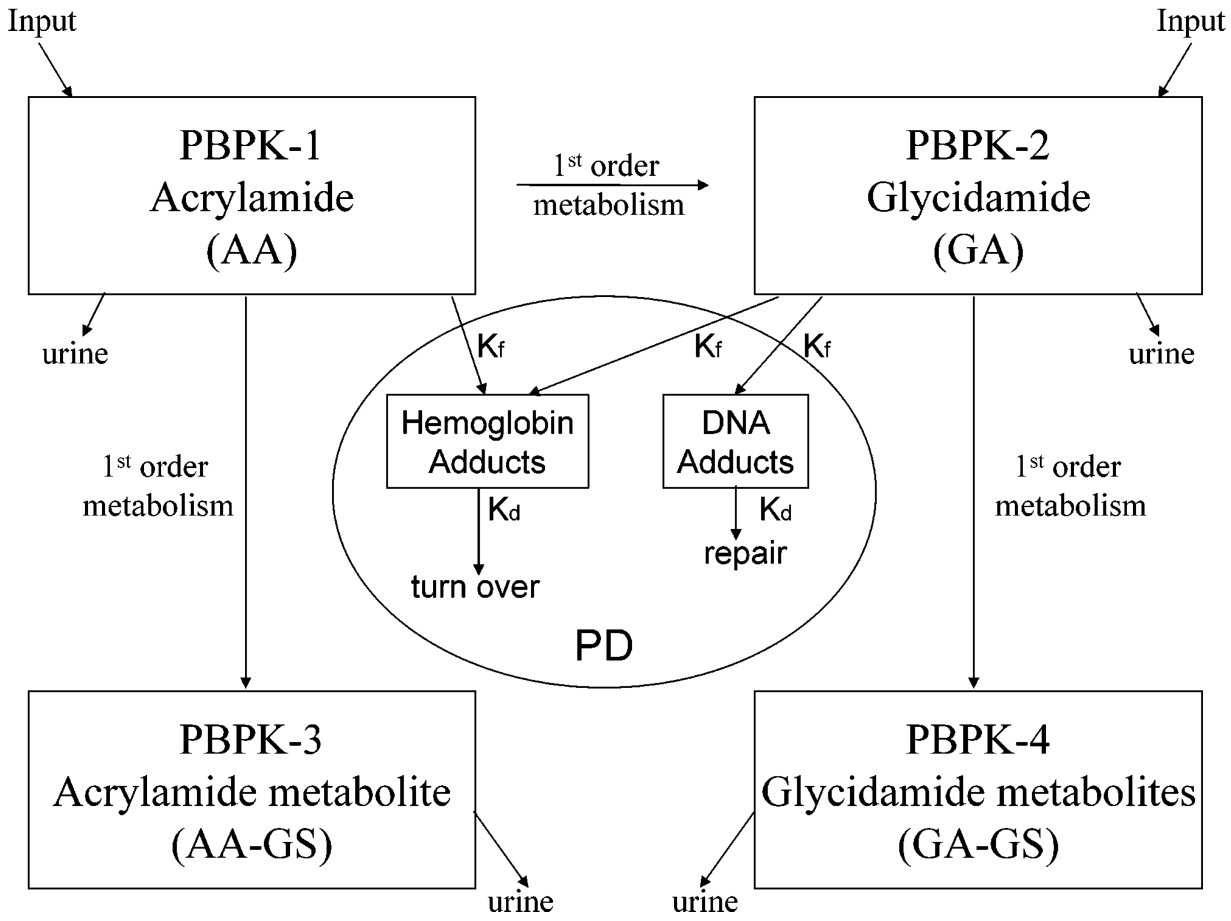


Source: Kirman et al. (2003, 087778).

Figure 3-3. Schematic of the Kirman et al. (2003, 087778) PBTK model for AA.

Young et al. (2007, 224545) also developed a PBTK/TD (toxicodynamic) model (Figure 3-4) 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. (2007, 224545) 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, 224344; Doerge et al., 2005, 224348; Doerge et al., 2005, 224355) and from the literature (Barber et al., 2001, 224414; Raymer et al., 1993, 224375; Sumner et al., 1992, 224339; Sumner et al., 2003, 224347); on published human urinary excretion data (Fennell et al., 2005, 224299; Fuhr et al., 2006, 224319) and on human Hb adduct data from a dietary exposure (Boettcher et al., 2005, 224446). Young et al. (2007, 224545) use the PBTK model to fit individual animal pharmacokinetic data, and then evaluate the resulting differences in parameter values (and distributions). The Young et al. (2007, 224545) model simulated liver DNA-adduct levels based upon data from Doerge et al. (2005, 224344), and was subsequently used to integrate the findings of rodent neurotoxicity and cancer into estimates of risks from human AA exposure through the diet (Doerge et al., 2008, 224362). The approach taken in Young et al. (2007, 224545) was to adjust the model parameter values to fit individual data sets, rather than develop a single set of parameters that best fit all of the data. For the

Young et al. (2007, 224545) model to be used for specific EPA application in deriving a toxicity value, additional work is needed to determine which individual parameter values would be the most appropriate to use for each derivation, or preferably, what set of parameters could be developed that would best fit all of the data.



Source: Young et al. (2007, 224545).

Figure 3-4. Schematic of the Young et al. (2007, 224545) PBTK model for AA.

Additional kinetic data have become available that can be used to further calibrate and test the predictive capability of an AA PBTK model and to further refine the parameter estimates. These data include time course serum data in F344 rats (Doerge et al., 2005, 224355; Kopp and Dekant, 2009, 224532) and in humans (Kopp and Dekant, 2009, 224532), additional adduct data in F344 rats (Tareke et al., 2006, 224387; Törnqvist et al., 2008, 224428) and humans (Hartmann et al., 2008, 224480), and additional urinary metabolite data in rats and humans (Boettcher et al., 2006, 224449; Boettcher et al., 2006, 224451; Doroshyenko et al., 2009, 224430; Hartmann et al., 2008, 224480; Heudorf et al., 2009, 224517; Kopp and Dekant, 2009, 224532). An AA PBTK model has not yet been published and peer reviewed that

incorporates and integrates all of these newer data sets. PBTK models are often the only viable alternative to uncertainty factors (UFs) by which to extrapolate the animal to human dose-response relationship (i.e., to derive human reference values). In this case, however, the Hb adduct and serum data, provide a means to conduct a direct extrapolation from rat to human of the area under the time-concentration in blood of AA or GA sufficient to derive the oral reference values for AA's potential noncancer and cancer effects (see discussion above on the use of adducts to estimate AUC and daily intake, and the discussion in Section 5 on the use of these data and equations to derive reference values).

The following discussion provides a general description of the Kirman et al. (2003, 087778), Walker et al. (2007, 224527), and Young et al. (2007, 224545) PBTK models. The reader is referred to the published articles for additional detailed information on model parameters and simulation results.

3.6.1. Kirman et al. (2003, 087778) PBTK Model

A diagram of the Kirman et al. (2003, 087778) model is presented in Figure 3-3. 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 Hb in red blood cells). Different routes of exposure to AA are represented in the Kirman et al. model including i.v., i.p., 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 GSH via 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 Hb, 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 implemented in Excel.

The model parameters values and 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 (Miller et al., 1982, 061351; Ramsey et al., 1984, 067937), or to urinary metabolite levels (Miller et al., 1982, 061351; Raymer et al., 1993, 224375; Sumner et al., 1992, 224339). 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, 061351) and Ramsey et al. (1984, 067937). 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.

3.6.2. Walker et al. (2007, 224527) PBTK Model

The original Kirman et al. (2003, 087778) 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 Hb binding data in rats and humans (Bergmark et al., 1991, 224423; Boettcher et al., 2005, 224446; Fennell et al., 2003, 224295; Fennell et al., 2005, 224299; Sumner et al., 2003, 224347) were used by Walker et al. (2007, 224527) to recalibrate the Kirman et al. (2003, 087778).

Walker et al. (2007, 224527) recalibrated the Kirman et al. (2003, 087778) PBTK model based on Hb adduct data as a surrogate for serum levels of AA and GA because the formation of Hb adducts occurs as a direct function of the blood concentration of the reactive agents and 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 of 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%. Hb adduct levels, however, provide a direct measure of the total amount of parent AA and GA metabolite in the blood over a given time period, which is quantified as the AUC (in amount-unit time/volume). AUC is the integral of "concentration" (e.g., mg or mmol/L)×"time" (e.g., minutes or hours). Under the reasonable assumption that the amount of parent or reactive toxicant in blood indicates the amount available to bind to tissue

macromolecules or DNA, Hb adducts provide a more relevant internal metric to use to calibrate a PBTK model for use in estimating the risk of AA-induced toxicity.

A caveat in the use of the model developed by Walker et al. (2007, 224527) or any updated model based on the currently available studies, is that the estimated serum AUCs are directly related to the value of the second order adduct formation rate, and at present there are only three estimates of this rate, all derived from in vitro studies (Bergmark et al., 1993, 224424; Fennell et al., 2005, 224299; Tareke et al., 2006, 224387) and only one human serum study (Kopp and Dekant, 2009, 224532), with an estimated AUC normalized to administered mg/kg bw in the Kopp and Dekant (2009, 224532) study that is not consistent with the normalized AUCs reported in Fennell et al. (2005, 224299) based on the in vitro rates. There is a clear data need for accurate human in vivo second order rate constants for AA and GA Hb adduct formation and elimination. Specifically studies are needed that measure all three of the critical variables needed to resolve these rate constants – administered dose, time course serum levels, and time course adduct levels.

3.6.3. Young et al. (2007, 224545) PBTK/TD Model

Young et al. (2007, 224545) published a PBTK model developed by the U.S. FDA's 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 AA, Young et al. (2007, 224545) represented the kinetics of AA, GA, AA bound to GSH, and GA bound to GSH in separate models coupled by input and output terms with urinary excretion represented in each model (Figure 3-4). 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. (2007, 224545) 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. (2007, 224545) model for rats and mice include AA serum levels in rats from an i.p. acute exposure (Raymer et al., 1993, 224375), plasma AA and GA levels, and AA and GA Hb adduct levels following relatively high (50 mg/kg bw) repeated i.p. dosing in rats for 11 days or 2.8 mM of AA in drinking water for 47 days (Barber et al., 2001, 224414) urinary excretion profile and AA and GA Hb 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 hours)

(Sumner et al., 2003, 224347); 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 exposure 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., 2005, 224344; Doerge et al., 2005, 224348; Doerge et al., 2005, 224355). The single and multiple oral data from Barber et al. (2001, 224414) were combined with the urinary elimination data of Sumner et al. (1992, 224339; 2003, 224347) and simulated with the model. The Raymer et al. (1993, 224375) data were also combined with the urinary elimination data of Sumner et al. (1992, 224339; 2003, 224347) and simulated in a similar manner. The NCTR tissue data (Doerge et al., 2005, 224344; Doerge et al., 2005, 224348; Doerge et al., 2005, 224355) 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. Values for the human parameters were calibrated against urinary excretion data (Fennell et al., 2005, 224299; Fuhr et al., 2006, 224319) and Hb adduct data from a dietary exposure (Boettcher et al., 2005, 224446).

Values for the metabolism and elimination of AA or GA, for AA or GA binding to Hb, 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 Hb, and the binding of GA to liver macromolecule are represented as first order (i.e., rate constants of min^{-1}). Although Young et al. (2007, 224545) 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. 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 sixfold 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. (2007, 224545) model to be applicable for use in the development of toxicity values for AA, 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.

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, 2002, 224402; JIFSAN, 2004, 224420), research programs (U.S. FDA, 2009, 224492), special journal issues (*Mutation Research* vol. 580, issues 1–2, 2005) (Tornqvist et al., 2005, 224638), hazard and exposure assessments (FAO/WHO, 2005, 224279; NTP/CERHR, 2004, 224300), and internet sites (FAO/WHO, 2009, 224281; U.S. EPA, 2009, 224475) 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 MOAs.

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

Numerous case reports of occupational exposure to AA involving both inhalation and dermal exposure report neurological impairment in humans from exposure to AA, but levels of exposure are generally not measured (Auld and Bedwell, 1967, 061310; Davenport et al., 1976, 224305; Donovan and Pearson, 1987, 224425; Dumitru, 1989, 356205; Fullerton, 1969, 224325; Garland and Patterson, 1967, 061324; Gjerløff et al., 2001, 224367; Igisu et al., 1975, 061355; Kesson et al., 1977, 224568; Mapp et al., 1977, 224611; Mulloy, 1996, 224604; Takahashi et al., 1971, 061400). Substances like AA that are highly reactive with short half-lives in the blood are more challenging to monitor for estimates of exposure. AA, however, forms adducts with Hb that persist throughout the life of the adducted red blood cell (estimated at around 120 days), and Hb adducts have been used as biomarker of exposure. There are two cross-sectional health surveillance studies of AA-exposed workers that correlate AA-Hb adduct levels and measures of neurological impairment in AA workers (Calleman et al., 1994, 202900; Hagmar et al., 2001, 224453).

A quantitative human study on the toxicokinetics of AA was conducted by Fennell et al. (2005, 224299) to evaluate metabolism and Hb 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

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS).

potential for adverse effects of AA on sperm), and had not used tobacco products for the past 6 months. The study was conducted in accordance with the 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 [1,2,3-13C]-AA, and, prior to the conduct of exposures in humans, a low-dose study protocol was evaluated in rats administered 3 mg/kg [1,2,3-13C]-AA 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 electrocardiogram (ECG), vital signs (including oral temperature, respiratory rate, and automated seated pulse and blood pressure), and 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 ECGs, neurological evaluations, abbreviated physical examinations, and subjective evaluations were conducted at 4 hours after each AA administration.

No adverse events were reported in the oral phase of the Fennell et al. (2005, 224299) study. With the dermal administration, one individual was observed to have 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 (LH) 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 recent discovery of AA in foods has prompted a number of studies to evaluate a potential association between dietary AA intake and cancer. Available epidemiology studies on increased risk of cancer from AA in food include a number of case-control studies (Michels et al., 2006, 224586; Mucci et al., 2003, 224597; Mucci et al., 2004, 224598; Mucci et al., 2005,

224600; Pelucchi et al., 2006, 224364; Pelucchi et al., 2007, 224369; Wilson et al., 2009, 224535) and numerous reports from several ongoing prospective studies (Hogervorst et al., 2007, 224520; Hogervorst et al., 2008, 224521; Hogervorst et al., 2008, 224522; Larsson et al., 2009, 224466; Larsson et al., 2009, 224461; Larsson et al., 2009, 224463; Larsson et al., 2009, 224483; Larsson et al., 2009, 224484; Mucci et al., 2006, 224601). These studies evaluated Swedish, Danish, Dutch, or Italian populations; available assessment of a U.S. population is restricted to the prospective study of (Wilson et al., 2009, 224536). Some of the tumor sites observed in animal studies (thyroid, testis, central nervous system [CNS]) have also not been evaluated, and there are limitations in some of the study methods and cohort sizes.

In addition two case-control studies have examined possible associations between AA-Hb adduct levels in red blood cells and risks for breast cancer (Olesen et al., 2008, 224303) and prostate cancer (Wilson et al., 2009, 224535).

Two cohort mortality studies (Collins et al., 1989, 224284; Sobel et al., 1986, 067940) with follow-up analyses (Marsh et al., 1999, 224577; Marsh et al., 2007, 224578; Swaen et al., 2007, 224357) evaluated increased risk for cancer in AA workers.

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

An important factor in evaluating epidemiology studies that relate dietary intake to effects concerns the characterization of the variability in AA internal dose relative to differences in diet composition and consumption rates. Hagmar et al. (2005, 224457) observed relatively narrow interindividual variation in AA adduct levels, and suggest that estimates of individual dietary AA intake will need to be very precise to be useful in cancer epidemiology. Hagmar et al. (2005, 224457) evaluated variation in dietary exposure to AA relative to measurement of AA Hb 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 Hb adduct level in the randomly selected group of nonsmokers was compatible with earlier studies (0.031 nmol/g globin). The variation in the average internal dose, measured as Hb 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 globin), 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 globin) of AA adducts. Women who were smokers and had high dietary exposure to AA had significantly higher AA adduct

levels compared to women who were smokers and had low dietary exposure ($p = 0.01$), however, no significant difference was found in men who were smokers.

4.1.1. Cohort Mortality Studies

Collins et al. (1989, 224284) conducted a cohort mortality study of all male workers (8,854, of which 2,293 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 [1,295 workers]; Warners, NJ [7,153 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. AA 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 >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, CNS, 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% CIs were not reported). Trend tests showed no increased risk of mortality due to cancer at several sites (digestive tract, respiratory system, prostate, CNS, or lymphopoietic system) with increasing level of exposure to AA.

The most recent updated report (Marsh et al., 2007, 224578) of the cohort of Collins et al. (1989, 224284) includes study periods of 1925–2002 for the 8,508 workers in the three facilities in the United States, and 1965–2004 for the 344 workers at the Botlek plant in the Netherlands (the original cohort of 346 included 2 females who were excluded in the follow-up). In the Dutch cohort, deficits in deaths were reported for all sites of *a priori* interest (Marsh et al., 2007, 224578). Among the workers at the three facilities in the United States (during which 4,650 deaths occurred among the 8,508 workers in the period of 1925–2002), excess and deficit overall mortality risks were observed for cancer sites implicated in experimental animal studies: brain and other CNS (SMR 0.67, 95% CI 0.40–1.05), thyroid gland (SMR 1.38, 95% CI 0.28–4.02), and testis and other male genital organs (SMR 0.64, 95% CI 0.08–2.30); and for sites selected in

the original report (Collins et al., 1989, 224284) of this cohort: respiratory system cancer (SMR 1.17, 95% CI 1.06–1.27), esophagus (SMR 1.20, 95% CI 0.86–1.63), rectum (SMR 1.25, 95% CI 0.84–1.78), pancreas (SMR 0.94, 95% CI 0.70–1.22), and kidney (SMR 1.01, 95% CI 0.66–1.46). None of the mortality excesses were statistically significant, except for respiratory system cancer, which Collins et al. (1989, 224284) attributed to muriatic acid exposure. Table 4-1 lists all of the observed deaths and SMRs for selected causes among the U.S. workers who died between 1950 and 2002. Table 4-2 lists the SMRs from observed deaths for selected cancer sites (rectum, pancreas, and kidney) for all U.S. workers who died between 1950 and 2002, according to the following exposure parameters and categories: duration of employment (<1, 1-, and 15+ years), time since first employment (<20, 20-, and 30+ years), duration of exposure (unexposed, 0.001-, 5-, and 20+ years), cumulative exposure (<0.001, 0.001-, 0.03-, and 0.30+ mg/m^3-years), and estimated mean exposure concentrations (unexposed, 0.001-, 0.02-, and 0.3+ mg/m^3). In these exploratory exposure-response analyses of rectal, pancreatic, and kidney cancers, no statistically significantly elevated SMRs were found.

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

	1925–1994			1995–2002			1925–2002		
Cause of death (ICDA–8)[a]	**Obs**	**SMR**	**95% CI**	**Obs**	**SMR**	**95% CI**	**Obs**	**SMR**	**95% CI**
All causes (000–999):	3,557	0.93[b]	0.90–0.96	1,093	0.95	0.89–1.00	4,650	0.93[b]	0.90–0.96
All malignant neoplasms (140–209)	913	1.06	0.99–1.13	291	0.97	0.86–1.08	1,204	1.04	0.98–1.10
Buccal cavity and pharynx (140–149)	24	0.99	0.63–1.47	8	1.67	0.72–3.28	32	1.10	0.75–1.56
Digestive organs and peritoneum (150–159)	240	1.08	0.95–1.22	68	0.92	0.75–1.23	308	1.05	0.94–1.18
Esophagus (150)	32	1.27	0.87–1.79	9	1.01	0.46–1.91	41	1.20	0.86–1.63
Stomach (151)	48	1.24	0.91–1.64	8	0.99	0.43–1.95	56	1.19	0.90–1.55
Large intestine (153)	72	0.97	0.76–1.22	33	1.29	0.89–1.81	105	1.05	0.86–1.27
Rectum (154)	26	1.31	0.86–1.93	4	0.94	0.26–2.40	30	1.25	0.84–1.78
Liver (155, 156)	13	0.72	0.38–1.24	4	0.45	0.12–1.16	17	0.63	0.37–1.02
Pancreas (157)	45	1.04	0.76–1.39	9	0.62	0.28–1.18	54	0.94	0.70–1.22
Respiratory system (160–163)	369	1.19[b]	1.07–1.32	110	1.08	0.89–1.31	479	1.17[b]	1.06–1.27
Larynx (161)	15	1.24	0.70–2.05	1	0.32	0.01–1.79	16	1.05	0.60–1.71
Lung (162, 163)	354	1.21[b]	1.08–1.34	109	1.12	0.92–1.35	463	1.18[b]	1.08–1.30
Bone (170)	2	0.69	0.08–2.50	1	2.30	0.06–12.83	3	0.90	0.19–2.63
Skin (172, 173)	10	0.67	0.32–1.23	4	0.74	0.20–1.89	14	0.69	0.38–1.15
Prostate (185)	73	0.98	0.77–1.24	38	0.93	0.66–1.28	111	0.97	0.79–1.16
Testis and other male genital organs (186,	1	0.36	0.01–1.99	1	2.96	0.07–16.51	2	0.64	0.08–2.30

	1925–1994			1995–2002			1925–2002		
Cause of death (ICDA–8)[a]	**Obs**	**SMR**	**95% CI**	**Obs**	**SMR**	**95% CI**	**Obs**	**SMR**	**95% CI**
187)									
Bladder (188)	29	1.30	0.87–1.87	10	1.09	0.52–2.00	39	1.24	0.88–1.70
Kidney (189)	23	1.16	0.73–1.74	4	0.57	0.16–1.47	27	1.01	0.66–1.46
Brain and other CNS (191, 192)	15	0.69	0.39–1.15	3	0.55	0.11–1.62	18	0.67	0.40–1.05
Thyroid gland (193)	3	2.10	0.43–6.14	0	–	0.00–4.89	3	1.38	0.28–4.02
All lymphopoietic tissue (200–209)	62	0.80	0.61–1.03	22	0.74	0.47–1.12	84	0.78[c]	0.63–0.97
Lymphosarcoma and reticulosarcoma (200)	6	0.62	0.23–1.35	0	–	0.00–14.14	6	0.60	0.22–1.31
Hodgkin's disease (201)	9	1.33	0.61–2.52	1	2.13	0.05–11.85	10	1.38	0.66–2.53
Leukemia and aleukemia (204–207)	23	0.75	0.48–1.12	9	0.79	0.36–1.51	32	0.76	0.52–1.08
Other lymphatic tissue (202, 203, 208)	23	0.76	0.48–1.14	12	0.68	0.35–1.20	35	0.73	0.51–1.02
Benign neoplasms (210–239)	10	1.01	0.49–1.86	2	1.11	0.14–4.02	12	1.03	0.53–1.79
Diabetes mellitus (250)	47	0.76	0.56–1.02	37	1.18	0.83–1.63	84	0.91	0.72–1.12
Diseases of the circulatory system (390–458)	1,569	0.91[b]	0.86–0.95	383	0.78b	0.70–0.86	1,952	0.88[b]	0.84–0.92
Nonmalignant respiratory disease (460–519)	196	0.76[b]	0.66–0.87	99	0.77b	0.62–0.93	295	0.76[b]	0.68–0.85
Cirrhosis of the liver (571)	83	0.96	0.76–1.19	9	0.79	0.36–1.50	92	0.94	0.76–1.15
All external causes of death (800–998)	251	0.72[b]	0.63–0.81	20	0.77	0.47–1.19	271	0.72[b]	0.64–0.81
Unknown causes (999.9)	202			108			310		

	1925–1994			1995–2002			1925–2002		
Cause of death (ICDA–8)[a]	**Obs**	**SMR**	**95% CI**	**Obs**	**SMR**	**95% CI**	**Obs**	**SMR**	**95% CI**
People (n)		8,508			4,565			8,508	
Person-yrs		288,126			32,219			320,345	

[a]Monson life table program ICD–8 categories, labels and codes for U.S. plants for 1925–1989; corresponding rates for 1990–2001 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. (2007, 224578).

Table 4-2. Observed deaths and SMRs for selected cancer sites by duration of employment, time since first employment, and measures of exposure to AA, all U.S. workers, 1950–2002 (compared with the local male populations)

	Rectum			Pancreas			Kidney		
	Obs	**SMR**	**95% CI**	**Obs**	**SMR**	**95% CI**	**Obs**	**SMR**	**95% CI**
Duration of employment (yrs)									
<1	8	0.63	0.27–1.24	22	0.82	0.51–1.24	10	0.73	0.35–1.34
1–	13	1.25	0.66–2.13	17	0.84	0.49–1.35	9	0.87	0.40–1.64
15+	7	1.05	0.42–2.12	15	1.17	0.66–1.93	8	1.20	0.52–2.37
Time since first employment (yrs)									
<20	3	0.71	0.15–2.06	4	0.66	0.18–1.68	2	0.58	0.07–2.09
20–	5	0.79	0.26–1.83	11	0.96	0.48–1.72	4	0.65	0.18–1.66
30+	20	1.04	0.64–1.61	39	0.92	0.65–1.26	21	1.00	0.62–1.52
Duration of exposure (yrs)									
Unexposed	21	0.85	0.52–1.29	38	0.78	0.55–1.08	19	0.78	0.48–1.22
0.001–	3	1.32	0.27–3.86	6	1.12	0.41–2.43	4	1.31	0.36–3.36
5–	2	1.15	0.14–4.14	6	1.55	0.57–3.38	3	1.37	0.28–4.00
20+	2	1.96	0.24–7.07	4	1.81	0.49–4.63	1	0.88	0.02–4.88
Cumulative exposure (mg/m^3-yrs)									
<0.001	21	0.85	0.52–1.29	38	0.78	0.55–1.08	19	0.78	0.47–1.22
0.001–	1	1.43	0.04–7.98	3	1.65	0.34–4.83	1	0.88	0.02–4.92
0.03–	4	2.44	0.67–6.25	4	0.94	0.26–2.40	4	1.56	0.42–4.00
0.30+	2	0.75	0.09–2.71	9	1.71	0.78–3.25	3	1.15	0.24–3.36
Mean intensity of exposure (mg/m^3)									
Unexposed	21	0.85	0.52–1.29	38	0.78	0.55–1.08	19	0.78	0.47–1.22
0.001–	4	2.96	0.81–7.58	5	1.34	0.44–3.14	2	0.86	0.10–3.10
0.02–	0	–	0.00–1.64	5	1.11	0.36–2.60	3	1.27	0.26–3.71
0.30+	3	2.08	0.43–6.09	6	1.85	0.68–4.03	3	1.77	0.37–5.18

Source: Marsh et al. (2007, 224578).

Although an earlier update analysis (Marsh et al., 1999, 224577) of the Collins et al. (1989, 224284) cohort reported a significant 2.26-fold risk (95% CI 1.03–4.29) for pancreatic cancer among workers with cumulative exposure to AA >0.30 mg/m^3-years, the excess in the most recent update (Marsh et al., 2007, 224578) was not statistically significant (SMR 1.71, 95% CI 0.78–3.25). Marsh et al. (2007, 224578) concluded that exposure to AA at the levels reported in their study sites "was not associated with elevated cancer mortality risks." 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 54% of the cohort had died through 2002). 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, additional important information will be gathered.

Sobel et al. (1986, 067940) conducted a mortality study on a cohort of 371 workers assigned to AA 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 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 CNS, 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, 061340); however, no statistically significantly increased incidences of cancer-related deaths were observed. Mortality from cancer among the entire cohort was slightly elevated (11 versus 7.9 expected) but was lower than expected when the workers with previous exposure to the organic dyes were excluded (4 deaths versus 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, 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, 224357) provided an update of the Sobel study cohort (of 371 AA workers) and expand the cohort to include employees hired since 1979. A total of 696 AA workers were followed from 1955 through 2001 to ascertain the long-term health effects of occupational exposure to AA among production and polymerization workers, and/or the cause of death. Exposure to AA was retrospectively assessed based on personal samples from the 1970s onwards and area samples over the whole study period. The study reported that fewer of the AA

workers died (n = 141) compared to an expected number of 172.1 (SMR 81.9, 95% CI 69.0–96.6). No cause-specific SMR for any of the investigated types of cancer was exposure related. The authors report more total pancreatic cancer deaths (n = 5) than expected (n = 2.3) (SMR 222.2, 95% CI 72.1–518.5); however, three of the five were in the low dose group, with no apparent dose-response relationship with AA exposure, and thus questionable support for an AA related carcinogenicity. Although these studies provide no good evidence of a cancer risk from occupational exposure to AA at production facilities, additional studies are needed to further evaluate the potential carcinogenicity in humans from exposure to AA.

4.1.2. Case-Control Studies

No statistically significant associations were found between high consumption of foods with high (300–1,200 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, 224597) of an existing population-based case-control study (Augustsson et al., 1999, 224397). Augustsson et al. (1999, 224397) 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 versus 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 that 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, 224598) 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 versus lowest quartile] = 0.7; 95% CI = 0.4–1.1), crispbreads (OR [highest versus lowest quartile] = 1.0; 95% CI = 0.6–1.6), and fried potatoes (OR [highest versus lowest quartile] = 1.1; 95% CI = 0.7–1.7), were associated with a higher risk of renal cell cancer. There was also no association between estimated daily AA intake through diet and cancer risk (OR [highest versus 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, 224600) 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. AA intake was determined from food frequency questionnaires (FFQs) 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 AA 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, 224600) 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, 224364) 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, 1,772 controls), esophagus (395 cases, 1,066 controls), large bowel (1,394 cases of colon cancer, 886 cases of rectal cancer, 4,765 controls),

larynx (527 cases, 1,297 controls), breast (2,900 cases, 3,122 controls), ovary (1,031 cases, 2,411 controls), and prostate (1,294 cases, 1,451 controls). All of the studies included incident, histologically confirmed cancer cases and controls admitted to the same network of hospitals for acute nonneoplastic conditions. ORs were derived from multivariate logistic regression models, adjusted for energy intake and other major covariates of interest. The ORs for the highest versus 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.

Pelucchi et al. (2007, 224369) subsequently reported the results of a case-control study to investigate the relation between dietary AA intake and renal cell cancer that was conducted in four areas of Italy between 1992 and 2004. The study design was similar to that of Pelucchi et al. (2006, 224364). Incident, histologically confirmed renal cell cancer cases were 767 patients (494 men, 273 women). Controls consisted of 1,534 subjects (988 men, 546 women) matched with cases by study center, sex, and age; controls were admitted to hospitals for acute nonneoplastic conditions, which were not related to known or potential risk factors for renal cell cancer or long-term dietary modifications. ORs for increasing quartiles of total AA intake (20.4–31.2, 31.2–44.1, and >44.1 µg-day) were 1.21 (95% CI 0.94–1.57), 1.14 (95% CI 0.86–1.51), and 1.20 (95% CI 0.88–1.63), respectively, compared to the lowest quartile (<20.4 µg-day); there was no trend in risk ($p = 0.35$). The study authors stated that with respect to estimated total AA intake, risk of renal cell cancer was consistent across strata of sex and age. Estimated average AA intake was 37 µg-day. With respect to consumption of selected foods containing AA and their relative contribution to estimated total AA intake (fried/baked potatoes, 29.6%; white bread, 28.6%; sweet biscuits, 15.0%; coffee, 12.4%; crackers, 6.5%), only white bread exhibited statistically significantly elevated ORs (1.49, 95% CI 1.18–1.87; 1.70, 95% CI 1.25–2.30) for weekly portions of 7–<21 and ≥ 21, respectively. The study authors indicated that the relationship between white bread consumption and renal cell cancer might be explained by a high glycemic content and consequent effect on levels of insulin-like growth factors. It was concluded that the study results confirm the results of Mucci et al. (2003, 224597; 2004, 224598) in which there was no significant association between food items containing elevated levels of AA and risk of kidney or renal cell cancer.

Wilson et al. (2009, 224535) conducted a case-control study to assess possible associations between AA and prostate cancer risk using two measures of AA exposure: intake from FFQs and AA-Hb adduct levels in blood samples. Dietary data were available for 1,499 prostate cancer cases and 1,118 controls from a Cancer of the Prostate in Sweden (CAPS)

population-based case-control study. AA-Hb adduct levels were measured in blood samples from a subset of 170 prostate cancer cases and 161 controls. Incident cases of prostate cancer were pathologically or cytologically verified; clinical data were available for 95% of the cases in the study. Controls were randomly selected from the Swedish Population Registry and were frequency matched to cases by five-year age groups and region of residence. No significant association was found between AA exposure (as measured by FFQ or AA-Hb adduct levels) and risk of prostate cancer. The FFQ OR for the highest versus the lowest quintile was 0.97 (95% CI: 0.75–1.27), with adjustments made for age, smoking, body mass index, zinc intake and energy intake. The AA-Hb adduct OR for the highest versus the lowest quintile was 0.93 (95% CI: 0.47-1.85), with adjustment for age, region, body mass index, laboratory batch, and smoking.

Michels et al. (2006, 224586) 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 1,569 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 and 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 self-administered 30-item FFQ. 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 were used as continuous variables. For 718 nurses, complete data on the frequencies of food intake were available, but for 1,433 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. ORs 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 1,569 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 to 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 and 1950, and the questionnaire was administered in 1997. The mothers in this validation study ranged in age from 60 to 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.

Olesen et al. (2008, 224303) conducted a nested case-control study to examine associations between breast cancer and AA exposure using AA-Hb and GA-Hb adduct levels in red blood cells as biomarkers. The study design included separate analyses for endocrine receptor (ER) positive and negative (ER+ and ER–) breast cancer cases. The study included 374 breast cancer cases and 374 age-matched controls selected from a cohort of 24,697 postmenopausal women participating in the Danish Diet, Cancer, and Health prospective cohort study. Information on cancer occurrence was obtained from the Danish Cancer Registry. ER status was obtained from the Danish Breast Cancer Co-operative Group.

The median age of the cases and controls at entry into the cohort was 57 years; the median length of follow-up was 4.2 years. ER status was obtained for 348 (93%) of the breast cancer cases; 269 were reported as ER+. Mean AA-Hb and GA-HB adduct levels were 47 and 26 pmol/g globin in the 374 breast cancer cases, 47 and 28 pmol/g globin in the 374 matched controls, 48 and 27 pmol/g globin in the 269 ER+ breast cancer cases, and 40 and 23 pmol/g

globin in the 79 ER– breast cancer cases. No significant association was found between AA-Hb or GA-Hb adduct levels and total breast cancer either with or without adjusting for smoking status. However, the study authors reported a statistically significant positive association between AA-Hb adduct level and ER+ breast cancer (estimated incidence rate ratios of 4.9, 95% CI 1.2–20) per 10-fold increase in AA-Hb adduct level in smokers and 2.7 (95% CI 1.1–6.6) per 10-fold increase in AA-Hb level after adjustment for smoking.

4.1.3. Prospective Studies for Cancer

Mucci et al. (2006, 224601) 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 (the interquartile range [Q25–70] = 18.7–29.9 µg-day). 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.

Wilson et al. (2009, 224536) evaluated possible associations between AA in food and risks of breast cancer in a cohort of 90,628 registered nurses from the Nurses' Health Study II (United States) who were premenopausal, had baseline diet information, were without a diagnosis of cancer before baseline in 1991, and had plausible energy intake. FFQs with more than 130 food items (including major AA-containing foods) were completed in 1991, 1995, 1999, and 2003. Newly diagnosed cases of breast cancer were identified in biennial follow-up questionnaires. Pathology reports confirmed 98% of the self-reported breast cancers. Information on ER/progesterone receptor (PR) status, available for 916 of the breast cancer cases, indicated that 597 were ER+/PR+ and 196 were ER–/PR–.

During 14 years (945,764 person-years) of follow-up, 1,179 cases of breast cancer were identified in the cohort of 90,628 premenopausal women. The ages at breast cancer diagnosis ranged from 26 to 56 years. Mean AA intakes in the lowest and highest quintiles were 10.8 and 37.8 µg-day, respectively. The major contributors to AA intake were French fries (23%), coffee (15%), cold breakfast cereal (12%), potato chips (9%), and other potatoes (5%). Women in the highest quintile of AA consumption tended to be current smokers and were less likely to exercise than women in the lowest quintile. After adjusting for potential confounders, the authors reported no association between estimated AA intake and risk of breast cancer. The relative risk (95% CI; *p* for trend) of premenopausal breast cancer was 0.92 (0.76–1.11; $p = 0.61$) for the

highest quintile versus the lowest quintile. Results were similar regardless of ER or PR status of the tumors, smoking status, and specific AA-containing food type.

Larsson and coworkers conducted a series of prospective studies to evaluate associations between exposure to AA in food and risks of breast cancer (Larsson et al., 2009, 224463), endometrial cancer (Larsson et al., 2009, 224484), and epithelial ovarian cancer (Larsson et al., 2009, 224483) in cohorts of Swedish women (n > 61,000 in each cohort); and colorectal cancer (Larsson et al., 2009, 224466) and prostate cancer (Larsson et al., 2009, 224461) in a cohort of 45,306 Swedish men. The cohorts were cancer free at enrollment in 1987–1990, completed FFQs at baseline and again in 1997, and were followed for averages of 17.4–17.7 years. The mean daily intake of AA at baseline was 24.6 µg (±7.6, SD) in the female cohorts and 36.1 µg (±9.6, SD) in the male cohort. After adjusting for potential confounders, the authors reported no association between estimated AA intake and risk of breast, endometrial, or epithelial ovarian cancer in the female cohorts, and colorectal cancer or prostate cancer in the male cohort. Comparing extreme quartiles, the adjusted relative risk (95% CI; *p* for trend) in the female cohorts were 1.17 (0.84–1.64; $p = 0.76$) for breast cancer, 0.96 (0.76–1.21; $p = 0.72$) for endometrial cancer, and 0.86 (0.63–1.16; $p = 0.39$) for total ovarian cancer. In the male cohort, the adjusted relative risks (95% CI; *p* for trend) were 0.95 (0.74–1.20; $p = 0.69$) for colorectal cancer, 0.97 (0.71–1.31; $p = 0.78$) for colon cancer, and 0.91 (0.62–1.34; $p = 0.78$) for rectal cancer. For prostate cancer in men, compared with the lowest quintile of acrylamide intake (mean, 23.7 µg/d), the multivariable relative risks (95% confidence interval) for the highest quintile (mean, 49.8 µg/d) were 0.88 (0.70–1.09) for total prostate cancer, 1.07 (0.87–1.32) for localized prostate cancer (n = 1,088), and 0.98 (0.78–1.22) for advanced prostate cancer (n = 951).

Hogervorst and coworkers selected the Netherlands Cohort Study on diet and cancer to evaluate associations between exposure to AA in food and risks of endometrial, ovarian, and breast cancer (Hogervorst et al., 2007, 224520); renal cell, bladder, and prostate cancer (Hogervorst et al., 2008, 224521); and gastrointestinal cancer (Hogervorst et al., 2008, 224522). At baseline (1986), the participants completed a self-administered questionnaire on diet and other cancer risk factors. A case-cohort approach was used in which cases were enumerated for the entire cohort (consisting of 58,279 men and 62,573 presumed menopausal women) to provide the numerator information for estimating incidence rates and randomly-sampled subcohorts (2,589 women in the study of endometrial, ovarian, and breast cancer; 5,000 cohort members for the studies of other cancer sites) from the entire cohort at baseline to provide the denominator information for estimating incidence rates.

Hogervorst et al. (2007, 224520), identified 327, 300, and 1,835 cases of endometrial, ovarian, and breast cancer, respectively during 11.3 years of follow-up. The estimated mean daily intake of AA was 21 ± 11.9 µg-day in the subcohort. The investigators found no increased risk of breast cancer, but reported increased risks of postmenopausal endometrial and ovarian

cancer with increasing dietary AA intake, particularly among never-smokers. Comparing the lowest quintile of AA intake (mean intake 8.9 µg-day) with the highest quintile (mean intake 40.2 µg-day), adjusted hazard rate ratios (HRs) (95% CI; *p* for trend) were 0.93 (0.73–1.19; *p* = 0.79) for breast cancer, 1.29 (0.81–2.07; *p* = 0.18) for endometrial cancer, and 1.78 (1.10–2.88; *p* = 0.02) for ovarian cancer. Among never-smokers, HRs were 1.10 (0.80–1.52; *p* = 0.55) for breast cancer, 1.99 (1.12–3.52; *p* = 0.03) for endometrial cancer, and 2.22 (1.20–4.08; *p* = 0.01) for ovarian cancer.

Hogervorst et al. (2008, 224521) identified 339, 1,210, and 2,246 cases of renal cell, bladder, and prostate cancer, respectively, during 13.3 years of follow-up. The estimated mean daily intake of AA was 21.8 ± 12.0 µg-day in the subcohort. The investigators found no increased risk of bladder or prostate cancer, but reported increased risk of renal cell cancer with increasing dietary AA intake. Comparing the lowest quintile of AA intake (mean intake 9.5 µg-day) with the highest quintile (mean intake 40.8 µg-day), adjusted HRs were 0.91 (0.73–1.15; *p* = 0.60) for renal cell cancer, 1.06 (0.87–1.30; *p* = 0.69) for prostate cancer, and 1.59 (1.09–2.30; *p* = 0.04) for ovarian cancer.

Hogervorst et al. (2008, 224522) identified 2,190, 563, 349, and 216 cases of colorectal, gastric, pancreatic, and esophageal cancer, respectively, during 13.3 years of follow-up. The estimated mean daily intake of AA was 21.7 ± 12.1 µg-day in the subcohort. This study found no significant association between AA intake and risk of colorectal, gastric, pancreatic, or esophageal cancer. Comparing the lowest quintile of AA intake with the highest quintile, adjusted HRs were 1.00 (0.84–1.20; *p* = 0.94) for colorectal cancer, 1.06 (0.78–1.45; *p* = 0.77) for gastric cancer, 0.98 (0.68–1.40; *p* = 0.75) for pancreatic cancer, and 0.83 (0.54–1.30; *p* = 0.68) for esophageal cancer.

4.1.4. Cross-Sectional Neurological Evaluations

He et al. (1989, 061330) 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 (ENMG) 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 $\chi 2$ test to analyze symptoms and clinical signs and the Student's t-test to assess ENMG 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 AA workers and nonexposed workers

Symptoms	AA 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]$p < 0.01$ (χ^2 test).

Source: He et al. (1989, 061330).

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 individuals 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 Hb 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, 202900) performed a cross-sectional analysis of Hb adduct formation and neurological effects in a group of 41 factory workers (34 males and 7 females, aged 18–42 years) who were exposed to AA (and acrylonitrile, from which AA 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, 224424) who detected GA adducts of Hb in AA-exposed workers indicating that the transformation of AA to GA occurs in humans, and by Deng et al. (1993, 224326). AA 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 a referent group for ENMG 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, 224326). Physical and neurological examinations and ENMG testing were similar to those described by He et al. (1989, 061330). 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 Hb adduct formed by the reaction of AA with 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
ENMG 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 1–3 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, 202900).

Statistical analyses included the $\chi 2$ 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, 31–40, 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 10–20% decrease in conduction velocity in the peroneal and sural nerves and 25–36% 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 Hb 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 Hb 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 hr)	AAVal[c] (nmol/g globin)	ANVal[d] (nmol/g globin)	$AccD_{AA}$[e] (mM/hr)	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 AA.
[b]Urinary mercapturic acid.
[c]Hb adduct between N-terminal valine and AA.
[d]Hb adduct between N-terminal valine and acrylonitrile.
[e]Predicted cumulative in vivo AA dose (based on rates of AA-Hb adduct formation in human globin hydrolysates and mean AA exposure concentrations measured in areas of polymerization and synthesis by station sampling) (see Section 3.1 and Bergmark et al. (1993, 224424) for additional information).
[f]Neurotoxicity index.

Source: Calleman et al. (1994, 202900).

A principal finding of the study of Calleman et al. (1994, 202900) was the strong correlation between Hb adduct levels of AA 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 (Hb 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 AA.
[b]Urinary mercapturic acid.
[c]Hb adduct between N-terminal valine and AA.
[d]Hb adduct between N-terminal valine and acrylonitrile.
[e]Predicted cumulative in vivo AA dose (based on rates of AA-Hb adduct formation in human globin hydrolysates and mean AA exposure concentrations measured in areas of polymerization and synthesis by station sampling) (see Section 3.1 and Bergmark et al. (1993, 224424) for additional information).
[f]Neurotoxicity index.

Source: Calleman et al. (1994, 202900).

Hagmar et al. (2001, 224453) 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 AA and NMA. 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 Hb 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 Hb adduct data relative to neurotoxic responses to AA in the Hagmar et al. (2001, 224453) study is that both AA and NMA form the same N-(2-carbamoylethyl)valine adduct in Hb. Fennell et al. (2003, 224295) measured levels of this adduct following separate exposure to equimolar doses of AA and NMA to rats and reported formation of 21 ± 1.7 nmol/g globin from AA and 41 ± 4.9 nmol/g globin 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 Hb with AA or with NMA, conclusions about AA exposure (with adducts as the surrogate for internal exposure) versus responses are confounded by not being able to reliably distinguish the AA internal dose from the NMA internal dose in humans.

Hb 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 Hb 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 Hb adduct levels.

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

Symptoms with trend test *p*-value <0.001	Hb adducts of AA (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, 224453).

The principal findings of the study of Hagmar et al. (2001, 224453) are the positive correlations between measures of exposure (Hb 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 no-observed-adverse-effect level (NOAEL), and 0.30–1.00 nmol/g globin was the lowest-observed-adverse-effect level (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 Hb adduct (Fennell et al., 2003, 224295) that was used as an internal measure of dose.

Myers and Macun (1991, 224605) 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 (1.6–2.5 times the REL).

Bachmann et al. (1992, 224401) 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, 224605). The study design was similar to that of Myers and Macun (1991, 224605) 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, 224605) and Bachmann et al. (1992, 224401) 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., Hb adduct levels), meaningful effect levels were not established.

4.1.5. 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 nervous system and CNS (Auld and Bedwell, 1967,

061310; Davenport et al., 1976, 224305; Donovan and Pearson, 1987, 224425; Dumitru, 1989, 356205; Fullerton, 1969, 224325; Garland and Patterson, 1967, 061324; Gjerløff et al., 2001, 224367; Igisu et al., 1975, 061355; Kesson et al., 1977, 224568; Mapp et al., 1977, 224611; Mulloy, 1996, 224604; Takahashi et al., 1971, 061400). 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 AA consists of one 90-day drinking water study in F344 rats (Burek et al., 1980, 061311) 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, 224307; Johnson et al., 1984, 067926; Johnson et al., 1986, 061340).

4.2.1.1. *Subchronic Studies*

4.2.1.1.1. ***Neurotoxic effects.*** Burek et al. (1980, 061311) 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 Hb 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 Hb 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 AA in drinking water for 90 days

Endpoint	Dose (mg/kg-day)					
	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
Endpoint	**Dose (mg/kg-day)**					
	0	**0.05**	**0.2**	**1**	**5**	**20**
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, 061311).

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, 061311) 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.*

4.2.1.2.1. *Johnson et al. (1984, 067926; 1986, 061340) study.* Johnson et al. (1984, 067926; 1986, 061340) 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, Hb, 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, 067932). 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, 061311) 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.

4.2.1.2.2. ***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 versus 20% in control females and 41% in 2.0 mg/kg-day males

versus 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.

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

Endpoint	Dose (mg/kg-day)				
	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, 061340).

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., 1984, 067926; Johnson et al., 1985, 067932; Johnson et al., 1986, 061340) 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.

4.2.1.2.3. ***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), 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. Within the context of the statistical power of this study, 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 AA in drinking water for 2 years

Tumor type	Dose (mg/kg-day)				
	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, 061340) (1986).

In summary, chronic exposure of male and female F34 rats to the highest dose of 2.0 mg/kg-day of AA in water (Johnson et al., 1986, 061340) 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.

4.2.1.2.4. ***Friedman et al. (1995, 224307) study.*** A second cancer bioassay in F344 rats exposed to AA in drinking water (Friedman et al., 1995, 224307; Tegeris Laboratories, 1989, 224400) 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 (Table 4-11). Ambiguities in the Johnson et al. (1986, 061340) 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.

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

Group	Males		Females	
	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, 224307); Tegeris Laboratories (1989, 224400).

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.

4.2.1.2.5. ***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% versus 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 g versus 408 g in controls, approximately 2.5% lower) to study end (375 g versus 412 g 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.

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 AA in drinking water for 2 years

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

[a]Number of sciatic nerves (percent 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, 224307); Tegeris Laboratories (1989, 224400).

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, 224307) 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.

4.2.1.2.6. ***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 (TVM) 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 AA 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, 224393).
[b]Significantly different from control, $p < 0.05$.
[c]The data reported in Table 4 in Friedman et al. (1995, 224307) list one follicular cell adenoma in the second control group; however, the raw data obtained in the Tegeris Laboratories (1989, 224400) 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, 224307).

Table 4-14. Incidences of tumors in female F344 rats exposed to AA 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, 224393).
[b]Significantly different from control, $p < 0.001$ as reported by Friedman et al. (1995, 224307).
[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, 224307).

These findings confirm the results of the earlier Johnson et al. (1986, 061340) 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, 061340) that were not reported as being replicated in the study of Friedman et al. (1995, 224307) 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, 224307) study data, Rice (2005, 224393) 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, 224393) 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, 1994, 018474) and by the simple epoxide carcinogen, ethylene oxide (IARC, 1999, 224635). Rice (2005, 224393) concluded that the primary brain tumors were underreported in the Friedman et al. (1995, 224307) 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, 224307) report tables and discussion, and concurs with the Rice (2005, 224393) conclusion that the CNS tumors be considered one of the tumor types replicated in the Friedman et al. (1995, 224307) 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, 224628) reevaluated reproductive tissue from the 38 male rats originally diagnosed with TVMs 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, 224307) and Tegeris Laboratories (1989, 224400). Using criteria specified by McConnell et al. (1992, 224613), 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 (PCNA) 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 (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 AA 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+
	6036	Malignant mesothelioma		L+++
	606	Focal mesothelial hyperplasia	Metastasis to hepatic serosa	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++

Dose (mg/kg-day)	Rat no.	Diagnosis[a]	Evidence of metastasis or invasion	Leydig cell neoplasia[b]
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, 224307).
[b]Leydig cell neoplasms occupying 25% (+), 50% (++), 75% (+++), or 100% (++++) of testes;
– denotes no neoplasm.

Source: Iatropoulos et al. (1998, 224628).

4.2.2. Inhalation Exposure

Information on the response to subchronic 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, 1953, 224514; Hazleton Laboratories, 1954, 061399) 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 AA 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, 061399). Results of periodic blood studies (hematocrit, Hb, 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, 224514). 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, 224300)).

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

A National Toxicology Program (NTP)-sponsored expert panel (NTP/CERHR, 2004, 224300) 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, 224289; Smith et al., 1986, 224276; Zenick et al., 1986, 061394), by gavage in rats (Sublet et al., 1989, 061380; Working et al., 1987, 224542), by i.p. injection in mice (Dobrzynska et al., 1990, 224341; Ehling and Neuhäuser-Klaus, 1992, 224391; Holland et al., 1999, 224523; Nagao, 1994, 224606; Shelby et al., 1986, 094659; Shelby et al., 1987, 088819), and by dermal application in mice (Gutierrez-Espeleta et al., 1992, 224413). The NTP/CERHR (2004, 224300) report summarized that the lowest effective doses of AA 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, 224276), 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, 224289), 15 mg/kg-day for 5 days by gavage in rats (Sublet et al., 1989, 061380), 75 mg/kg i.p. in mice (single dose) (Ehling and Neuhäuser-Klaus, 1992, 224391)), and 25 mg/kg-day for 5 days applied dermally to mice (Gutierrez-Espeleta et al., 1992, 224413). 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 antifertilization effect may be due to nongenetic actions, the doses needed to elicit the antifertilization effects were generally higher than that needed to elicit the postimplantation genetic effects, and thus the antifertilization effects are of limited utility for predicting human risk.

The following discussion presents details of the oral studies, including two-generation/dominant lethal studies (Chapin et al., 1995, 224265; Tyl, 2000, 224456) and dominant lethal studies (Smith et al., 1986, 224276; Sublet et al., 1989, 061380; Tyl et al., 2000, 224459; Working et al., 1987, 224541; Working et al., 1987, 224542; Zenick et al., 1986,

061394). The results for other reproductive function endpoints are also discussed (Sakamoto and Hashimoto, 1986, 224442; Sakamoto et al., 1988, 061365; Zenick et al., 1986, 061394).

4.3.1.1. *Tyl et al. (2000, 224456) Two-Generation/Dominant Lethal Study*

Tyl et al. (2000, 224456) 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, analysis of variance (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 three high-dose females and two 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.

AA 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 and 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.

Table 4-16. Changes in reproductive parameters in F344 rats exposed to AA 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)				
Number of males impregnating	17	24	22	21
Number of females pregnant	20	24	26	18
Number of implantations/dam	10.4 ± 2.5	10.0 ± 3.6	10.2 ± 2.2	6.8 ± 3.1[a]
Number of 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)				
Number of males impregnating	23	25	25	27
Number of females pregnant	23	25	27	23
Number of implantations/dam	11.3 ± 1.5	10.0 ± 3.4	10.5 ± 2.1	6.8 ± 3.3[a]
Number of 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. (2000, 224456).

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.

4.3.1.2. *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.

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

Parameter	AA dose (mg/kg-day) in the drinking water			
	0.0	0.5	2.0	5.0
Number of males paired	30	30	30	30
Number of females paired	60	60	60	60
Number of fecund males[a]	29 (96.7%)	30 (100.0%)	30 (100.0%)	30 (100.0%)
Number of fertile males[b]	28 (93.3%)	29 (96.7%)	29 (96.7%)	29 (96.7%)
Number of plug- or sperm-positive females	57 (95.0%)	56 (93.3%)	59 (98.3%)	57 (95.0%)
Number of 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%)
Number of corpora lutea/dam	11.8 ± 2.1[d]	11.5 ± 1.1	11.8 ± 1.1	11.4 ± 1.2
Number of 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. (2000, 224456).

4.3.1.3. *Chapin et al. (1995, 224265) Two-Generation/Dominant Lethal/Grip Strength Study*

Chapin et al. (1995, 224265) 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 one 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 one 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 $p < 0.05$.

AA 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, versus 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.

AA 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 versus 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 g versus 37.7 ± 0.9 g in controls).

4.3.1.4. *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 (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 AA in the drinking water

	AA 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, 224265).

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/g 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/g 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. AA 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. AA treatment did not increase the incidence of grossly visible lesions or histopathologic findings in examined nerve tissues of male or female F1 parental mice.

4.3.1.5. *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 (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 AA in drinking water on grip strength of mice

	AA 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 (g)[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 wks of treatment (mean ± SEM, n = 10).
[b]Grip strength measured at F1 parental treatment wk 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, 224265).

In summary, the results presented by Chapin et al. (1995, 224265) identified 30 ppm AA 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.

4.3.1.6. *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, 224276). 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 χ^2 statistics. Effects on pre- and postimplantation loss were analyzed using Kruskal-Wallis ANOVA with Mann-Whitney U-test for posthoc 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 AA 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, 224276).

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, 224542; 1987, 224541) 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, 061380) 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% versus 73% in controls) at week 3 but not at weeks 2 or 4. Sublet et al. (1989, 061380) 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. (2000, 224459) observed significantly decreased fertility and increased postimplantation losses following mating of untreated female rats with males that had been administered AA at 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.

4.3.1.7. *GA 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, 224322) administered ABT, an inhibitor of CYP450 metabolism, to reduce the levels of the epoxide GA. 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, 224300) 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. (2005, 224351), 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. (2005, 224354) 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.

4.3.1.8. *Other Reproductive Function Studies*

4.3.1.8.1. ***Zenick et al. (1986, 061394) reproductive function study.*** Zenick et al. (1986, 061394) 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)[7] 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

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

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 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 AA in the drinking water

Parameter	AA concentration (ppm)		
	0 (n = 15)	50 (n = 15)	100 (n = 11)[a]
Sperm count (× 10^6)			
Baseline	46 ± 12[b]	45 ± 19	43 ± 14
Wk 9	56 ± 18	36 ± 23	14 ± 20[c]
Sperm motility (%)			
Baseline	43 ± 9.1	39 ± 9.2	41 ± 6.3
Wk 9	41 ± 11.3	46 ± 11.2	[d]
Sperm morphology (% normal)			
Baseline	96 ± 2.7	96 ± 2.3	95 ± 1.8
Wk 9	94 ± 3.6	96 ± 2.0	[d]
Seminal plug weight (mg)			
Baseline	115 ± 20	100 ± 38	111 ± 20
Wk 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-min trial.
[b]Mean ± SD.
[c]Significantly different from control, $p < 0.05$.
[d]Sperm recovered from the uterus of only one 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 wk 6 due to high mortality.

Source: Zenick et al. (1986, 061394).

In a female reproduction assessment phase, Zenick et al. (1986, 061394) 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, 061394) 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).

4.3.1.8.2. ***Sakamoto and Hashimoto (1986, 224442) reproductive function study.*** Sakamoto and Hashimoto (1986, 224442) 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.

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

Effects observed following 13 d 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 d of delivery				
Treatment (mM)	**Calculated dose (mg/kg-day)**	**Fertility rate**	**Number of offspring/dam**	**Offspring body weight (g)**
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)**	**Fertility rate**	**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]$p < 0.05$ versus 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, 224442).

The results identify 0.6 mM AA (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, 224442). 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.

4.3.1.8.3. ***Sakamoto et al. (1988, 061365) histology of testicular lesions.*** Sakamoto et al. (1988, 061365) 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 mice 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 AA. 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 AA. 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 AA in either age group. Histologic abnormalities in the testes of prepubertal animals treated with 150 mg/kg AA 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, 224542; 1987, 224541) 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, 061380) 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% versus 73% in controls) at week 3 but not at weeks 2 or 4. Sublet et al. (1989, 061380) 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. (2000, 224459) observed significantly decreased fertility and increased postimplantation losses following mating of untreated female rats with males that had been administered AA at 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, 024984) 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 (Field et al., 1990, 224302; Wise et al., 1995, 224539; Zenick et al., 1986, 061394) and mice (Field et al., 1990, 224302) and neurobehavioral changes in the offspring of female Sprague-Dawley rats exposed on GDs 6–10 to 15 mg/kg-day, but not to 10 mg/kg-day (Wise et al., 1995, 224539) and in adolescent F344 rats exposed during gestation and lactation and extending through 12 weeks of age at an average dose of 6 mg/kg-day, but not at 1.3 mg/kg-day (Garey and Paule, 2007, 224337). 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, 224302). 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., 1999, 224311). The results of these studies are summarized in Section 4.7.1, and discussed below, except for the Zenick et al. (1986, 061394) 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).

4.3.2.1. *Field et al. (1990, 224302) Developmental Toxicity Study—Gestational Exposure*

Field et al. (1990, 224302) administered AA (in distilled water) to groups of timed-mated Sprague-Dawley rat dams (29–30/group) in 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). AA 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 gavage administration of AA 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
Percentage of resorptions/litter	3.5 ± 1.1	5.5 ± 1.5	11.7 ± 3.9	3.4 ± 1.6
Percentage of litters with resorptions	32.1	46.2	58.6[c]	24.0
Mean male fetal bw (g)/litter	1.05 ± 0.02	1.03 ± 0.02	1.02 ± 0.01	0.89 ± 0.02[c]
Mean female fetal bw (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, 224302).

4.3.2.2. *Wise et al. (1995, 224539) Developmental Neurotoxicity Study—Gestational Exposure*

Wise et al. (1995, 224539) 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, 224539). 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.

4.3.2.3. *Husain et al. (1987, 061336) Developmental Neurotoxicity Study—Lactational And Postnatal Exposure*

Husain et al. (1987, 061336) 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.

4.3.2.4. *Friedman et al. (1999, 224311) Developmental Neurotoxicity Study—Lactational Exposure*

Friedman et al. (1999, 224311) administered AA to female Wistar rats (15/group) with litters at 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, χ^2 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 g, whereas a net mean weight gain of 47 g 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.

4.3.2.5. *Garey and Paule (2007, 224337) Developmental Neurotoxicity Study—Exposure During Gestation, Lactation, and through 12 Weeks of Age*

Garey and Paule (2007, 224337) evaluated performance in an operant test of cognitive motivation in adolescent male and female F344 rats exposed to AA during gestation, lactation, and through 12 weeks of age. Pregnant F344 rats were exposed by gavage throughout gestation

to AA doses of 0, 0.1, 0.3, 1.0, or 5.0 mg/kg-day. On PNDs 1–22, offspring received the same gavage dose received by their dam. On PNDs 22–85, offspring were provided AA in drinking water at concentrations (0, 1, 3, 10, and 50 ppm) designed to maintain similar administered doses (based on an assumed drinking water intake of 10% of bw-day). Average daily doses between PNDs 40 and 85 are estimated at approximately 0, 0.2, 0.5, 1.6, or 7.8 mg/kg-day, from reported average water intake values for offspring between PNDs 40 and 85 (15.5% of bw-day) and the reports that average water intakes for high-dose offspring between PND 40 and 50 was about 20% of bw-day and average daily AA dose was 20 mg/kg-day during this period. Offspring (one male and one female/litter; eight or nine/sex/exposure group) were trained between 3 and 6 weeks of age to operate levers to obtain food pellets. Offspring were subsequently evaluated in a progressive ratio task of motivation to obtain food in 14 10-minute sessions distributed between weeks 6 and 12 of age. In each session, food was obtained with a progressively greater number of actions. For example, the first food pellet ("reinforcer") was obtained after one press of the lever; the second pellet was obtained after two presses; and the third with three presses. Measured behavioral endpoints were number of food pellets obtained per session (i.e., "number of food reinforcers earned"), post-reinforcement pause (average duration from reinforcer delivery to the next lever press), and response rate (number of presses/second over the entire 10-minute test session). No significant ($p < 0.05$) differences were found between control and exposed groups in drinking water intake or body weight throughout the testing period. Offspring in the high-dose group showed significant ($p < 0.05$) decreases, compared with control values, in average reinforcers earned per session (5.5 ± SEM 0.2 versus 7.2 ± SEM 0.2), and response rate (0.041 ± SEM 0.002 versus 0.065 ± SEM 0.003). No significant changes in these endpoints, compared with controls, were found in the other exposure groups. No significant effect of exposure was found on post-reinforcement pause in any exposure group. The high dose in this study, (about 6 mg/kg-day—an average of the gavage dose and the estimated drinking water dose during testing) is a LOAEL for effects on measures of cognitive motivation; the NOAEL is 1.3 mg/kg-day.

4.3.2.6. *Other Developmental Toxicity Studies*

Genotoxic effects observed in the germ cells of mice following i.p. injection of 125 mg/kg AA included a weakly positive result for sperm head DNA dealkylation and a positive result for sperm head protamine alkylation (Sega et al., 1989, 224477). 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, 224442).

Edwards (1976, 224385) 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, 224385), 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 g/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 g pregnant rat, 12 g/rat-day feed containing 400 ppm AA represents a daily dose of 16 mg/kg-day). Fetal weights were reduced by AA treatment (AA 2.4 ± 0.05 g, control 3.2 ± 0.05 g, mean ± SEM). (The *p*-value reported by the authors using the Student t-test was >0.2; however, the t-test performed by the Center for the Evaluation of Risks to Human Reproduction [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, 224385) 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, 224439) administered AA in the feed to female Sprague-Dawley CD rats at 0, 25, or 50 ppm for 2 weeks prior to mating, and on GDs 0–19. AA 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, or pup weights. Albert Einstein College of Medicine (1980, 224363) 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, 061391) 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, as well as 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 AA-treated or control dams during postnatal lactation

Intestinal enzyme	Effect[a]	PND		
		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, 061391).

A study by Rutledge et al. (1992, 224429) 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, 224529) 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

Five heritable translocation studies (Adler, 1990, 224296; Adler et al., 1994, 224314; Adler et al., 2004, 224343; Generoso et al., 1996, 224346; Shelby et al., 1987, 088819) and two specific mouse locus mutagenicity assays (Ehling and Neuhäuser-Klaus, 1992, 224391; Russell et al., 1991, 224406) are available. These studies all found positive results following exposure of male mice to 40–100 mg/kg i.p. doses of AA, but do not provide information for possible effects at lower exposure levels. No experiments have studied the potential for AA to induce heritable germ cell effects in the female germ line. The heritable germ cell effects in male mice are consistent with the extensive evidence supporting dominant lethal effects in male murine test animals. In addition, there are two reports of increased incidence of male-mediated stable chromosomal aberrations (CAs) in two-cell mouse embryos following exposure of male mice to 50 mg/kg AA and mating to unexposed females (Marchetti et al., 1997, 224573; Marchetti et al., 2009, 224574). These studies found correlations between stable CAs and the percentages of offspring with reciprocal translocations.

The seven heritable germ cell studies in mice are briefly discussed below, and the results, as tabulated by Favor and Shelby (2005, 224283), are included in Tables 4-25, 4-26, and 4-27. These studies are also listed in Appendix B, Table B-1 summarizes the mutagenicity assay results. Recent reviews and discussions regarding the results of available heritable germ cell studies for AA include Besaratinia and Pfeifer (2007, 224436), Carere (2006, 224267), Exxon (2006, 224398), Shipp et al. (2006, 224488), Favor and Shelby (2005, 224283), and NTP/CERHR (2004, 224300).

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

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, 224346)
	–	9,890[e,f]		5 (0.05)[f]		Adler et al. (2002, 224336)
50 AA i.p.	7–16	362[f]		2 (0.55)[f]		Adler et al. (1994, 224314)
100 AA i.p.	7–16	367[f]		10 (2.72)[f]		Adler et al. (1994, 224314)
100 GA i.p.	3.5–7.5	669		135 (20.17)		Generoso et al. (1996, 224346)
5×40 AA i.p.	7–10	162		39 (24.07)		Shelby et al. (1987, 088819)
5×50 AA i.p.	7–10	125		49 (39.20)		Shelby et al. (1987, 088819)
5×50 AA i.p.	7–11	57	48	17 (29.82)	6 (12.5)	Adler (1990, 224296)
5×50 AA i.p.	36–42	556	449	2 (0.36)	0 (0)	Adler (1990, 224296)
5×50 AA dermal	1.5–8.5	258	217	28 (10.85)	13 (5.99)	Adler et al. (2004, 224343)

[a]5×40 and 5 ×50 represent 40 or 50 mg AA/kg on 5 consecutive d.
[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, 088819) and Generoso et al. (1996, 224346).
[e]Laboratory historical control used for statistical comparisons of the translocation frequencies reported by Adler (1990, 224296) and Adler et al. (1994, 224314; 2004, 224343).
[f]Both male and female F1 animals were tested but not reported separately.

Source: Favor and Shelby (2005, 224283).

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

Mating interval (d posttreatment)	Number of offspring	Number of mutations[a]
1–7	113	0 (0)
8–14	1,506	2 (0.13)
15–21	5,077	1 (0.02)
22–28	5,191	0 (0)
29–35	5,312	0 (0)
36–42	5,353	1 (0.02)
43–49	6,419	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. (1991, 224406); table from Favor and Shelby (2005, 224283).

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

Dose (mg/kg)	Mating interval (d posttreatment)	Number of offspring	Number of mutations[a]
Historical control	–	248,413	22 (0.01)
100	1–4	1,362	0 (0)
	5–8	2,226	1 (0.04)
	9–12	2,421	2 (0.08)
	13–16	2,453	0 (0)
	17–20	2,574	0 (0)
	21–42	2,925	0 (0)
	>42	23,489	6 (0.03)
125	1–4	771	0 (0)
	5–8	1,924	2 (0.10)
	9–12	1,948	1 (0.05)
	13–16	2,419	0 (0)
	17–20	2,598	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 d 21 to assay for effects on spermatogonia (i.e., for effects ≥ 43 d posttreatment).

Sources: Data from Ehling and Neuhäuser-Klaus (1992, 224391); table from Favor and Shelby (2005, 224283).

4.4.1. Heritable Translocation Studies

Shelby et al. (1987, 088819) 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 17/8,095 in the historical control. All 10 of the semi-sterile males sampled from the 5×50 treatment for cytogenetic analysis of spermatocytes had translocations.

Adler (1990, 224296) administered AA 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 versus female, $p < 0.05$). In the second mating interval (36–42 days after treatment), 1,005 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, 224314) administered AA 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, 5/9,890. Clustering was not apparent, as indicated by the fact that all translocations were unique.

Adler et al. (2004, 224343) 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, 224296). The calculated ratio of end effects in this study of i.p. vs. dermal exposure is 0.39.

Favor and Shelby (2005, 224283) summarized the cytogenetic analysis from the Adler et al. (1994, 224314; 2004, 224343) and Adler (1990, 224296) 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 carried a translocation among three chromosomes plus a reciprocal translocation between two chromosomes.

Generoso et al. (1996, 224346) (1996) administered a single i.p. dose of GA 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.

4.4.2. Specific Locus Studies

Russell et al. (1991, 224406) 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). AA 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 showed that 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 CA-type events (deletions and/or translocations), and AA is not mutagenically active in stem cell spermatogonia. Russell et al. (1991, 224406)) 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, 224391) 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, 224391) 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, 224406) 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, 224406), 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, 224391) observed a significant increase in the frequency of specific-locus mutations following exposure of spermatogonia to AA. Favor and Shelby (2005, 224283) reevaluated the mating intervals to more directly compare the results and noted that in the results of Russell et al. (1991, 224406) 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, 224391) 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, 224406) (higher total accumulated dose, 50 mg AA/kg on 5 consecutive days) and Ehling and Neuhäuser-Klaus (1992, 224391) (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, 224391). One mutation resulted from a conception 43 days posttreatment and represented an exposure at the differentiating spermatogonial stage. Russell et al. (1991, 224406) 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, 224391) all had conceptions much later (70, 181, 201, 234, and 436 days posttreatment) and represented exposures of stem-cell spermatogonia.

4.4.3. Synthesis and Evaluation of Heritable Germ Cell Effects

Available heritable germ cell studies clearly demonstrate AA-induced heritable germ cell effects. These findings are of particular concern because the human relevance has not been determined. In the absence of experimental data from which to assess the potential for AA to act as a human germ cell mutagen, the animal data must be considered potentially relevant to humans.

Animal studies did not include adequate assessment of dose-response relationships for the heritable germ cell effects, and doses employed in the various studies were relatively high. Single or repeated i.p. doses of AA or GA ranged from 40 to 125 mg/kg and one study employed five daily dermal applications of AA at 50 mg/kg. Heritable translocations appeared at high frequency at the lowest doses tested, which indicates that lower doses may have also elicited heritable translocations. Well-designed animal studies are needed to assess dose-response relationships for AA- and GA-induced heritable germ cell effects, particularly in the low dose region that is expected to be more relevant to human exposure.

The results of each of the above-described heritable germ cell studies suggest that AA and/or GA act as clastogenic agents (Favor and Shelby, 2005, 224283). Possible mechanisms involved include (1) covalent modifications of protamines associated with DNA by AA or GA, and (2) direct alkylation of DNA by GA or a combination of both MOAs (Besaratinia and Pfeifer, 2004, 224427; Besaratinia and Pfeifer, 2007, 224436; Carere, 2006, 224267; Dearfield et al., 1995, 224315; Doerge et al., 2005, 224344; Moore et al., 1987, 224589; Schmid et al., 1999, 224458; Segerbäck et al., 1995, 224485).

Limited information is available regarding specific mechanisms of AA-induced heritable germ cell effects in laboratory animals. Demonstrations that GA binds more strongly than AA to DNA and some indication that the genetic damage in germ cells of mice is dependent on metabolism of AA to GA by CYP2E1 (Ghanayem et al., 2005, 224354) led Carere (2006, 224267) to suggest that GA-DNA adducts may be responsible for gene mutations observed in the laboratory animal germ cell studies.

Sega et al. (1989, 224477) 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. The involvement of protamine binding as a mechanism of AA-induced heritable translocations is

suggested because both AA-induced dominant lethal mutations and heritable translocations appear to be late-stage germ cell effects and because AA has been shown to exert effects on the synaptoneural complex and on the spindle. Furthermore, AA exhibits a relatively stronger binding to proteins than does GA. Based on these observations, Carere (2006, 224267) suggested that AA-protamine binding may explain some chromosomal effects in germ cells.

It is critical to determine mechanisms whereby AA induces heritable germ cell effects and the critical germ cell stages at which the heritable germ cell effects occur because, as Favor and Shelby (2005, 224283) 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, 061323) and Tilson and Cabe (1979, 224408) 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, 224365) 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, 224335) 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, 061347). Fullerton and Barnes (1966, 061323) 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, 224592). AA 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, 061325; Hashimoto et al., 1981, 061328), cats (McCollister et al., 1964, 061347; Post and McLeod, 1977, 224374), dogs (Hersch et al., 1989, 061331; Satchell and McLeod, 1981, 061368), and monkeys (Eskin et al., 1985, 224376; Maurissen et al., 1983, 061346; McCollister et al., 1964, 061347).

4.5.2. Other Cancer Studies

The potential of AA 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., 1984, 202896). AA was dissolved in distilled water for oral and injection routes and in ethanol for dermal applications. AA 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 4-28.

Table 4-28. AA 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
i.p.	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 wks with acceptable nonautolyzed tissues.
bSignificantly different ($p < 0.05$) from the vehicle initiation/TPA promotion group by Fisher's exact test.

Source: Bull et al. (1984, 202896).

Incidences were also reported for the number of skin tumor-bearing mice/total mice in each group (Bull et al., 1984, 202896). 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 4-29.

Table 4-29. AA initiation of skin tumor masses >1 mm 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
i.p.	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. (1984, 202896).

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 at oral doses of 0, 12.5, 25, or 50 mg/kg-day, 3 times/week for 2 weeks (Bull et al., 1984, 202897). Two weeks later, 2.5 µg TPA in acetone was applied to the shaved backs, 3 times/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 (an asterisk indicates significantly difference [$p < 0.05$] 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., 1984, 202897). 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., 1984, 202897). 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. (1984, 202896) 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. AA 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/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. AA 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 (an asterisk 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. (1984, 202896) 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, 224403) 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 3 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, 224403) 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.

Jin et al. (2008, 224426) assessed the potential for AA to induce thyroid tumors in female CD1 mice administered AA in drinking water. The study comprised both short-term (2-month) and longer-term (up to 8 months) experiments. Each experiment included six groups of mice (20–30/group); groups 1–3 received drinking water without AA and groups 4–6 were exposed to AA in the drinking water. In addition, groups 2 and 5 received thyroxine in the drinking water to depress activity of the thyroid and groups 3 and 6 received methimazole that causes thyroid activation. Concentrations of AA in the drinking water were adjusted to deliver AA at an intended dose of 3 mg/kg-day, although the concentration was increased during the later portion of the longer-term experiment. The exposures designed to alter activity of the thyroid produced the intended results. In the longer-term experiment, mice receiving thyroxine treatment with or without AA exposure died or were sacrificed moribund after 6 months. Peripheral neuropathy was noted in mice receiving AA in the longer-term experiment, particularly later in the experiment when AA concentrations were increased. There were no indications of AA-induced effects on thyroid weights or thyroid tumorigenesis, including those mice with hyper- or hypo-stimulated thyroids. Although the AA in the drinking water of the mice resulted in AA doses comparable to those associated with thyroid tumors in rats treated for 2 years (Friedman et al., 1995, 224307), the mice were only treated and examined for up to 8 months (Jin et al., 2008, 224426).

Ølstørn et al. (2007, 224309) assessed the tumorigenicity of subcutaneous administration of AA or GA during early perinatal periods in the intestine of C57BL/6J Min/+ mice and their wild type. The Min/+ mice are heterozygous for a mutation in the tumor suppressor gene (Apc), which leads to the development of multiple intestinal neoplasms, particularly in the small intestine. The study consisted of two experiments. In the first experiment, Min/+ and wild type mice were subcutaneously injected with AA or GA (0 , 10, or 50 mg/kg-bw) at 1 and 2 weeks postpartum and sacrificed after 8 (Min/+) or 32 (wild type) weeks. Respective numbers of mice included in the control though high-dose AA groups were: 19, 19, and 17 for Min/+ mice, and

31, 12, and 24 for WT mice. Respective group sizes for the low- and high-dose GA groups were: 26 and 21 for Min/+ mice and 24 and 24 for WT mice. At the 50 mg/kg dose level, GA (but not AA) induced significantly ($p < 0.05$) increased number of small intestinal tumors per mouse in the Min/+ mice (1.3-fold increase compared with control value) and significantly ($p < 0.05$) increased incidence of WT mice with intestinal neoplasms (25 vs. 3% in controls). In the second experiment, Min/+ and wild type mice were exposed to AA or GA either in utero via single subcutaneous injections of their dams at 1 week prior to birth, postpartum subcutaneous injections at 1 and 2 weeks, or both. In the second experiment, the number of small intestinal and colonic tumors per mouse in the Min/+ mice correlated positively with the number of GA injections. The results indicate that early life subcutaneous exposure of Min/+ and WT C57BL/J6 mice to 50 mg/kg GA, but not 50 mg/kg AA, elicited tumorigenic responses in the intestine. The tumorigenic response to GA, but not to AA, in the first experiment of this study could potentially be explained by relatively low expression of CYP2E1 (and thus low capability to convert AA to GA) during the early life exposure period.

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

4.6.1. Studies on the Hypothalamus-Pituitary–Thyroid Axis

Both of the available chronic oral exposure studies for AA in F344 rats 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, 224307; Johnson et al., 1986, 061340). 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 thyroid stimulating hormone (TSH) from the pituitary, which, if sustained, may induce thyroid follicular cell hyperplasia that may progress to neoplasia (U.S. EPA, 1998, 030018). These findings have led to several investigations of effects of AA on hypothalamus-pituitary-thyroid axis endpoints. To date, there is no clear and consistent 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 T_4 or TSH (Khan et al., 1999, 224565). 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, 224565).

In an unpublished study, blood levels of T_3, T_4, 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 4-30) (Friedman et al., 1999, 224381). A significant decrease in T_3 and T_4 in high dose males is reported at 28 days, but T_4 in high-dose males increased at 14 days, and overall, there is inadequate support for a consistent, significant change in blood levels of T_3, T_4, or TSH.

Table 4-30. Circulating thyroid hormone levels in F344 rats following exposure to AA in drinking water for 14 or 28 days

Dose (mg/kg-day)		T_3 (ng/dL)		T_4 (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. (1999, 224381).

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 (EIC, 2002, 224394; Klaunig, 2000, 594245 as cited in EIC 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 EIC, 2002, 224394) are shown in Table 4-31. 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.

Table 4-31. Plasma TSH, bromodeoxyuridine (BrdU) incorporation in thyroid, and PCNA expression in thyroid in male Sprague-Dawley rats exposed to AA 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 EIC (2002, 224394).

Source: Klaunig (2000, 594245) as cited in EIC (2002, 224394).

Bowyer et al. (2008, 224470) examined a number of endpoints indicative of disruption of the hypothalamus-pituitary-thyroid axis in male F344 rats (70 days of age) exposed for 14 days to AA in drinking water delivering nominal doses of 0, 2.5, 10, or 50 mg/kg-day. Based on twice weekly measurement of body weight and water intake, average measured doses for the 2.5-, 10-, and 50-mg/kg-day groups were 93–100, 99–100, and 85–88% of nominal values. The following endpoints were evaluated: (1) expression of genes related to thyroid hormone production and cell proliferation in the hypothalamus, thyroid, and pituitary (using cDNA array and RT-PCR analysis after isolation of total RNA from tissues from 20 rats per group); (2) levels of neurotransmitters (and metabolites) in the brain and pituitary (from 10 rats per group) that affect hormone homeostasis (dopamine, 3-methoxytyramine [3MT], homovanillic acid [HVA], 3,4,-dihydroxyphenylacetic acid [DOPAC]), serotonin, 5-hydroxytryptamine [5HT], and 5-hydroxyindoeacetic acid [5HIAA]); (3) serum levels of pituitary and thyroid hormones (from 10 rats/groups): TSH, T4, T3, and thyrotropin-releasing hormone (TRH); and (4) histology of pituitary and thyroid glands. The low- and mid-dose groups showed no obvious effect on locomotory activity or body weight gain. The high-dose group showed clear signs of locomotor impairment (lethargy and hind limb paralysis) and decreased body weight (92–93% of control values). No clear exposure-related effects were found on levels of dopamine and its metabolites (DOPAC, 3MT, and HVA) or 5HT and 5HIAA in the hypothalamus or pituitary. AA exposure caused a significant ($p < 0.05$) decrease in serum T4 only at the high dose, but had no effect on serum T3 or TSH. Exposed and control rats showed no difference in response to a challenge dose of TRH increase in serum levels of TSH and T4, 30 minutes following a challenge i.p. dose

of 2.5 mg/kg TRH. No significant exposure-related effects were found on mRNA levels in hypothalamus or pituitary for TRH, TSH, or thyroid hormone receptor α and β. Control and high-dose rats showed no significant changes in mRNA levels for other important pituitary hormones including growth hormone, opiomelancorticotropin, vasopressin, and LH. Hematoxylin- and eosin-stained sections of thyroid and pituitary tissue from control and high-dose rats showed no evidence for exposure-related changes in cell morphology (i.e., hypertrophy, hyperplasia, karyomegaly, or degeneration). Indices for cell proliferation in pituitary and thyroid tissues (Mki67 mRNA and ki-67 protein levels) were not increased in exposed rats, compared with controls. Expression of genes in the thyroid, which are typically increased in response to anti-thyroid effects (e.g., thyroglobulin, thyroid peroxidase), were not significantly increased in high-dose rats, compared with controls. This study found no evidence that 14-day exposures of F344 rats to oral AA doses of 2.5, 10, or 50 mg/kg-day disrupt the hypothalamus-pituitary-thyroid axis. Bowyer et al. (2008, 224470) concluded that these negative findings are important to understanding AA's MOA in producing thyroid tumors in rats with chronic oral AA exposure (Section 4.2.1.2), because chronically elevated TSH levels with resultant thyroid follicular cell hyperplasia are strongly associated with exposure to other compounds that induce rodent thyroid tumors by a nongenotoxic mechanism.

4.6.2. Genotoxicity Studies

Appendix B (Table B-1) summarizes results of numerous published mutagenicity tests for AA 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, 224322; Shelby et al., 1987, 088819), oral exposure of mice (Chapin et al., 1995, 224265; Sakamoto and Hashimoto, 1986, 224442), or rats (Smith et al., 1986, 224276; Sublet et al., 1989, 061380; Tyl, 2000, 224456; Tyl et al., 2000, 224459; Working et al., 1987, 224541; Working et al., 1987, 224542; Zenick et al., 1986, 061394), and dermal exposure of mice (Gutierrez-Espeleta et al., 1992, 224413) 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.[8] Heritable germ cell studies in male mice were consistently positive for heritable translocations (Adler, 1990, 224296; Adler et al., 1994, 224314; Adler et al., 2004, 224343; Generoso et al., 1996, 224346; Shelby et al., 1987, 088819) and specific mouse locus (Gutierrez-Espeleta et al., 1992, 224413; Russell et al., 1991, 224406). 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 4.4.

[8] It is further acknowledged that male-mediated dominant lethal effects can be mediated by effects on altered male mating performance and sperm motility and/or morphology, as well as effects on genetic integrity of the sperm (Perreault, 2003, 224370).

Manjanatha et al. (2006, 224572) 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 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 ± 0.3] × 10–6 and [2.2 ± 0.5] × 10–6 in females and males, respectively). The high doses of AA and GA also produced significant 2–2.5-fold increases in liver cII MFs ($p \leq 0.05$; control MFs = [26.5 ± 3.1] × 10–6 and [28.4 ± 4.5] × 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. (2005, 224354) 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 AA (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 MN 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., 2005, 224351) (discussed in Section 4.3.1).

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 CAs in bone marrow cells yielded both positive (Adler et al., 1988, 224301; Cihák and Vontorková, 1988, 224270)) and negative (Krishna and Theiss, 1995, 224525; Shiraishi, 1978, 224490) results. In one study, male B6C3F1 mice were administered deionized water (control) or AA at doses ranging from 0.125 to 24 mg/kg-day via gavage for 28 days and evaluated for MN response in bone marrow cells [reticulocytes (RETs) and normochromatic erythrocytes (NCEs)] by flow cytometry (Zeiger et al., 2009, 224546). AA significantly ($p < 0.05$) induced MN at ≥ 4 mg/kg-day in RETs, and ≥ 6 mg/kg-day in NCEs. MN were not induced at lower doses of AA (0.125–2 mg/kg-day).

Similar assays of mouse spleen lymphocytes, splenocytes, and spermatogonia were all negative for CAs (Adler, 1990, 224296; Adler et al., 1988, 224301; Backer et al., 1989, 224404; Kligerman et al., 1991, 006236). Significant increases in CAs were observed in spermatocytes of mice that had been administered an i.p. dose of 100 mg/kg (Adler, 1990, 224296), 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, 224458). Consistent with AA induction of CAs in sperm, the frequency of zygotes with CAs 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, 224573). Tests were positive for early cleavage stages of mouse zygotes (Pacchierotti et al., 1994, 224316) and embryos (Valdivia et al., 1989, 224501), positive for polyploidy or aneuploidy (Shiraishi, 1978, 224490), and negative for spindle disturbances (Adler et al., 1993, 224310) in mouse bone marrow cells.

AA-induced increases in MN were seen in bone marrow cells, reticulocytes, spleen lymphocytes, and splenocytes of mice and spermatids of rats and mice (Adler et al., 1988, 224301; Backer et al., 1989, 224404; Cihák and Vontorková, 1988, 224270; Cihák and Vontorková, 1990, 224271; Collins et al., 1992, 224280; Kligerman et al., 1991, 006236; Knaap et al., 1988, 224547; Lähdetie et al., 1994, 224500; Paulsson et al., 2002, 224334; Russo et al., 1994, 224409; Xiao and Tates, 1994, 224543; Zeiger et al., 2009, 224546) but not in rat bone marrow cells (Krishna and Theiss, 1995, 224525; Paulsson et al., 2002, 224334). 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, 224404). Tests for heritable translocations and reciprocal translocations in male mice yielded positive results (Adler et al., 1994, 224314; Shelby et al., 1987, 088819).

AA was found to induce chromosomal alterations (CAs, cell division aberration, chromosome enumeration, polyploidy, spindle disturbances) in a number of in vitro mammalian cell test systems at concentrations as low as 0.01–1 mg/mL (Adler et al., 1993, 224310; Knaap et al., 1988, 224547; Martins et al., 2007, 224580; Moore et al., 1987, 224589; Tsuda et al., 1993, 224441; Warr et al., 1990, 224530). In human hepatoma G2 cells, concentrations of ≥ 0.625 mM

induced MN (Jiang et al., 2007, 224388)); however, a test for MN in spermatids collected from Sprague-Dawley rats yielded negative results at concentrations up to 0.05 mg/mL (Lähdetie et al., 1994, 224500).

Evidence for AA-induced DNA damage and repair includes positive results in a spore rec assay (Tsuda et al., 1993, 224441), DNA breakage in vitro (Jiang et al., 2007, 224388) and in vivo in mice following i.p. injection of AA at doses ≥ 25 mg/kg (Dobrzynska, 2007, 224338; Sega and Generoso, 1990, 224465), oxidative DNA damage in human hepatoma G2 cells (Jiang et al., 2007, 224388), in vitro unscheduled DNA synthesis (UDS) in human mammary epithelial cells (Butterworth et al., 1992, 202898), and in vivo UDS in male mouse germ cells (Sega et al., 1990, 224482). Testing for UDS in male rats in vivo/in vitro yielded positive results in spermatocytes and negative results in hepatocytes (Butterworth et al., 1992, 202898).

AA tested positive for sister chromatid exchange (SCE) in mammalian cells both in vitro (Knaap et al., 1988, 224547; Martins et al., 2007, 224580; Tsuda et al., 1993, 224441) and in vivo (Backer et al., 1989, 224404; Kligerman et al., 1991, 006236; Russo et al., 1994, 224409). Both positive (Banerjee and Segal, 1986, 224412; Park et al., 2002, 224330; Tsuda et al., 1993, 224441) and negative (Abernethy and Boreiko, 1987, 224278; Kaster et al., 1998, 224588) 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 (Hashimoto and Tanii, 1985, 224504; Jung et al., 1992, 224612; Knaap et al., 1988, 224547; Lijinsky and Andrews, 1980, 017361; Müller et al., 1993, 224602; Tsuda et al., 1993, 224441; Zeiger et al., 1987, 073869). A fluctuation test in *Klebsiella pneumoniae* was also negative for mutagenicity (Knaap et al., 1988, 224547).

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, 224547), but positive results were obtained when *D. melanogaster* larvae were fed concentrations ≥ 1 mM (Tripathy et al., 1991, 224435). 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, 224419; Knaap et al., 1988, 224547; Tripathy et al., 1991, 224435).

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, 224417; Knaap et al., 1988, 224547; Mei et al., 2008, 224585; Moore et al., 1987, 224589). This response was seen both with and without metabolic activation. Negative results were obtained for gene mutation at the HPRT locus in Chinese hamster V79H3 cells at the highest concentration tested (7 mM) without activation (Tsuda et al., 1993, 224441); however, positive results were obtained for gene mutation at the HPRT locus in human promyelocytic leukemia HL-60 and NB4 cells at 700 mg/L without activation (Ao et al., 2008, 224395).

Additional studies on the genotoxic potential of GA include positive results to *Salmonella typhimurium* strains TA100 and TA1535 (Hashimoto and Tanii, 1985, 224504) and mouse lymphoma cells (Barfknecht et al., 1988, 224417) but not *K. pneumoniae* (Voogd et al., 1981, 018782). GA induced UDS in mouse spermatids in vivo (Sega et al., 1990, 224482), in human epithelial cells in vitro (Butterworth et al., 1992, 202898), in one of two tests for UDS in rat hepatocytes in vitro (Barfknecht et al., 1988, 224417; Butterworth et al., 1992, 202898), and in (C3H/RL×C57BL)F1 male mice given single i.p. injections of 150 mg/kg GA (Generoso et al., 1996, 224346). GA (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, 224346). GA 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, 224346). Synthetic GA induced a similar frequency for MN 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, 224340). 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.

4.6.2.1. *Formation of DNA Adducts and Oxidative Stress*

GA forms DNA adducts in mice and rats (Figure 3-2) (Doerge et al., 2005, 224344; Gamboa da Costa et al., 2003, 194572; Segerbäck et al., 1995, 224485). 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 AA (Gamboa da Costa et al., 2003, 194572; Sega et al., 1990, 224482; Segerbäck et al., 1995, 224485).

Doerge et al. (2005, 224344) measured DNA adducts following a single i.p. administration of AA and GA to adult B6C3F_1 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.

AA has been observed to form DNA adducts in vitro, but the formation rate is very slow (Solomon et al., 1985, 224306)).

Besaratinia and Pfeifer (2004, 224427) treated normal human bronchial epithelial cells and BB 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. AA and GA 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. AA-DNA adduct formation was saturable, whereas the formation of most GA-DNA adducts was dose-dependent. GA treatment dose-dependently increased the frequency of cII mutations relative to control treatment ($p < 0.001$). GA 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 ($p = 0.038$). 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 ($p < 0.001$). 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.

Martins et al. (2007, 224580) conducted a study that examined, side-by-side, the cytogenic damage induced by AA and GA in V79 Chinese hamster cells, and compared this damage with the extent of GA-DNA adduct formation in AA- and GA-treated cells. At the highest concentration tested (2 mM), AA weakly induced CAs and significantly ($p < 0.01$) induced SCEs. The levels of N7-(2-carbamoyl-2-hydroxyethyl) guanine (N7-GA-Gua), a well-characterized GA-DNA adduct, were only detectable in AA-treated cells at 2 mM. In contrast, treatment with equimolar doses of GA produced a twofold higher clastogenic response. In GA-treated cells, both the induction of SCEs and N7-GA-Gua levels increased linearly in response to GA concentrations. The strong correlation ($r = 0.987$, $p = 1.25\times10^{-12}$) between N7-GA-Gua levels and SCE induction in both AA- and GA-treated cells provided evidence that the metabolic conversion of AA to GA and the ensuing formation of DNA adducts may play a critical role in the induction of SCEs. In support, the depurination of DNA is thought to generate abasic sites, which are vulnerable to DNA breakage. Therefore, the higher clastogenicity of GA compared to AA is likely due to higher levels of GA-DNA adduct formation, and AA probably induces SCEs after its conversion to GA. Although these results support the hypothesis that the genotoxicity of AA is based on its ability to form GA-DNA adducts, another mechanism must also be at play since the induction of CAs did not correlate with N7-GA-Gua levels.

Additional research by Mei et al. (2008, 224585) supports the hypothesis that AA and GA exert their mutagenic effects through different mechanisms. In an in vitro mouse lymphoma assay, mouse lymphoma cells (L5178Y/Tk+/-) treated with AA or GA were examined for the frequency and types of mutations incurred and for GA-DNA adduct formation. A significant increase in mutation frequency was observed at concentrations ≥ 12 mM for AA and ≥ 2 mM for GA. In GA-treated cells, GA-DNA adducts of both adenine and guanine were formed in a linear dose-response manner; however, no GA-DNA adducts were detected in cells treated with 8–20 mM AA. DNA analyses of the mutants revealed that the types of mutations incurred by AA and GA were significantly different ($p < 0.018$). The majority of AA and GA mutants displayed loss of heterozygosity (LOH) at the Tk locus; however, AA mutants more frequently contained mutations that resulted in LOH of more than half of the chromosome. Given these differences, the authors concluded that AA and GA, although both clastogenic, may induce mutations through different mechanisms. DNA breakage after GA treatment likely involves GA-DNA adduct formation, whereas AA may incur DNA damage via oxidative stress. In support, AA is known to interact with GSH, a nucleophile that protects cells from oxidants. In addition, AA has been shown to interact with nucleophiles in DNA, lipids, and proteins and to be involved in the production of reactive oxygen species.

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 (Section 4.5.2) (Spencer and Schaumburg, 1974, 061376; Spencer and Schaumburg, 1977, 224313; Tilson, 1981, 061388). 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 g AA crystals (Donovan and Pearson, 1987, 224425). 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, 224318; Igisu et al., 1975, 061355). 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 4-32). The studies in Table 4-32 provided the information needed to characterize the dose-response relationships for noncancer effects.

Table 4-32. Noncancer effects in animals repeatedly exposed to AA by the oral route

Reference/species	Exposure conditions (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effect
Burek et al. (1980, 061311) F344 rat, male and female	0, 0.05, 0.2, 1, 5, or 20 90 d 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 and skeletal muscle
Johnson et al. (1986, 061340) F344 rat, male and female	0, 0.01, 0.1, 0.5, or 2.0 2 yrs 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 wks Other nonneoplastic lesions
Friedman et al. (1995, 224307) F344 rat, male and female	0, 0.1, 0.5, or 2.0 (M) 0, 1.0, or 3.0 (F) 2 yrs in DW	0.5 (male) 1.0 (female) 2.0 (male) 3.0 (female) 0.5 (male) 1.0 (female) 0.5 2.0 (male) 3.0 (female)	2.0 (male) 3.0 (female) ND ND 2.0 (male) 3.0 (female) 2.0 ND ND	Degenerative nerve changes (LM) Hindlimb foot splay Decreased body weight (8–9%) Early mortality after 60 wks Other nonneoplastic lesions
Tyl et al. (2000, 224456) F344 rat, male and female	0, 0.5, 2.0, or 5.0 Two generations in DW	2.0 ND ND 5.0 (female) 0.5 (male)	5.0 5.0(M) 0.5(M) ND (female) 2.0 (male)	Male-mediated 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, 224265) CD-1 mouse, male and female	0, 0.8, 3.1, or 7.5 Two generations in DW	3.1 7.5 3.1 7.5 3.1 (female)	7.5 ND 7.5 ND 7.5 (female)	Male-mediated implantation losses (F0&F1) Degenerative nerve changes (F1, LM) Mild grip strength deficits (F1&F2) Hindlimb foot splay Decreased body weight (8%, F1 only)
Zenik et al. (1986, 061394) Long-Evans rat, male	0, 4.6, 7.9, or 11.9 10 wks in DW; mated w/ nonexposed female	ND 4.6	7.9 7.9	Male-mediated implantation losses Hindlimb foot splay
Zenick et al. (1986, 061394) Long-Evans rat, female	0, 5.1, 8.8, or 14.6 9 wks in DW; mated w/ nonexposed male	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, 224276) Long-Evans rat, male	0, 1.5, 2.8, or 5.8 80 d in DW; mated w/ nonexposed female	1.5 5.8 5.8	2.8 ND ND	Male-mediated postimplantation losses Peripheral nerve changes (LM) Hindlimb foot splay

Reference/species	Exposure conditions (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effect
Sakamoto et al. (1986, 224442) ddY mouse, male	0, 3.3, 9.0, 13.3, or 16.3 4 wks in DW; mated w/ nonexposed female	9.0 13.3 13.3	13.3 16.3 16.3	Male-mediated decreased fetuses/dam Slight hindlimb weakness Decreased sperm counts, abnormal sperm morphology
Sakamoto et al. (1986, 224442) ddY mouse, female	0, 18.7 4 wks in DW; mated w/ nonexposed male	18.7 ND	ND 18.7	Female reproductive performance Slight hindlimb weakness
Field et al. (1990, 224302) Sprague-Dawley rat, female	0, 2.5, 7.5, or 15 GDs 6–20 by gavage	7.5 15 15	15 ND ND	Decreased maternal weight gain Fetal malformations or variations Hindlimb splay, maternal
Field et al. (1990, 224302) CD-1 mouse, female	0, 3, 15, or 45 GDs 6–17 by gavage	15 45 15	45 ND 45	Decreased maternal weight gain Fetal malformations or variations Hindlimb splay, maternal
Wise et al. (1995, 224539) Sprague-Dawley rat, female	0, 5, 10, 15, or 20 GDs 6–10 by gavage	10 10 ND 10	15 15 5 15	Decreased maternal weight gain Hindlimb splay, maternal Decreased body weight in offspring Increased overall horizontal activity, decreased auditory startle response in offspring
Friedman et al. (1999, 224311) Wistar rat, female	0, 25 (maternal doses) PNDs 0–21 by gavage	ND 25 25	25 ND ND	Hindlimb foot splay, maternal Degenerative nerve changes (LM), maternal Hindlimb foot splay in offspring
Garey and Paule, 2007 (2007, 224337) F344 rat, male and female adolescents	Gavage to dams during gestation; PNDs 1–21 to offspring at same gavage dose; PNDs 22–85 in drinking water. Average estimated doses for offspring: 0, 0.1, 0.4, 1.3, or 6.	6 1.3	ND 6	Offspring body weight Decreased cognitive motivation in adolescent offspring

DW = drinking water; LM = light microscopy; ND = not determined; EM = electron microscopy

Table 4-32 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, 061311) or 2 mg/kg-day (Johnson et al., 1986, 061340) or 2 or 3 mg/kg-day (Friedman et al., 1995, 224307) 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 (Burek et al., 1980, 061311; Friedman et al., 1995, 224307; Johnson et al., 1986, 061340) (Table 4-32). Although studies selected for inclusion in Table 4-32 only examined rats and mice, Table 4-33 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 4-33. Neurological effects following exposure to AA in species other than the rat and mouse

Reference/species (strain)	Exposure conditions (dose, route, duration)	Effect
McCollister et al. (1964, 061347) Cats (n = 2)	Single 100 mg/kg i.p. dose	After 24 hrs, one was unconscious and was sacrificed, the other had severe neurotoxicity.
Post and McLeod (1977, 224374) Cats (2–3 kg)	15 mg/kg in food for up to 16 wks	Progressively increasing neurotoxicity; by 12–16 wks, severe poisoning, reduction in conduction velocity, damage to large and small myelinated fibers in peripheral nervous system.
Hersch et al. (1989, 061331) Dogs (greyhound, 22–30 kg)	5.7 mg/kg-day via ingested capsule for 6–7 wks	Progressive, but reversible dysfunction of the pulmonary stretch receptors and their myelinated vagal afferents.
Satchell and McLeod (1981, 061368) Dogs (greyhound)	7 mg/kg-day in feed for 8 wks	Sensorimotor peripheral neuropathy and megaesophagus suggesting an axonopathy of the vagus nerve.
Eskin et al. (1985, 224376) Monkeys (macaque)	10 mg/kg-day in juice, 5 d/wk for 6–10 wks	Axonal swellings with neurofilament accumulation in the distal optic tract and lateral geniculate nucleus.
Maurissen et al. (1983, 061346) Monkeys (pigtail)	10 mg/kg-day in juice, 5 d/wk until appearance of mild toxicity (n = 4; average for 54 d; 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 mo after dosing.
McCollister et al. (1964, 061347) Monkeys (5.1 kg)	Total of 200 mg/kg of 4 consecutive 50 mg/kg i.v. doses	Death.
Gilbert and Maurissen (1982, 061325) Mice (Balb/c)	25.8 mg/kg-day (250 ppm) AA in drinking water for 12 d (total estimated dose 310 mg/kg)	Decreased retention time and increased hindlimb splay.
Hashimoto et al. (1981, 061328) Mice (ddY strain)	1/5–1/2 of the LD_{50} (107 mg/kg) administered by gavage twice wkly for 8–10 wks	241 mg/kg was the total dose for half maximal inhibition of rotarod performance.

LoPachin et al. (2002, 224564) 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 4-32 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 4-32. Changes in foot splay have been observed in most chronic or less-than-lifetime oral studies, but at levels above the lowest dose associated with histologic signs of peripheral nerve damage (1–3 mg/kg-day). Only one study 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, 2000, 224456) (Table 4-32). In the same study, however, Tyl et al. (2000, 224456) did not observe hindlimb foot splay in the F1-generation rats exposed to doses as high as 5 mg/kg-day, nor was this endpoint reported in F344 rats exposed to drinking water doses as high as 2–3 mg/kg-day for 2 years (Friedman et al., 1995, 224307; Johnson et al., 1986, 061340) or 5 mg/kg-day for 90 days (Burek et al., 1980, 061311). Although adverse behavioral effects are not currently sufficiently supported to be designated the most sensitive endpoint, there is a clear research need for additional neurobehavioral studies with protocols and endpoints suitable for quantifying low dose-response relationships, and efforts are ongoing to address this data need.

AA 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, 224265; Sakamoto and Hashimoto, 1986, 224442; Smith et al., 1986, 224276; Zenick et al., 1986, 061394) (Table 4-32). Dominant lethality testing (Chapin et al., 1995, 224265; Smith et al., 1986, 224276; Tyl, 2000, 224456; Tyl et al., 2000, 224459) and crossover trials (Chapin et al., 1995, 224265; Sakamoto and Hashimoto, 1986, 224442; Zenick et al., 1986, 061394) indicate male-mediated reproductive effects (Table 4-32). 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, 061311). 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, 224442) or i.p. exposures (Adler, 1990, 224296; Adler et al., 1994, 224314; Adler et al., 2000, 224322; Adler et al., 2004, 224343; Ehling and Neuhäuser-Klaus, 1992, 224391; Generoso et al., 1996, 224346; Russell et al., 1991, 224406; Sega et al., 1989, 224477; Shelby et al., 1987, 088819). 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, 224442) or female Long-Evans rats to doses up to 14.6 mg/kg-day (Zenick et al., 1986, 061394) did not adversely affect reproductive performance variables such as fertility or implantation when the animals were bred with nonexposed males (Table 4-32). 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, 061394).

Comparing the study LOAEL values listed in Table 4-32 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 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, 224539) and decreased performance in an operant test of cognitive motivation in adolescent F344 rats exposed during gestation and lactation and extending through 12 weeks of age at an average dose of 6 mg/kg-day, but not at 1.3 mg/kg-day (Garey and Paule, 2007, 224337). 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, 224302) (Table 4-32). 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., 1999, 224311).

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 (Friedman et al., 1995, 224307; Johnson et al., 1986, 061340; Tyl, 2000, 224456), CD-1 mice (Chapin et al., 1995, 224265), and Long-Evans rats (Zenick et al., 1986, 061394). More pronounced decreases in body weight were seen at higher doses, but these also produced overt neurotoxicity (e.g., Burek et al., 1980, 061311).

4.7.2. Inhalation

Numerous reports have associated human exposure to AA with neurological impairment (Auld and Bedwell, 1967, 061310; Bachmann et al., 1992, 224401; Calleman et al., 1994, 202900; Davenport et al., 1976, 224305; Donovan and Pearson, 1987, 224425; Dumitru, 1989, 356205; Fullerton, 1969, 224325; Garland and Patterson, 1967, 061324; Gjerløff et al., 2001, 224367; Hagmar et al., 2001, 224453; He et al., 1989, 061330; Igisu and Matsuoka, 2002, 224318; Igisu et al., 1975, 061355; Kesson et al., 1977, 224568; Mapp et al., 1977, 224611; Mulloy, 1996, 224604; Myers and Macun, 1991, 224605; Takahashi et al., 1971, 061400). 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 Hb 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, 202900) or increased incidences of self-reported symptoms alone (Hagmar et al., 2001, 224453). 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 Hb) 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, 224514)

4.7.3. Mode-of-Action Information

4.7.3.1. *Neurotoxic Effects*

Since experimental AA neuropathy was first reported (Hazleton Laboratories, 1953, 224514), AA has been extensively studied in an effort to understand its toxicological properties and MOA for the functional deficits observed in animal studies, including alterations in gait, hindfoot splay, impaired mobility and righting reflex, and decreased grip strength (Dixit et al., 1981, 224335; Fullerton and Barnes, 1966, 061323; McCollister et al., 1964, 061347; Moser et al., 1992, 224592; Tilson and Cabe, 1979, 224408). Similar muscle weakness and functional impairments have been observed in humans exposed to AA (Calleman et al., 1994, 202900; Hagmar et al., 2001, 224453; He et al., 1989, 061330).

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 Schaumburg, 1974, 061376; Spencer and Schaumburg, 1977, 224313). 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 (Cavanagh, 1964, 224266; Spencer et al., 1979, 224317), interruption of axonal protein transport (Pleasure et al., 1969), disruption of axon cytoskeleton (Lapadula et al., 1989, 224486), diminished axolemma Na+,K+-ATPase activity (LoPachin and Lehning, 1994, 224557), and reduction of fast anterograde axonal transport capacity (Harris et al., 1994, 224464; Harry, 1992, 224479; Padilla et al., 1993, 224323; Sickles, 1991, 224494).

Recent investigations serve as basis for two hypothetical MOAs for AA neurotoxicity, disruption of nitric oxide signaling at the nerve terminal and fast axonal transport disruption. A third MOA hypothesis, which has received less research support, proposes that AA effects on nerves may involve enhanced lipid peroxidation and decreased antioxidant status (decreased GSH levels and anti-reactive oxygen species enzyme activity).

4.7.3.1.1. ***Disruption of nitric oxide signaling at the nerve terminal hypothesis.*** The hypothesis that AA-induced neurotoxicity occurs at the nerve terminals as a primary site of action possibly due to disruption of neuronal nitrous oxide (NO) signaling has been supported by the work of LoPachin and colleagues (LoPachin and Barber, 2006, 224552; LoPachin et al., 2008, 224560). AA is a conjugated α,β-unsaturated carbonyl derivative in the type-2 alkene chemical class. Because electrons in pi orbitals of a conjugated system are mobile, the α,β-unsaturated carbonyl structure of AA is characterized as a soft electrophile according to the hard-soft, acid-base principle (reviewed in Pearson and Songstad, 1967, 224360). Characteristically, soft electrophiles will preferentially form Michael-type adducts with soft nucleophiles, which in biological systems are primarily sulfhydryl groups on cysteine residues (Hinson and Roberts, 1992, 224518; LoPachin and DeCaprio, 2005, 224556) Free sulfhydryl groups can exist in the reduced thiol-state or in the anionic thiolate-state, and recent research indicates that the highly nucleophilic thiolate is the preferential adduct target for AA (see also Friedman et al., 1995, 224307; LoPachin et al., 2007, 224569). Based on the pKa of cysteine (pH 8.5), at physiological pH (7.4), the thiolate state exists only in unique protein motifs called catalytic triads, where proton shuttling through an acid-base pairing of proximal amino acids (e.g., aspartic acid and lysine) regulates the protonation and deprotonation of the cysteine sulfhydryl group. Indeed, both mass spectrometric and kinetic data have demonstrated the selective adduction of cysteine residues on many neuronal proteins (Barber and LoPachin, 2004, 224415; Barber et al., 2007, 224416). Furthermore, it is now recognized that the redox state or nucleophilicity of cysteine sulfhydryl groups within catalytic triads can determine the functionality of these proteins (reviewed in LoPachin and Barber, 2006, 224552; Stamler et al., 2001, 224327). In contrast to AA, the epoxide metabolite GA, is a hard electrophile that preferentially forms adducts with hard nucleophiles such as nitrogen, carbon, and oxygen. Nucleotide residues of DNA contain abundant hard nucleophilic targets, which is consistent with the formation of GA adducts on adenine and guanine bases in AA-intoxicated animals (Doerge et al., 2005, 224344; Doerge et al., 2008, 224362; Ghanayem et al., 2005, 224351).

Based on the observation that the processes affected (e.g., neurotransmitter release and storage) and corresponding kinetics (Km, Vmax) were similar in synaptosomes exposed in vitro to AA and those isolated from AA-intoxicated rats (Barber and LoPachin, 2004, 224415; LoPachin et al., 2004, 224570; LoPachin et al., 2006, 224567), LoPachin and colleagues have reasoned that the parent compound, AA, is responsible for neurotoxicity. Moreover, cysteine thiolate groups have clear regulatory functions in many critical neuronal processes (LoPachin and Barber, 2006, 224552), whereas protein valine, lysine, and histidine residues, which are the likely hard nucleophilic targets for a hard electrophile such as GA, have unclear functional and therefore toxicological relevance. Quantitative morphometric and silver stain analyses of the peripheral nervous system and CNS of AA-intoxicated animals support the hypothesis that axon degeneration is an epiphenomenon related to dose-rate; i.e., degeneration occurs at lower but not

higher dose-rates. In contrast, some studies indicate that nerve terminal degeneration occurs regardless of dose-rate and in correspondence with the onset and development of neurological deficits (Crofton et al., 1996, 145493; Lehning et al., 2003, 224548; Lehning et al., 2003, 224549; LoPachin and Lehning, 1994, 224557; reviewed in LoPachin et al., 2002, 224562), suggesting the nerve terminals as a primary site of action. Subsequent neurochemical studies showed that both in vitro and in vivo AA exposure produced early disruptions of neurotransmitter release, reuptake, and vesicular storage (Barber and LoPachin, 2004, 224415; LoPachin et al., 2004, 224570; LoPachin et al., 2006, 224567; LoPachin et al., 2007, 224566). Further, proteomic analyses indicated that the inhibition of presynaptic function was due to the formation of cysteine adducts on proteins that regulate neurotransmitter handling; e.g., Cys 264 of *N*-ethylmaleimide sensitive factor, Cys 254 of v-ATPase (Barber and LoPachin, 2004, 224415; Barber et al., 2007, 224416; Feng and Forgac, 1992, 224292; reviewed in LoPachin and Barber, 2006, 224552; LoPachin et al., 2007, 224569; LoPachin et al., 2007, 224566; see LoPachin et al., 2009, 224561). The anionic sulfhydryl state, which is only found in the catalytic triads of regulatory proteins, is an acceptor for NO and, therefore, has led to the hypothesis that AA-induced neurotoxicity results from disruption of neuronal NO signaling (LoPachin and Barber, 2006, 224552; LoPachin et al., 2008, 224560).

4.7.3.1.2. ***Fast axonal transport disruption hypothesis.*** Sickles et al. (2002, 224497) provide support for AA neurotoxicity resulting from inhibition of the movement of materials in fast axonal transport from both AA and GA. According to this "kinesin/axonal transport" hypothesis, toxicant inhibition of kinesin leads to reductions in the axonal delivery of macromolecules eventually producing a deficiency of the essential proteins required to maintain axon structure, function, or both. Distal axons and nerve terminals are particularly vulnerable to transport defects based upon an exceptionally large axonal volume (as much as 1,000 times the volume of the neuron cell body) and the dependence of these distal regions on long distance transport (100-fold longer length than diameter of the cell body). This regional sensitivity is consistent with the previously identified distal spatial distribution of toxicant-induced damage (Cavanagh, 1964, 224266).

Microtubule motility assays using purified kinesin from bovine brain identified a dose-dependent inhibition of kinesin as well as a less sensitive effect on microtubules (Sickles et al., 1996, 224496). Preincubation of either kinesin or taxol-stabilized microtubules with AA produced a reduction in the affinity between kinesin and microtubules, recognized as a reduced number of microtubules bound or locomoting on an absorbed bed of kinesin. Microtubules that were locomoting did so in a less directed or staggering type of progression. The inhibitions were proposed to be due to covalent adduction, presumably through sulfhydryl alkylation by AA, although adduction of other amino acid residues such as valine was possible. The non-neurotoxic analog, propionamide, had no effect. Other investigators have identified kinesin

inhibition by sulfhydryl reagents such as N-ethylmaleimide and ethacrynic acid (Walker et al., 1997, 224528). As with AA, inhibition by these sulfhydryl reagents produced the characteristic staggering movement of microtubules. The reaction was slow and temperature-dependent, suggesting a sterically hindered cysteine residue as an important adduct target. Additional studies have demonstrated a comparable effect of GA on kinesin (Sickles, unpublished data). The predicted outcome of such an effect would be reduced quantity of flow, precisely the outcome from several experiments where rate of transport versus quantity could be discriminated (Sickles, 1989, 224491; Sickles, 1989, 224493; Stone et al., 1999, 224328).

Fast axonal transport has been studied in a variety of model systems using diverse techniques. A comprehensive survey of AA effects on fast anterograde and retrograde axonal transport (Sickles et al., 2002, 224497) revealed that all studies measuring fast transport within 24 hours of AA exposure demonstrated significant reductions, whereas longer postexposure delay was not associated with changes in transport. Furthermore, a reduction in transport quantity (but not rate) has been reported within 20 minutes of exposure. The duration of this effect was 16 hours, with full recovery at 24 hours (Sickles, 1991, 224494). Quantitation of transport after multiple dosings (i.e., 4, 7, or 10 doses) had a similar effect on transport in the proximal sciatic nerve (Sickles, 1991, 224494). The changes in transport were not due to an effect on protein synthesis and exposure of only the axons confirmed that the target was axonal (Sickles, 1989, 224491; Sickles, 1989, 224493; Sickles, 1992, 224495). Collectively, these results suggest action on a target that is replaced via the fast transport system, which is consistent with kinesin as that target. The actions of AA on fast axonal transport were independent of effects on axonal neurofilaments, as similar reductions were observed in wild-type and transgenic mice lacking axonal neurofilaments (Stone et al., 1999, 224328; Stone et al., 2000, 224333). The same results were observed using radiolabeling of proteins in mouse optic nerves and differential interference microscopy of isolated sciatic nerve axons. Other recent studies have identified a parallel inhibition of retrograde axonal transport by AA (Sabri and Spencer, 1990, 224434), although it is unclear whether this effect is due to inhibition of cytoplasmic dynein, the retrograde axonal transport motor, or whether this is a result of indirect effects of kinesin motor inhibition (Brady et al., 1990, 224473).

The predicted outcome from axonal transport compromise is a reduction in vital macromolecules in the distal axons and an accumulation of transported material within the axon. Morphological studies have consistently identified accumulations of tubulovesicular profiles and neurofilaments in axons of AA-intoxicated animals (Spencer and Schaumburg, 1977, 224313), which are morphological elements transported via kinesin along microtubules. Other studies have identified reduced synaptic vesicles in neuromuscular junctions (DeGrandchamp and Lowndes, 1990, 224320; DeGrandchamp et al., 1990, 224321). A reduction in GAP-43 in the terminal neurites of cultured primary spinal cord neurons following AA exposure has been observed (Clarke and Sickles, 1996, 224275). Future studies are required to quantitate

reductions in specific axonal compartments using a variety of neurotoxic and non-neurotoxic dosing regimens in vivo to confirm the loss of physiologically or structurally important macromolecules.

Additional supportive data for the axonal transport hypothesis come from several studies of kinesin knockouts as well as similarity to human diseases. While most knockouts are lethal, low level mutations of kinesin motors in Drosophila have identified an identical spatial pattern of dysfunction and morphological similarity in axonal pathology (Gho et al., 1992, 224358; Hurd and Saxton, 1996, 224273) as with AA intoxication. The group of neurological disorders classified as hereditary spastic paraplegias has a spatial pattern of ataxia, spasticity, and muscle weakness as observed with AA intoxication. Some of these types have been associated with mutations in kinesin motors (Reid et al., 2002, 224390), while others are the result of either axonal or glial protein mutations. However, the common theme is alteration in axonal transport (Gould and Brady, 2004, 224407; Reid, 2003, 224379).

4.7.3.1.3. ***Reactive oxygen species hypothesis.*** Zhu et al. (2008, 224559) provide some data supporting a third MOA for AA induced neurotoxicity involving enhancement of lipid peroxidation and decreased antioxidative capacity, as well as depletion of neural GSH levels and antioxidant enzyme activities, resulting in the key sequence of events of increased levels of reactive oxygen species, damage to cellular macromolecules, and subsequent degeneration of neural tissues. In this study, adult male Wistar rats were given AA (40 mg/kg i.p., 3 times/week) for 2, 4, 6, and 10 weeks. Time-dependent changes in levels of malondialdehyde (an indicator of lipid peroxidation) and reduced GSH and enzyme activity levels of GSH peroxidase, GSH reductase, superoxide dismutase, and anti-reactive oxygen species were examined along with several electrophysiological indices (nerve conduction velocity and compound action potential duration, amplitude, and latency). Time-dependent decreased GSH levels and anti-reactive oxygen species activities and increased malondialdehyde levels in sciatic nerve preparations were highly correlated ($p < 0.05$, $|r| > 0.80$) with changes in electrophysiological indices of AA-induced neurotoxicity.

4.7.3.1.4. ***Summary and data needs.*** The respective adduct chemistries of AA and GA are well understood and could have fundamental implications for neurotoxicity regardless of the proposed mechanism; i.e., kinesin inhibition (Sickles et al., 2002, 224497) or blockade of NO signaling (LoPachin and Barber, 2006, 224552; LoPachin et al., 2008, 224560; LoPachin et al., 2009, 224561). Accordingly, an obvious data gap in the current mechanistic understanding of AA neurotoxicity is the relative roles of the parent compound and GA. Thus, although early research suggested that GA produced neurotoxicity both in whole animal (Abou-Donia et al., 1993, 224288) and in vitro (Harris et al., 1994, 224464) model systems, other studies using similar models failed to find neurotoxic effects associated with the GA metabolite (Brat and Brimijoin, 1993, 224474; Costa et al., 1992, 224285; Costa et al., 1995, 224287; Moser et al., 1992,

224592). Clearly, resolving the relative roles of AA vs. GA is an important issue that will require more research. Although the adduct chemistry of these toxicants has been reasonably defined, the precise molecular mechanisms and sites of neurotoxicity have not yet been clearly resolved.

4.7.3.2. *Reproductive Effects*

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 (Costa et al., 1992, 224285). 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 (Adler et al., 2000, 224322; Perreault, 2003, 224370; Sega et al., 1989, 224477; Tyl and Friedman, 2003, 224450; Tyl et al., 2000, 224459).

Sega et al. (1989, 224477) 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, 224322), 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, 224553) 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. (2005, 224351; 2005, 224354) 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, 224485)). The mechanistic proposals presented by Tyl and Friedman (2003, 224450) 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, 224315).

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, 2005, 086237), 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. In addition, CNS tumors were observed in both of the chronic F344 rat bioassays. The elevation of the incidence for CNS tumors was significant in the one bioassay and of uncertain statistical significance in the other. There are no animal data on the carcinogenicity following chronic inhalation exposure to AA. EPA's *Guidelines for Carcinogen Risk Assessment* (2005, 086237) 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. In the case of AA, there is evidence of rapid, nearly complete absorption from the oral route and rapid distribution throughout the body (Kadry et al., 1999, 224596; Miller et al., 1982, 061351) and evidence that the elimination kinetics of radioactivity from oral or i.v.

administration of radiolabeled AA in rats is similar (Miller et al., 1982, 061351). In addition, there is similar flux of AA through metabolic pathways following either single dose oral or single 6 hr inhalation exposures in rats (Sumner et al., 2003, 224347) and while there are some route-to-route differences in the relative amounts of AA to GA, the differences are within twofold of each other. For these reasons, AA is considered likely to be carcinogenic to humans by all routes of exposure.

The potential for AA carcinogenicity from dietary exposure has been assessed in a number of case-control studies (Michels et al., 2006, 224586; Mucci et al., 2003, 224597; Mucci et al., 2004, 224598; Mucci et al., 2005, 224600; Pelucchi et al., 2006, 224364; Pelucchi et al., 2007, 224369; Wilson et al., 2009, 224535) and several prospective studies (Hogervorst et al., 2007, 224520; Hogervorst et al., 2008, 224521; Hogervorst et al., 2008, 224522; Larsson et al., 2009, 224466; Larsson et al., 2009, 224461; Larsson et al., 2009, 224463; Larsson et al., 2009, 224483; Larsson et al., 2009, 224484; Mucci et al., 2006, 224601; Wilson et al., 2009, 224536). Two cohort mortality studies (Collins et al., 1989, 224284; Sobel et al., 1986, 067940) with follow-up analyses (Marsh et al., 1999, 224577; Marsh et al., 2007, 224578; Swaen et al., 2007, 224357) have assessed associations with inhalation and dermal exposure to AA in the workplace. In addition, two case-control studies examined relationships between AA-Hb adducts and risks for breast cancer (Olesen et al., 2008, 224303) and prostate cancer (Wilson et al., 2009, 224535). These studies are judged as providing limited or no evidence of carcinogenicity in humans.

In most of the case-control studies and prospective studies, 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, pharyngeal, esophageal, laryngeal, ovarian, or prostate cancer. 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. One case-control study reported a slightly increased risk of breast cancer later in life associated with the consumption of French fries during preschool (Michels et al., 2006, 224586). Olesen et al. (2008, 224303) reported a significant positive association between AA-Hb adduct levels in red blood cells and ER+ breast in a case-control study of Danish women (374 cases and 374 controls) only after adjusting for smoking; no significant association was found between AA-Hb or GA-Hb adduct levels and total breast cancer either with or without adjustment for smoking. Increased risks of postmenopausal endometrial, ovarian, and renal cell cancer with increasing dietary AA intake were reported in prospective studies of a Dutch population (Hogervorst et al., 2007, 224520; Hogervorst et al., 2008, 224521). Each of these studies were limited by uncertainties in exposure assessments.

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 one follow-up assessment, an increased risk for pancreatic cancer was reported in a subgroup with the highest cumulative AA exposure (Marsh et al., 1999, 224577). However, no

increased risk for pancreatic cancer was observed in the most recent follow-up analysis of this cohort (Marsh et al., 2007, 224578).

The majority of the data support a mutagenic MOA for AA carcinogenicity. AA has been reported to induce gene mutations and CAs 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, UDS in a variety of test systems, and formation of DNA adducts. DNA adducts of GA have been observed in studies of in vivo AA exposure of rodents and in vitro AA exposure of human cells. Demonstration of AA tumor-initiation activity by several routes of exposure in mice provides additional support for a mutagenic MOA. Furthermore, the multiple-site characteristic of AA carcinogenicity in rats is consistent with other carcinogenic agents that are thought to act through mutagenic MOAs involving DNA alkylation. 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 a MOA are limited or lacking.

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

Cohort mortality studies of AA workers at several locations in the United States and the Netherlands (Collins et al., 1989, 224284; Marsh et al., 1999, 224577; Marsh et al., 2007, 224578) and a location in Michigan (Sobel et al., 1986, 067940; Swaen et al., 2007, 224357) have not found statistically significant increased risks for cancer-related deaths compared with national cancer mortality rates in whole-cohort analyses.

Numerous case-control studies (Michels et al., 2006, 224586; Mucci et al., 2003, 224597; Mucci et al., 2004, 224598; Mucci et al., 2005, 224600; Pelucchi et al., 2006, 224364; Pelucchi et al., 2007, 224369; Wilson et al., 2009, 224535) and prospective studies (Hogervorst et al., 2008, 224522; Larsson et al., 2009, 224466; Larsson et al., 2009, 224461; Larsson et al., 2009, 224463; Larsson et al., 2009, 224483; Larsson et al., 2009, 224484; Mucci et al., 2006, 224601; Wilson et al., 2009, 224536) 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, pharyngeal, esophageal, laryngeal, ovarian, or prostate cancers. These studies predominantly evaluated Swedish, Danish, Dutch, or Italian populations; available assessment of a U.S. population is restricted to the prospective study of Wilson et al. (2009, 224536). 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 a slightly increased risk of breast cancer later in life associated with the consumption of French fries during preschool (Michels et al., 2006, 224586), 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.

Increased risks of postmenopausal endometrial and ovarian cancer (Hogervorst et al., 2007, 224520) and renal cell cancer (Hogervorst et al., 2008, 224521) with increasing dietary AA intake were reported in prospective studies of a Dutch population, but estimations of dietary AA levels in foods on the market at baseline in 1986 were based on food samples analyzed since 2001 and questionnaires did not include details regarding specifics of food preparation. Olesen et al. (2008, 224303) reported a significant positive association between AA-Hb adduct levels in red blood cells and ER+ breast after adjusting for smoking, but this study is limited by the relatively small number of subjects (374 cases and 374 controls) and uncertainty regarding extrapolation of AA exposure as assessed by a few months of AA-Hb adduct measurements to a lifetime of exposure.

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 ($\geq$ 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, 224577). 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. Furthermore, no increased risk for pancreatic cancer was observed in the most recent follow-up analysis of this cohort (Marsh et al., 2007, 224578).

Limitations in the epidemiology studies include small cohort size and limited follow-up period (Sobel et al., 1986, 067940; Swaen et al., 2007, 224357); large proportion of short-term workers in the cohort, low exposures, incomplete smoking habit information, and incomplete follow-up period (Collins et al., 1989, 224284; Marsh et al., 1999, 224577; Marsh et al., 2007, 224578); 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). The single case-control study (Michels et al., 2006, 224586) and two prospective studies (Hogervorst et al., 2007, 224520; Hogervorst et al., 2008, 224521) that showed positive associations from estimated dietary consumption of AA had questionable data on diet composition and AA content in the diet.

Cancer studies in test animals include two 2-year drinking water administration studies in F344 rats (Friedman et al., 1995, 224307; Johnson et al., 1986, 061340), 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., 1984, 202896; Bull et al., 1984, 202897), and a lung adenoma i.p. administration assay in strain A/J mice (Bull et al., 1984, 202896). The results from the two chronic oral exposure studies in rats are presented in Table 4-34.

Table 4-34. Incidence of tumors with statistically significant increases in 2-year bioassays with F344 rats exposed to AA 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, 061340); 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]	–
Adrenal pheochromocytoma	3/60	–	7/59	7/60	5/60	–	10/60[e]	–
Johnson et al. (1986, 061340); females								
Follicular cell adenoma/carcinoma	1/58	–	0/59	1/59	1/58	–	5/60[f]	–
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[e]	–
CNS tumors of glial origin	1/60	–	2/59	1/60	1/60	–	9/61[e]	–
Oral cavity malignant+benign	0/60	–	3/60	2/60	3/60	–	8/60[e]	–
Uterus adenocarcinoma	1/60	–	2/60	1/60	0/59	–	5/60[f]	–
Clitoral adenoma, benign	0/2	–	1/3	3/4	2/4	–	5/5[f]	–
Pituitary gland adenoma	25/59	–	30/60	32/60	27/60	–	32/60[f]	–
Friedman et al. (1995, 224307); 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, 224307); 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, 224307) and not those reported in the reevaluation study by Iatropoulos et al. (1998, 224628).
[d]The data reported in Table 4 in Friedman et al. (1995, 224307) lists one follicular cell adenoma in the second control group; however, the raw data obtained in the Tegeris Laboratories (1989, 224400) 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 significantly ($p < 0.05$) different from control, Fisher's Exact test.
[f]Statistically significantly ($p < 0.05$) different from control, after Mantel-Haenszel mortality adjustment.

Sources: Friedman et al. (1995, 224307); Johnson et al. (1986, 061340).

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, 224307; Johnson et al., 1986, 061340). Data from both studies are sufficient to describe relationships between administered dose levels and cancer responses. The Friedman et al. (1995, 224307) 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, 061340) were not replicated in the second bioassay (Friedman et al., 1995, 224307). With respect to the CNS tumors, Friedman et al. (1995, 224307) 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, 224393), and seven cases of a morphologically distinctive category of primary brain tumor described as "malignant reticulosis" were reported but excluded from the authors' analysis (Tables 4-13 and 4-14). The Friedman et al. (1995, 224307) study therefore provides some support for AA induced CNS tumors, 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. The data for the female uterine adenocarcinomas and pituitary gland adenomas observed in Johnson et al. (1986, 061340) were not as strong as for the other tumor types because the statistical significance of the elevated incidences in the high-dose group was only demonstrated after Mantel-Haenszel mortality adjustment, there was no clear evidence for a trend for increasing risk with increasing exposure level, and in the case of the pituitary gland adenomas, there were very high control group levels (42% incidence), as well as incidence levels, in all of the dose groups, suggestive of a causal agent(s) other than AA. The increased incidence of clitoral adenomas is also less persuasive because it is based on differences in a very small number of animals ($n \leq 5$).

Results from the mouse skin tumor initiation assays add considerable weight to the evidence for AA 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., 1984, 202896; Bull et al., 1984, 202897). 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., 1984, 202896). These findings provide evidence that AA can initiate tumor development in mice, a process that is thought to involve a mutagenic MOA. These findings are consistent with the positive findings for AA and GA genotoxicity in numerous tests.

Other evidence of the carcinogenicity of AA 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/week for 8 weeks (Bull et al., 1984, 202896) 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., 1984, 202897).

As discussed in Section 4.6.2 and tabulated in Appendix B, AA 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 CAs in in vitro exposed mammalian cells (Moore et al., 1987, 224589; Tsuda et al., 1993, 224441; Warr et al., 1990, 224530); in vitro cell transformation of Syrian hamster embryo cells (Park et al., 2002, 224330); CAs or MN in bone marrow of mice given i.p. injections of 50–100 mg/kg (Adler et al., 1988, 224301; Cihák and Vontorková, 1988, 224270; Cihák and Vontorková, 1990, 224271); formation of DNA adducts of GA following i.p. injection of 50 mg/kg of AA in mice and rats (Segerbäck et al., 1995, 224485); and dominant lethal mutations in mice given one to five i.p. injections of 40–125 mg/kg AA (Shelby et al., 1987, 088819), in rats exposed to 2.8 mg/kg-day in drinking water for 80 days (Smith et al., 1986, 224276), and in mice exposed to five consecutive dermal doses of 50–125 mg/kg AA (Gutierrez-Espeleta et al., 1992, 224413). In addition, in vitro exposure to AA induced MN and DNA damage in human hepatoma G2 cells (Jiang et al., 2007, 224388) and DNA adducts of GA in human bronchial epithelial cells (Besaratinia and Pfeifer, 2004, 224427).

In addition, the epoxide metabolite of AA, GA, has been shown to be mutagenic to *S. typhimurium* strains TA100 and TA1535 (Hashimoto and Tanii, 1985, 224504) and mouse lymphoma cells (Barfknecht et al., 1988, 224417) but not to *K. pneumoniae* (Voogd et al., 1981, 018782). GA induced UDS in mouse spermatids in vivo (Sega et al., 1990, 224482), human epithelial cells in vitro (Butterworth et al., 1992, 202898), in one of two tests for UDS in rat hepatocytes in vitro (Barfknecht et al., 1988, 224417; Butterworth et al., 1992, 202898), and in (C3H/RL×C57BL)F1 male mice given single i.p. injections of 150 mg/kg GA (Generoso et al., 1996, 224346). GA (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, 224346). GA 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, 224346).

4.8.3. Mode-of-Action Information for Carcinogenicity

The 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, 224307; Johnson et al., 1986, 061340); (2) the CNS tumors reported in the Johnson et al. (1986, 061340) study, supported by the brain tumor data in Friedman et al. (1995, 224307), 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., 1984, 202896; Bull et al., 1984, 202897); and (4) the lung adenomas following i.p. doses of AA in A/J mice (Bull et al., 1984, 202896).

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 AA-induced carcinogenicity (Besaratinia and Pfeifer, 2005, 224433; Besaratinia and Pfeifer, 2007, 224436; Dearfield et al., 1995, 224315; Moore et al., 1987, 224589; Schmid et al., 1999, 224458; Segerbäck et al., 1995, 224485). AA has been reported to induce genotoxicity (gene mutations and some types of CAs [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:

- AA is metabolized by CYP2E1 to the DNA-reactive epoxide, GA;
- AA and GA are genotoxic in the BB 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.
- GA is mutagenic in short-term bacterial assays.
- GA is mutagenic in male and female mouse somatic cells following oral exposure and in male mouse germ cells (heritable translocations) following i.p. exposure.
- AA induces heritable translocations in male mouse germ cells following i.p. or dermal administration, and specific locus mutations in male germ cells following i.p. 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 2.8–13.3 mg/kg-day range, which is near the range of chronic dose levels associated with carcinogenic effects in rats (0.5–3 mg/kg-day).

4.8.3.1.1. ***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.

4.8.3.1.2. ***Strength, consistency, and specificity of the association between exposure to AA and mutagenic activity that could lead to the formation of tumors.*** There is ample evidence in the literature for the ability of AA and GA (administered via different routes of exposure) to induce a variety of genotoxic effects in mammalian cells (Adler et al., 1994, 224314; Besaratinia and Pfeifer, 2007, 224436; Dearfield et al., 1995, 224315; Doerge et al., 2005, 224344; Ehling and Neuhäuser-Klaus, 1992, 224391; Gamboa da Costa et al., 2003, 194572; Generoso et al., 1996, 224346; Ghanayem et al., 2005, 224351; Knaap et al., 1988, 224547; Moore et al., 1987, 224589; Rice, 2005, 224393; Russell et al., 1991, 224406; Segerbäck et al., 1995, 224485).

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 SCE or MN frequencies and subsequent cancer incidence or mortality (Bonassi et al., 2004, 224455; Hagmar et al., 1998, 224438). Other measures, such as UDS 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 AA'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 BB mouse to either AA and GA, and similar mutagenicity spectra between AA and GA (Manjanatha et al., 2006, 224572);
2. formation of GA-DNA adducts at similar specific locations within the cII gene in BB 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, 224427);

3. detection of DNA adducts of GA in various mouse and rat tissues following single i.p. administration of AA or GA (Doerge et al., 2005, 224344; Segerbäck et al., 1995, 224485);
4. demonstration that AA-induced germ and somatic cell mutations in male mice require CYP2E1-mediated epoxidation of AA (Ghanayem et al., 2005, 224351; Ghanayem et al., 2005, 224354);
5. positive results for GA in *S. typhimurium* strains TA100 and TA1535 (Hashimoto and Tanii, 1985, 224504);
6. detection of heritable translocations in mice following single i.p. injections of GA doses of 100–150 mg/kg (Generoso et al., 1996, 224346); 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, 224417; Knaap et al., 1988, 224547; Moore et al., 1987, 224589).

The results of Manjanatha et al. (2006, 224572) studies on significantly increased in vivo mutation frequencies in the 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.

AA and GA react with nucleophilic sites in macromolecules (including Hb and DNA) in Michael-type additions (Bergmark et al., 1991, 224423; Bergmark et al., 1993, 224424; Segerbäck et al., 1995, 224485; Solomon et al., 1985, 224306). Solomon et al. (1985, 224306) conducted in vitro studies for the reaction of AA with calf thymus DNA and with various deoxynucleosides including dAdo, dCyd, dGua, and dThd, and demonstrated the formation of

2-formamidoethyl and 2-carboxyethyl adducts via Michael addition. AA 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 AA concentrations.

Segerbäck et al. (1995, 224485) reported much higher rates of DNA-adduct formation from AA-generated GA than from AA 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 GA 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, 224427) treated normal human bronchial epithelial cells and BB mouse embryonic fibroblasts (that carry a lambda phage cII transgene) in vitro with AA, its primary epoxide metabolite (i.e., 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. AA and GA 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. AA-DNA adduct formation was saturable, whereas the formation of most GA-DNA adducts was dose-dependent for all doses tested. GA 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 ($p = 0.038$). 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 ($p < 0.001$). 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. (2005, 224344) 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. DNA adducts were measured following a single i.p. administration of either AA or GA to adult B6C3F_1 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. (2005, 224351) 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. (2005, 224354) 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, 224346) 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 (e.g., 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, 224417; Knaap et al., 1988, 224547; Moore et al., 1987, 224589). Moore et al. (1987, 224589) evaluated activity of AA without exogenous activation in L5178Y/Tk(+/-)-3.7.2C mouse lymphoma cells at the thymidine kinase locus, and noted that 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 cannot be definitively attributable to GA related DNA mutations or AA related chromosomal alterations.

AA and 15 of its analogues have been tested for mutagenicity in five TA strains of *S. typhimurium* (Hashimoto and Tanii, 1985, 224504). AA 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 GA (the other two were N,N-diglycidyl AA 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 AA, via conversion to its active epoxide metabolite, GA, can form DNA adducts, point mutations, and frameshift mutations that persist in viable mammalian (including human) somatic cells.

4.8.3.1.3. ***Mutations occur in target tissues where tumors have been observed.*** Doerge et al. (2005, 224344) provide the strongest evidence that AA-induced mutagenicity (via GA) 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 nontarget 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, 224550) 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. AA 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 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. AA 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.

4.8.3.1.4. ***Dose-response concordance and temporal relationship.*** Empirical support for dose-response and temporal concordance between AA-induced genotoxic events and tumor development comes from studies of DNA adduct formation in liver of rodents following repeated oral exposure to doses at or below the dose range of the chronic rat bioassays (0.5–3 mg/kg-day). N7-GA-guanine adducts increased to apparent steady-state levels in livers of male and female F344 rats by 14 days of repeat dosing of approximately 1 mg AA/kg-day in drinking water

(Doerge et al., 2005, 224344)). A similar temporal pattern for increased liver levels of N7-GA-guanine adducts was reported for male and female B6C3F1 mice exposed to 1 mg/kg-day doses in drinking water for up to 40 days (Doerge et al., 2005, 224344). In another study, N7-GA-guanine adduct levels in livers increased with increasing dose level in mice exposed for 28 days to gavage doses of AA ranging from 0.125 to 24.0 mg/kg-day; some evidence for saturation at the higher dose levels was evident (Zeiger et al., 2009, 224546). In this study, significantly ($p < 0.05$) increased MN were observed in peripheral blood reticulocytes at AA doses $\geq$ 6 mg/kg-day and in normochromatic erythrocytes at $\geq$ 4 mg/kg-day (Zeiger et al., 2009, 224546). These studies, which examined levels of DNA adducts at doses in the range of the chronic bioassays, did not examine cancer target tissues (e.g., CNS, thyroid), but, as discussed earlier, single i.p. doses of 50 mg/kg AA increased N7-GA-guanine adducts in the brain, thyroid, testes, and mammary gland tissue of F344 rats (Doerge et al., 2005, 224344).

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.

AA'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., 1984, 202896; Bull et al., 1984, 202897). Oral administration of AA (3 times/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., 1984, 202896). 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., 1984, 202897). 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, 2000, 224456); 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, 224265); 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, 224276); 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, 224442). There is currently insufficient information, however, to determine if the events leading to the dominant lethals are relevant 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 CAs 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, 224276) 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 (Chapin et al., 1995, 224265; Smith et al., 1986, 224276; Tyl, 2000, 224456). 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, 224386) attempted dose-response modeling of AA in vivo genotoxicity data to extrapolate the response for CAs or SCE 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 [BMR] 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 BMR 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. (2005, 224344; 2005, 224348) data demonstrate 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.

4.8.3.1.5. ***Biological plausibility and coherence.*** The multiple-site characteristic of AA carcinogenicity in rats (i.e., CNS, thyroid, scrotal sac, mammary gland) is consistent with other carcinogenic agents that are thought to act through mutagenic MOAs involving alkylation of DNA. For example, inhalation exposure to ethylene oxide, a DNA reactive epoxide, induced lung, Harderian gland, uterine, mammary, and lymph tumors in mice and leukemia, brain tumors, peritoneal mesotheliomas in the region of the testes, and subcutaneous fibromas in rats (IARC, 2007, 224636). Similarly, inhalation or oral exposure of rats to acrylonitrile, which is metabolized to a DNA reactive epoxide, induced cancer in a range of tissues, including CNS, Harderian gland, gastrointestinal tract, and mammary glands in rats (IARC, 1999, 224635). Inhalation exposure of rats to 1,3-butadiene, which is metabolized to DNA-reactive epoxides, induced tumors of the pancreas and testes in males and tumors of the thyroid, uterus, Zymbal gland, and mammary glands in females (IARC, 2007, 224637). In addition, oral exposure to NMA, which is metabolized to AA and GA (Fennell et al., 2003, 224295), induced tumors in the liver, lung, and Harderian gland in mice (NTP, 1989, 224294). Thus, it is biologically plausible that the formation of GA-DNA adducts is a key event in the carcinogenicity of AA.

The fact that GA-DNA adducts have been 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 and coherent with other cancer agents acting through DNA-reactive epoxides, key events that have not been firmly established for a mutagenic MOA for AA include AA-induced DNA adducts in target tissues at tumor-inducing exposure levels and AA-induced DNA adducts in cancer-critical genes in target tissues (e.g., proto-oncogenes/tumor-suppressor genes). Thus, further research is needed to better assess dose-response and temporal concordance between AA-induced DNA adduct formation, mutations in cancer-critical genes, and tumor responses.

Results from investigations of AA effects on hypothalamus-pituitary-thyroid endpoints in rats provide no clear and consistent evidence to support an alternative cancer MOA involving hormonal dysregulation by AA or its metabolites (see Section 4.8.3.2 for further discussion). These results, especially the negative results from an examination of a comprehensive suite of hypothalamus-pituitary-thyroid endpoints in rats exposed to 50 mg/kg-day for 14 days (Bowyer et al., 2008, 224470) (Section 4.8.3.2), are consistent with a mutagenic MOA for AA.4.8.3.2), are consistent with a mutagenic MOA for AA.

4.8.3.1.6. ***Human relevance.*** A mutagenic MOA involving the key events of AA metabolic activation to GA, and GA modification of DNA leading to mutation of cancer-critical genes is reasonably expected to be relevant to humans. Observations of GA-Hb adducts (Bjellaas et al., 2007, 224444; Bjellaas et al., 2007, 224443; Chevolleau, et al., 2007, 224269; Vesper et al., 2007, 224511; Vesper et al., 2008, 224512) and GA urinary metabolites (Heudorf et al., 2009, 224517; Urban et al., 2006, 224476) demonstrate that internal exposure to GA, the mutagenic AA metabolite, occurs in the general population experiencing low levels of AA exposure. In addition, human cells exposed to AA or GA have been shown to develop mutations in a cancer-critical gene. Besaratinia and Pfeifer (2004, 224427) exposed normal human bronchial epithelial cells for 4 hours to AA or GA and detected GA-DNA adducts within the tumor suppressor p53 gene, using terminal transferase-dependent polymerase reaction to map the formation of DNA adducts within the gene.

4.8.3.1.7. ***Conclusion.*** There is evidence from a variety of studies of GA'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 CAs 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, UDS 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 (Besaratinia and Pfeifer, 2004, 224427; Dearfield et al., 1995, 224315; Doerge et al., 2005, 224344; Moore et al., 1987, 224589; Schmid et al., 1999, 224458; Segerbäck et al., 1995, 224485). 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., 2005, 224351; Ghanayem et al., 2005, 224354), and the similar mutation spectra that AA and GA produced in the BB male and female mice (Manjanatha et al., 2006, 224572)).

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, 224307) chronic rat bioassay, whereas GA-DNA adducts have been observed in a much wider array of tissues.

4.8.3.2. *Other Possible MOAs—Disruption of Hormone Levels or Signaling*

An alternative MOA via disruption of hormone levels or hormone signaling has also been suggested for the AA-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) (Dourson et al., 2008, 224432; EIC, 2002, 224394; Haber et al., 2009, 224431; Klaunig, 2008, 224554; KS Crump Group, 1999, 224622; KS Crump Group, 1999, 224623; Shipp et al., 2006, 224488). 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 (Dourson et al., 2008, 224432; EIC, 2002, 224394; Klaunig, 2008, 224554; KS Crump Group, 1999, 224622; KS Crump Group, 1999, 224623; Shipp et al., 2006, 224488).

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 (Ali et al., 1983, 061319; Friedman et al., 1999, 224381; Khan et al., 1999, 224565; Uphouse et al., 1982, 224472). 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 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 TVMs 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, there is no clear and consistent evidence to support the disruption of thyroid hormone homeostasis in AA-exposed rats. Although there is one published report of changes in thyroid follicular cell colloid area and cell height in female F344 rats exposed to 2 or 15 mg/kg-day for up to 7 days without any changes in circulating levels of TSH

or T_4 (Khan et al., 1999, 224565), another study found no morphological changes or evidence of increased cell proliferation in male F344 rats exposed to 50 mg/kg-day for 14 days (Bowyer et al., 2008, 224470). Bowyer et al. (2008, 224470) (2008) found elevations of serum T4 levels at 50 mg/kg-day, but not at 2.5 or 10 mg/kg-day, and no changes in serum levels of T3 or TSH at any of these dose levels. Other unpublished 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 (EIC, 2002, 224394; Friedman et al., 1999, 224381; Klaunig, 2000, 594245 as cited in EIC 2002). 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.

4.8.3.2.1. *TVMs*

Description and identification of key events. The events in the proposed hormonal pathway MOA for AA-induced formation of TVMs are 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, 224277; Cook et al., 1999, 001577; Prentice and Meikle, 1995, 085738); 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 (Ali et al., 1983, 061319; Friedman et al., 1999, 224381; Khan et al., 1999, 224565; Uphouse et al., 1982, 224472). These studies were instigated because it is well known that dopamine plays a predominant role in hypothalamic suppression of pituitary secretion of prolactin (Neumann, 1991, 224608; Yamada et al., 1995, 224544), and AA has been demonstrated to increase striatal dopamine receptors in rats (Agrawal et al., 1981, 224356; Agrawal et al., 1981, 224361; Bondy et al., 1981, 224468; Uphouse et al., 1982, 224472). 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., 1999, 224381). 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., 1999, 224381) 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, 224565).

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, 224472). The decrease in prolactin levels was statistically significant in rats that were not handled for 3 minutes-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 (8–10 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, 061319).

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

AA 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 et al., 1981, 224361; Agrawal et al., 1981, 224356; Bondy et al., 1981, 224468; Uphouse and Russell, 1981, 224469). In contrast, 24 hours postdosing, 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, 224468). Bondy et al. (1981, 224468) 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, 061304). 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, 224468). 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 (Agrawal

et al., 1981, 224356; Ali, 1983, 224378; Ali et al., 1983, 061319; Rafales et al., 1983, 061363). 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., 1981, 224356). 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, 061319).

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, 224378). 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, 061363).

AA-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, 061363).

Dose-response concordance. Only a few studies are available to support a dose-response relationship of AA 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., 1999, 224381). 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, 061319).

Temporal relationship. If AA-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, 224366) 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 (Goodman et al., 1979, 224373; Solleveld et al., 1984, 224298; Tanigawa et al., 1987, 224366). 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, 224392; Turek and Desjardins, 1979, 224445). 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, 224418).

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, 224519). 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, 224628) in male rats originally assessed as having TVMs (Friedman et al., 1995, 224307) provides some support for the proposal that AA-induced mesotheliomas in F344 rats may not be relevant to humans (Shipp et al., 2006, 224488). 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% of the testes occupied by Leydig cell neoplasia (Iatropoulos et al., 1998, 224628).[9] 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 (Clegg et al., 1997, 224277; Cook et al., 1999, 001577). Several other mechanisms involving elevations of LH or other disruptions of the hypothalamic-

[9] In another study of the tunica vaginalis testis mesotheliomas reported in Friedman et al. (1995, 224307), it was concluded, based on light and electron microscopy, that tumors in the AA-exposed rats did not differ morphologically from tumors in the control rats (Damjanov and Friedman, 1998, 089567). This study, however, did not specifically compare morphological features of Leydig cell tumors between AA-exposed and control rats.

pituitary-testis axis could possibly result in an adverse human response (Clegg et al., 1997, 224277; Cook et al., 1999, 001577).

Conclusion. In summary, there is some evidence to suggest that AA can promote or enhance age-related decreases in serum prolactin and testosterone in older male F344 rats (Ali et al., 1983, 061319; Friedman et al., 1999, 224381; Khan et al., 1999, 224565; Uphouse et al., 1982, 224472) and that this enhancement may lead to the development of TVMs due to larger adjacent Leydig cell tumors (Iatropoulos et al., 1998, 224628). 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 TVMs 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.

4.8.3.2.2. *Mammary gland fibroadenomas.*

Description and identification of key events. The events in the proposed hormonal pathway MOA for AA 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, 224488).

Experimental support for the hormonal pathway MOA in female rats.

Strength, consistency, and 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., 1999, 224381) 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, 224565).

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

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, 224488) 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, 224499) 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, 224608); and because the dopamine agonist, SKF-38393, acting at D1 dopamine receptors in rat ovary cells, stimulates progesterone secretion (Mori et al., 1994, 224591) but does not appear to stimulate progesterone secretion in human ovary cells (Mayerhofer et al., 1999, 224583).

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).

4.8.3.2.3. *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 (Dourson et al., 2008, 224432; EIC, 2002, 224394; KS Crump Group, 1999, 224622; KS Crump Group, 1999, 224623).

Experimental Support for the Hormonal Pathway MOA in Male and Female Rats.

Strength, consistency, and specificity of association. Both of the available chronic exposure studies of F344 rats 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, 224307; Johnson et al., 1986, 061340). Chemicals that alter thyroid hormone homeostasis by interfering with synthesis or secretion of T3 or 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 possibly progressing to neoplasia (U.S. EPA, 1998, 030018).

As discussed in more detail in Section 4.6.1, there is no clear and consistent evidence to support the hypothesis that AA induces sustained follicular cell proliferation by altering thyroid

hormone homeostasis. Khan et al. (1999, 224565) reported statistically significant changes in follicular cell colloid area and cell height in female F344 rats exposed to 2 or 15 mg/kg-day AA for 2 or 7 days without any significant changes in plasma levels of TSH or T4, but Bowyer et al. (2008, 224470) found no morphological changes or evidence of increased cell proliferation (mRNA levels for the Mki67gene and protein levels of Ki67) in the thyroid and pituitary of male F344 rats exposed to 50 mg/kg-day AA for 14 days. Bowyer et al. (2008, 224470) found statistically significant elevations of serum T4 levels in male rats at 50 mg/kg-day, but not at 2.5 or 10 mg/kg-day, and no changes in serum T3 or TSH at any dose level. In unpublished studies, blood levels of T3, T4, or TSH were not consistently changed in male and female F344 exposed to up to 25 mg/kg-day AA in drinking water for up to 28 days (Friedman et al., 1999, 224381), and blood levels of TSH and indices of cell proliferation in the thyroid (BrdU incorporation into DNA) were not changed in male Sprague-Dawley rats exposed to 2 or 15 mg/kg-day for up to 28 days (EIC, 2002, 224394; Klaunig, 2000, 594245 as cited in EIC 2002). Klaunig and Kamendulis (2005, 224550) 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. In summary, there is a lack of consistent evidence for AA alteration of thyroid hormone homeostasis. The negative findings in the comprehensive study by Bowyer et al. (2008, 224470) of hypothalamus-pituitary-thyroid axis endpoints in male F344 rats exposed to AA for 14 days are particularly noteworthy as not being consistent with the hypothesis that thyroid hormone dysregulation is a key event in AA induction of thyroid tumors.

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, 1998, 030018)).

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 Hypothesized 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 AND LIFE STAGES

4.9.1. Possible Childhood Susceptibility

4.9.1.1. *Neurotoxicity*

No human data are available regarding age-related differences in susceptibility to AA-induced neurotoxicity. Animal studies provide conflicting results. Some reports indicate that young animals may be less susceptible than older ones (Fullerton and Barnes, 1966, 061323; Kaplan et al., 1973, 224594), whereas other reports present evidence that young animals may be more sensitive.

Fullerton and Barnes (1966, 061323) 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, 224594) 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, 224350) administered 50 mg/kg of AA to 1-day-old and adult rats 3 times/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, 224540) 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 versus 15.6 ± 4.0 days in 8-week-old mice) and more rapid progression of AA-induced neuropathy.

4.9.1.2. *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, 2005, 088823), 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 IUR (Section 5.4.5) 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 subpopulations <16-years of age or for lifetime exposures that begin in early life. 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 da Costa et al. (2003, 194572) 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 2,000 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.

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, 224307; Johnson et al., 1986, 061340). 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, 224616). 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 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 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, 224551; Lipscomb et al., 2003, 192847), 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 AA in humans.

AA-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 (Sections 4.3.1 and Table 4-32). Part of the gender difference may be due to the AA or GA alkylation of sperm protamines late during spermiogenesis and resultant genetic damage (Adler et al., 2000, 224322; Generoso et al., 1996, 224346; Perreault, 2003, 224370; Sega et al., 1989, 224477; Sublet et al., 1989, 061380). Other modes may involve neurotoxic actions impairing copulatory behavior (Zenick et al., 1986, 061394) and sperm motility (Sublet et al., 1989, 061380; Tyl et al., 2000, 224459), both of which are key determinants of male reproductive performance (see Section 4.3 for a more detailed discussion).

AA-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 (Burek et al., 1980, 061311; Friedman et al., 1995, 224307; Johnson et al., 1985, 067932; Johnson et al., 1986, 061340). 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, 061311; Fullerton and Barnes, 1966, 061323).

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, 224307; Johnson et al., 1986, 061340). 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 AA on other potentially susceptible populations.

Variability in internal dose following exposure to AA in the diet or the environment is also an area of active research. Genetic polymorphisms in the AA metabolizing P-450 enzyme CYP2E1 have been identified in humans (Hanioka et al., 2003, 224460) and studied for the impact of a susceptible population to alcohol toxicity (Verlaan et al., 2004, 224503) and to acrylonitrile, a chemical with similar metabolism to AA (Thier et al., 2002, 224405). The polymorphisms result in differences in the Vmax of the enzyme (Hanioka et al., 2003, 224460) 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, 224503) and that a slower CYP2E1-mediated metabolism of acrylonitrile might result in higher acrylonitrile-Hb adducts (and lower N-(cyanoethyl)valine adducts from the metabolite) (Thier et al., 2002, 224405). As discussed for childhood susceptibility, however, the delivery rate of the substrate relative to the values of Km and Vmax for CYP2E1 is an important determinant of the total amount metabolized (or parent compound cleared) (Lipscomb, 2004, 224551; Lipscomb et al., 2003, 192847).

Estimates of daily AA intake in a nonsmoking general population in Germany based on Hb adduct levels in blood and mercapturic acid excretion in urine indicated that children take up approximately 1.3–1.5 times more AA per kg of body weight than adults. The ratio GAMA/AAMA was also significantly higher in the group of young children (6–10 years) with a median level of 0.5 (Hartmann et al., 2008, 224480). In this same study there were no observed gender-related differences in internal exposure and metabolism.

Vesper et al. (2008, 224512) reported highly variable estimates of AA exposure in subgroups of the European Prospective Investigation into Cancer and Nutrition (EPIC) study population (510 subjects from 9 European countries, randomly selected and stratified by age, gender, and smoking status) based upon levels of Hb adducts of AA (HbAA) and its primary metabolite glycidamide (HbGA). A large variability in AA exposure and metabolism between individuals and country groups was observed with HbAA and HbGA values ranging between 15-623 and 8-377 pmol/g of Hb, respectively. Both adducts differed significantly by country, sex, and smoking status.

Clearly, any assessment of health effects for a potentially susceptible population must consider the impact of factors such as age, country of origin, BMI, alcohol consumption, sex, and

smoking status on the internal dose, as well as the susceptibility of any subpopulation to the biological activity of AA or GA at a target site. It is important to note, however, that since both the parent AA and the metabolite GA have adverse effects, different catalytic activities of CYP2E1 or other factors that lead to high variability in the internal dose may result in different spectra of adverse effects, providing an even greater challenge to simple classification schemes for susceptible subpopulations

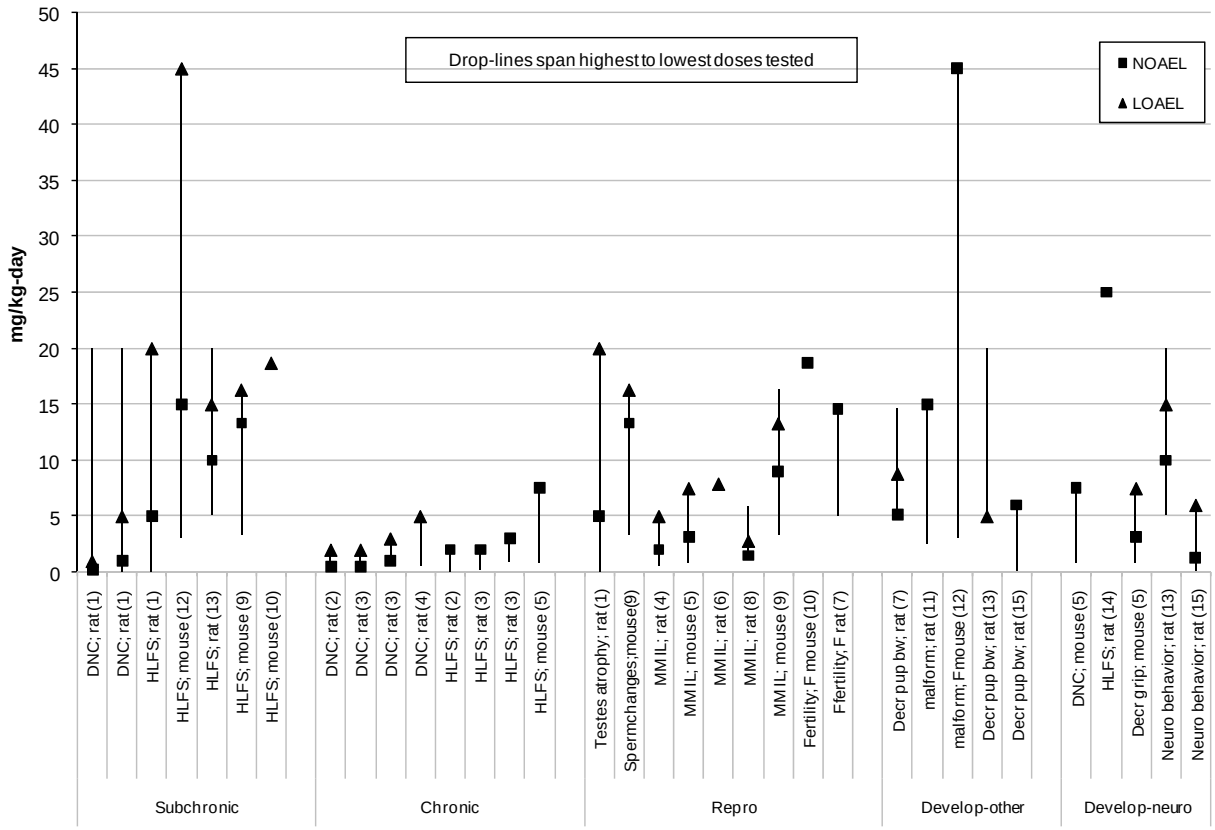
5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RFD)

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

As discussed in Section 4.7.1, 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 Figure 5-1 and Table 5-1): 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.

[10] Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS).



Decr = decreased; Develop = developmental study; DNC = degenerative nerve changes; HLFS = hindlimb foot splay; MM IL = male mediated implantation losses; Malform = fetal malformations and variations; Neuro = neurological; Repro = reproductive study.

References: 1-Burek et al. (1980, 061311); 2-Johnson et al. (1986, 061340); 3-Friedman et al. (1995, 224307); 4-Tyl et al. (2000, 224456); 5-Chapin et al. (1995, 224265); 6,7-Zenick et al. (1986, 061394); 8-Smith et al. (1986, 224276); 9,10-Sakamoto and Hashimoto (1986, 224442); 11,12-Field et al. (1990, 224302); 13-Wise et al. (1995, 224539); 14-Friedman et al. (1999, 224311); 15-Garey and Paule (2007, 224337).

Figure 5-1. AA oral exposure: selected NOAELs and LOAELs.

Table 5-1. AA oral exposure: selected NOAELs and LOAELs (mg AA/kg-day)

Effect/study	Lowest dose tested	NOAEL	LOAEL	Highest dose tested	Reference
Subchronic					
DNC; male and female rat, EM, 90 day	0.05	0.2	1	20	Burek et al. (1980, 061311)
DNC; male and female rat, LM, 90 day	0.05	1	5	20	Burek et al. (1980, 061311)
HLFS; male and female rat, 90 day	0.05	5	20	20	Burek et al. (1980, 061311)
HLFS; female mouse, GDs 6–20	3	15	45	45	Field et al.(1990, 224302)
HLFS; female rat, GDs 6–17	5	10	15	20	Wise et al. (1995, 224539)
HLFS; male mouse, 4 wks before mating	3.3	13.3	16.3	16.3	Sakamoto and Hashimoto (1986, 224442)
HLFS; female mouse, 4 wks before mating	–	–	18.7	18.7	Sakamoto and Hashimoto (1986, 224442)
Chronic					
DNC; male and female rat, LM, 2 yrs	0.01	0.5	2	2	Johnson et al. (1986, 061340)
DNC; male rat, LM, 2 yrs,	0.1	0.5	2	2	Friedman et al. (1995, 224307)
DNC; female rat, LM, 2 yrs	1	1	3	3	Friedman et al. (1995, 224307)
DNC; male rat, LM, two-generation	0.5	–	5	5	Tyl et al. (2000, 224456)
HLFS; male and female rat, 2 yrs	0.01	2	–	2	Johnson et al. (1986, 061340)
HLFS; female rat, 2 yrs	0.1	2	–	2	Friedman et al. (1995, 224307)
HLFS; female rat, 2 yrs	1	3	–	3	Friedman et al. (1995, 224307)
HLFS; male and female mouse, two generations	0.8	7.5	–	7.5	Chapin et al. (1995, 224265)
Reproductive effects					
Testes atrophy; male rat	0.05	5	20	20	Burek et al. (1980, 061311)
Sperm changes; male mouse	3.3	13.3	16.3	16.3	Sakamoto and Hashimoto (1986, 224442)
Male-mediated implantation losses					
MMIL; male rat	0.5	2	5	5	Tyl et al. (2000, 224456)
MMIL; male mouse	0.8	3.1	7.5	7.5	Chapin et al. (1995, 224265)

Effect/study	Lowest dose tested	NOAEL	LOAEL	Highest dose tested	Reference
Subchronic					
MMIL; male rat	7.9	–	7.9	7.9	Zenick et al. (1986, 061394)
MMIL; male rat	1.5	1.5	2.8	5.8	Smith et al. (1986, 224276)
MMIL; male mouse	3.3	9	13.3	16.3	Sakamoto and Hashimoto (1986, 224442)
Fertility; female mouse, 4 wks before mating	18.7	18.7	–	18.7	Sakamoto and Hashimoto (1986, 224442)
Fertility; female rat, 9 wks before mating	5.1	14.6	–	14.6	Zenick et al. (1986, 061394)
Developmental effects - other					
Decreased pup bw; rat	5.1	5.1	8.8	14.6	Zenick et al. (1986, 061394)
Malformations; rat	2.5	15	–	15	Field et al.(1990, 224302)
Malformations; mouse	3	45	–	45	Field et al.(1990, 224302)
Decreased pup bw; rat	5	–	5	20	Wise et al. (1995, 224539)
Decreased pup bw; rat	0.1	6	–	6	Garey and Paule (2007, 224337)
Developmental effects - neurological					
DNC; mouse	0.8	7.5	–	7.5	Chapin et al. (1995, 224265)
HLFD; rat	25	25	–	25	Friedman et al. (1999, 224311)
Decreased grip strength; mouse	0.8	3.1	7.5	7.5	Chapin et al. (1995, 224265)
Neurobehavior; rat, GDs 6–10	5	10	15	20	Wise et al. (1995, 224539)
Neurobehavior; rat, gestation, lactation, to PND 85	0.1	1.3	6	6	Garey and Paule (2007, 224337)

DNC = degenerative nerve changes; HLFS = hindlimb foot splay; MMIL = male mediated implantation losses

As shown in Figure 5-1 and Table 5-1, the most sensitive effects noted in animals are degenerative peripheral nerve changes ("DNC" in Figure 5-1) and male-mediated implantation losses (i.e., male-mediated dominant lethal mutations ["MMIL" in Figure 5-1]). The lowest observed exposure levels associated with peripheral nerve changes are: 1 mg/kg-day for ultrastructural changes associated with degenerative nerve changes (0.2 mg/kg-day NOAEL) in male F344 rats exposed for 90 days (Burek et al., 1980, 061311) and 2 mg/kg-day for degenerative nerve changes detected by light microscopy (0.5 mg/kg-day NOAELs) in male

F344 rats exposed for 2 years in two separate bioassays (Friedman et al., 1995, 224307; Johnson et al., 1986, 061340) . 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, 224276); 5 mg/kg-day (2.0 mg/kg-day NOAEL) in F0 and F1 F344 rats exposed over 10 weeks plus breeding (Tyl, 2000, 224456); 7.5 mg/kg-day (3.1 mg/kg-day NOAEL) in F0 and F1 CD-1 mice exposed over 14 weeks of breeding (Chapin et al., 1995, 224265); and 13.3 mg/kg-day (9.0 mg/kg-day NOAEL) in ddY mice exposed for 4 weeks (Sakamoto and Hashimoto, 1986, 224442). Testicular atrophy in rats and sperm abnormalities in mice have been observed only at oral doses > 15 mg/kg-day, and female fertility and reproductive performance in rats were unaffected at doses in the 15–20 mg/kg-day range (Figure 5-1).

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 5-1). 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 5-1; HLFS in Figure 5-1). As discussed in Section 4.7.1, an exception is one report that exposure to 0.5 mg/kg-day AA induced hindlimb splaying in F0 male F344 rats (Tyl, 2000, 224456), but this report is not consistent with other findings and was not included in Figure 5-1. In the same study, Tyl et al. (2000, 224456) did not observe hindlimb foot splay in the F1-generation rats exposed to doses as high as 5 mg/kg-day, nor was this endpoint reported in the other F344 rats exposed to drinking water doses as high as 2–3 mg/kg-day for 2 years (Friedman et al., 1995, 224307; Johnson et al., 1986, 061340) or 5 mg/kg-day for 90 days(Burek et al., 1980, 061311) (Table 5-1 and Figure 5-1). No increases in fetal malformations or variations were observed in rats or mice following maternal exposure to oral doses as high as 15 or 45 mg/kg-day, respectively (Field et al., 1990, 224302) (Table 5-1 and Figure 5-1). Neurobehavioral assessments of rat offspring found evidence for subtle effects at ≥ 15 mg/kg-day (decreased auditory startle response) (Wise et al., 1995, 224539) and 6 mg/kg-day (decreased cognitive motivation) (Garey and Paule, 2007, 224337); NOAELs for these effects were 10 and 1.3 mg/kg-day, respectively (Table 5-1 and Figure 5-1).

In conclusion, 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.

AA 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. As discussed in Section 4.4, dose-response relationships for heritable germ cell effects in animals (exposed dermally or by i.p. injection to AA) are not well described, particularly at dose levels below 50 mg/kg-day, and possible associations

between human exposure to AA and altered sperm characteristics have not been adequately studied. Thus, although the available data indicate that degenerative nerve changes are the critical effect in animals from chronic oral exposure to AA, additional research may find that heritable germ cell effects may occur at chronic oral exposure levels comparable to those inducing degenerative nerve cell changes.

Two chronic (2-year) drinking water studies (Friedman et al., 1995, 224307; Johnson et al., 1986, 061340) were 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 et al. (1986, 061340) study. These studies are the most appropriate datasets to derive the chronic RfD, rather than the subchronic study by Burek et al. (1980, 061311), primarily due to more appropriate durations of exposure (lifetime versus 90 days) and greater numbers of animals/exposure group (a range of 20 to 88/sex/group in the chronic studies versus 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, 061311) used a more sensitive electron microscopic technique to detect degenerative nerve changes versus the light microscopy used in the 2-year bioassays. The chronic drinking water study by Johnson et al. (1986, 061340) 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, 061311) study evaluated sciatic nerves from only three rats/exposure group (about 150 fields/rat)[11], 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 (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, 0.2, or 1 mg/kg-day groups, equivocal to very slight degeneration in 15/20 rats at 5 mg/kg-day, and moderate to severe degeneration in 20/20 rats at 20 mg/kg-day. The 1 mg/kg-day LOAEL, however, was for only very slight changes that were reversible by day 25 posttreatment, and the

[11] 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, respectively. Approximately 150 fields were examined for each rat; 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.

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). The raw data for the ultrastructural changes in the subchronic study were not available for benchmark analysis, but the results clearly support the findings from the chronic studies.

The two chronic studies provided sufficient data for benchmark analysis, and reproducible 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. These studies are lacking functional testing of neurotoxicity and use a relatively insensitive measure (peripheral axonopathy detected by light microscopy) as the primary index of neurotoxicity, and leave yet unanswered the possibility that lower doses might result in adverse terminal degeneration or other functional deficit prior to the onset of axonal degeneration.

5.1.2. Methods of Analysis—Including Models (BMD, Equivalent AUCs, In Vivo Rate Constants, Etc.)

The methods and models used to derive an RfD include benchmark dose (BMD) models; methods to estimate the serum AUC based upon Hb adduct levels, serum time-course data; and second order rate constants for adduct formation; and a model developed by EPA that estimates rat in vivo adduct formation rate constants based on rat adduct time-course data and various measures of rat serum in vivo AUCs for a given dose rate.

All available models in the EPA Benchmark Dose Software (BMDS version 1.3.1) (U.S. EPA, 2002, 537121) 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, 224307; Johnson et al., 1986, 061340). The modeled data are shown in Table 5-2. The BMR predicted to affect 5% of the population, BMR5, was selected for the POD. A BMR of 5% extra risk was selected for the following reasons: (1) this effect level is considered to be a minimal biologically significant change given the critical effect of degenerative nerve changes; (2) the $BMDL_5$ remained near the range of observation; and (3) the 5% extra risk level is supportable given the relatively large number of animals used in the principal studies.

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

Reference	Dose (mg/kg-day)							
	0	0	0.01	0.1	0.5	1.0	2.0	3.0
Johnson et al. (1986, 061340) (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.[d] (1995, 224307) (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. Incidences for severe degeneration with dose levels in parentheses (in mg/kg-day) were 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, 061340) study, as assessed by a $\chi 2$ goodness-of-fit test. The log-logistic model was the best fitting model for the male rat data as assessed by the Akaike's Information Criterion (AIC). The probit model was the best fitting model for the female rat data as assessed by the AIC. The log-logistic model was thus selected to estimate a BMD from the Johnson et al. (1986, 061340) data. The probit model was selected to estimate the BMD for the female rat data. Table 5-3 (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, 061340) 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 5-3. 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 AA 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, 061340). See Appendix C for model description and results.

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, 224307) study, as assessed by a $\chi 2$ 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 5-4 (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, 224307) 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 5-4. 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 AA 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, 224307). See Appendix C for model description and results.

5.1.3. RfD Derivation—Including Application of Uncertainty Factors

The results of BMD analysis for the male and female rats were similar in the Johnson et al. (1986, 061340) and Friedman et al. (1995, 224307) studies with BMDLs of 0.49 and 0.46 mg/kg-day, respectively, for female rats, and 0.27 and 0.57 mg/kg-day, respectively, for male rats. The lowest of the BMDLs is from the Johnson et al. (1986, 061340) study for male rats

(0.27 mg/kg-day for 5% extra risk for mild-to-moderate lesions), and was chosen as the POD for the most sensitive effect to derive the RfD.

As discussed in Section 3.5, the internal dose (area under a time-concentration curve, AUC) of AA or GA in a rat can be derived for an external exposure to the $BMDL_5$ of 0.27 mg/kg-day. In the external review draft of the Toxicological Review for AA (U.S. EPA, 2007, 418811), a PBTK model was used to estimate the AA-AUC or the GA-AUC following a given external dose of AA. More recent kinetic data on Hb adducts and serum AUCs for AA or GA following a specified administered dose (Doerge et al., 2005, 224348; Doerge et al., 2005, 224355; Doerge et al., 2005, 224344; Fennell et al., 2005, 224299; Tareke et al., 2006, 224387) have become available that require an update of the PBTK model, but that are also sufficiently detailed and appropriate for a direct estimate of the AA-AUC or GA-AUC precluding the need to use a PBTK model for this purpose. EPA reviewed and evaluated these kinetic data, and concluded that the recent data are insightful and that a direct estimate of the AUC in test animals and humans based on these data can be used to derive an HED for an oral exposure (or a human equivalent concentration [HEC] for an inhalation exposure) . The HED is the estimate of the daily human oral intake of AA needed to produce an internal serum level in humans that is comparable to the internal level in the test animals at the oral exposure POD (in this case, the $BMDL_5$).

For the neurotoxicity endpoint, the internal dose metric that is used to derive an HED is the AA-AUC in blood. The HED is further adjusted with uncertainty factors (UFs) to derive the RfD. The sequence of equations needed to derive the rat AUC for the $BMDL_5$ of 0.27 mg AA/kg-day, and to use that AUC to derive the HED are as follows:

(1) Estimate the rat serum AUC_{BMDL5} (μM-hr) for AA based on the daily dose of the $BMDL_5$ as the POD in mg AA/kg-day (or in mg AA/kg/day in the equations below).

$$Rat\ AUC_{BMDL5}(\mu M - hr) = BMDL_5(mg\ AA/kg\text{-}day) \times Conversion\ Factor\left(\frac{\mu M - hr_{F344rat}}{mg\ AA/kg\text{-}day}\right)$$

Equation-8

where the conversion factor of $\mu M\text{-}hr_{F344\ rat}$/mg AA/kg-day is the measured (or estimated) AUC values in rats normalized to a daily administered dose of AA in mg/kg-day.

(2) Estimate of the daily HED (mg AA/kg-day) needed to generate a comparable AUC in humans to that of the rat AUC_{BMDL5}.

$$HED\,(mg\ AA/kg\text{-day}) = Rat\ AA - AUC_{BMDL5}\,(\mu M - hr) \div Conversion\ Factor\,(\frac{\mu M - hr_{human}}{mg\ AA/kg\text{-day}})$$

Equation-9

where the conversion factor of $\mu M\text{-}hr_{human}$/mg AA/kg-day is the measured (or estimated) AUC values in humans normalized to a daily administered dose of AA (mg/kg-day).

The tables discussed in Section 3-5 (Tables 3-5 through 3-7) that summarize the second order rate constants and various measured or estimated μM-hr AUCs per mg AA/kg bw for F344 rats and humans are reproduced below for convenience as Tables 5-5 through 5-7.

Table 5-5. Second-order rate constants for reaction of AA or GA with the N-terminal valine residue of Hb

Source	Second-order rate constant for formation of Hb adducts (L/g globin/hr)×10^{-6}					
	Male rat	Female rat	Pooled male and female rat data	Gender not specified rat	Pooled rat and mouse data	Human
AAVal in vivo adduct formation rate[a]						
Based on all rat and mice Tareke et al. (2006, 224387) adduct data and measured serum AUCs in Doerge et al. (2005, 224348; 2005, 224355) single-dose studies					7.5	
Based on gender-specific rat Tareke et al. (2006, 224387)adduct data and measured serum AUCs in Doerge et al. (2005, 224355) single-dose studies	8.9	5.9				
Based on all rat Tareke et al. (2006, 224387)adduct data and measured serum AUCs in Doerge et al. (2005, 224355) single-dose studies			7.5			
AAVal in vitro rate adduct formation rate						
As reported by Fennell et al. (2005, 224299)	3.82					4.27
As reported by Bergmark et al. (1993, 224424)						4.4
As reported by Tareke et al. (2006, 224387)				2.9		7.4
As reported by Törnqvist et al. (2008, 224428)				4.6		
GAVal in vivo adduct formation rate[a]						
Based on all rat and mice Tareke et al. (2006, 224387) adduct data and measured serum AUCs in Doerge et al. (2005, 224348; 2005, 224355) single-dose studies					32.5	
Based on gender-specific rat Tareke et al. (2006, 224387) adduct data and measured serum AUCs in Doerge et al. (2005, 224355) single-dose studies	38.4	30.7				
Based on all rat Tareke et al. (2006, 224387) adduct data and measured serum AUCs in Doerge et al. (2005, 224355) single-dose studies			34.0			
GAVal in vitro rate adduct formation rate						
As reported by Fennell et al. (2005, 224299)	4.96					6.72
As reported by Bergmark et al. (1993, 224424)				12.0[b]		11.0
As reported by Tareke et al. (2006, 224387)				9.5		59.0
As reported by Törnqvist et al. (2008, 224428)				13.6		

[a]See Appendix E for a description of the derivation of the in vivo adduct formation rates.
[b]Bergmark derived the rat GAVal residue such that kval = (GAVal *kcys)/GA cys; the human GAVal adduct was measured directly.

Table 5-6. Measured and estimated AA-AUCs normalized to dose in humans and F344 rats

	AA-AUC in µM-hr per mg AA/kg bw[12]			
	Male rat	Female rat	Gender not specified rat	Human
AA in humans				
Measured				
Kopp and Dekant (2009, 224532); human serum data AA (single dose of 20 µg/kg, n = 3F,3M)				2.83
Estimated using human adduct data and test animal in vivo rate constants				
Fennell et al. (2005, 224299); human adduct data (single oral exposure) and in vivo rate constants derived from Tareke et al. (2006, 224387) adduct data for all rat and mice in Doerge et al. (2005, 224348; 2005, 224355) single-dose AUCs				140.1
Estimated using human adduct data and human in vitro rate constants				
Fennell et al. (2005, 224299); human adduct data (single oral exposure) and Fennell et al. (2005, 224299) in vitro rate constants				246.0
Fennell et al. (2005, 224299); human adduct data (single oral exposure) and Bergmark et al. (1993, 224424) in vitro rate constants				238.8
Estimated using human adduct data and rat in vitro rate constants				
Fennell et al. (2005, 224299); human adduct data (single oral exposure) and Törnqvist et al. (2008, 224428) in vitro rate constants				228.5
AA in F344 rats				
Measured				
Doerge et al. (2005, 224355) ; time course data from a single dietary exposure	18.0	15.0		
Doerge et al. (2005, 224355); time course data from a single gavage exposure	24.0	45.0		
Tareke et al. (2006, 224387) adduct data for the Doerge et al. (2005, 224344) 49-day drinking water study, and gender-specific in vivo derived rate constants from Tareke et al. (2006, 224387) and Doerge et al. (2005, 224355)	23	37.7		
Tareke et al. (2006, 224387) adduct data for the Doerge et al. (2005, 224344) 49-day drinking water study, and nongender in vivo derived rate constants from Tareke et al. (2006, 224387) and Doerge et al. (2005, 224355)	27.4	29.7		
Estimated using rat adduct data and rat in vitro rate constants				
Törnqvist et al. (2008, 224428); adduct data from a 7-day drinking water study and in vitro rate constants	34.0	48.0		
Fennell et al. (2005, 224299); adduct data (single dose gavage) and in vitro rate constant			80.2	

[12] Note: The reported AUCs were based on a daily administered dose. In the equations below, units are in µM-hr per mg AA/kg-day

Table 5-7. Measured and estimated GA-AUCs normalized to dose in humans and F344 rats

	GA-AUC in µM-hr per mg AA/kg bw[13]			
	Male rat	**Female rat**	**Gender not specified rat**	**Human**
GA in humans				
Estimated using human adduct data and test animal in vivo rate constants				
Fennell et al. (2005, 224299); human adduct data (single oral exposure) and in vivo rate constants derived from Tareke et al. (2006, 224387) adduct data for all rat and mice in Doerge et al. (2005, 224348; 2005, 224355) single dose AUCs				12.5
Estimated using human adduct data and human in vitro rate constants				
Fennell et al. (2005, 224299); human adduct data (single oral exposure) and Fennell et al. (2005, 224299)in vitro rate constants				60.4
Fennell et al. (2005, 224299); human adduct data (single oral exposure) and Bergmark et al. (1993, 224424) in vitro rate constants				37.0
Estimated using human adduct data and rat in vitro rate constants				
Fennell et al. (2005, 224299); human adduct data (single oral exposure) and Törnqvist et al. (2008, 224428) in vitro rate constants				29.9
GA in F344 rats				
Measured				
Doerge et al. (2005, 224355); time course data from a single dietary exposure	19.0	15.0		
Doerge et al. (2005, 224355); time course data from a single gavage exposure	13.0	44.0		
Estimated using rat adduct data and rat in vivo rate constants				
Tareke et al. (2006, 224387)adduct data for the Doerge et al. (2005, 224344) 49-day drinking water study, and gender-specific in vivo derived rate constants from Tareke et al. (2006, 224387)and Doerge et al. (2005, 224355)	14.4	32.3		
Tareke et al. (2006, 224387)adduct data for the Doerge et al. (2005, 224344) 49-day drinking water study, and nongender in vivo derived rate constants from Tareke et al. (2006, 224387)and Doerge et al. (2005, 224355)	16.2	29.2		
Estimated using rat adduct data and rat in vitro rate constants				
Törnqvist et al. (2008, 224428); adduct data from a 7-day drinking water study and in vitro rate constants	18.0	34.0		
Fennell et al. (2005, 224299); adduct data (single dose gavage) and in vitro rate constant			52.1	

[13] Note: The reported AUCs were based on a daily administered dose. In subsequent equations, units are in µM-hr per mg AA/kg-day

5.1.3.1. *Choice of the Rat AUC/mg AA/kg bw Conversion Factor to Derive The Rat AUC_{BMDL5}*

The best AUC conversion factor to use to derive a value for the rat AA-AUC following exposure to the $BMDL_5$ from the Johnson et al. (1986, 061340) study would be a value that was determined directly from in vivo serum measurements following a known dose of AA from a similar dosing regimen (i.e., a drinking water exposure). For the F344 rat, the only directly measured AUC values from serum time course data for male and female rats are from single doses of AA administered by i.v., gavage, or in the diet (Doerge et al., 2005, 224355). Some serum data are available from the Doerge et al. (2005, 224344) drinking water study in F344 rats, however, the published report only provides average serum levels apparently based on point samples from a limited number of days during the 42 day dosing period, and taken at unknown times relative to the intake of AA in the drinking water. Because of the rapid clearance of AA and GA from the blood, a single point sample with unknown time relative to intake is not sufficiently accurate to derive an AUC. A daily AUC from the Doerge et al. (2005, 224344) drinking water study, however, can be derived from the measured Hb adduct levels reported by Tareke et al. (2006, 224387) divided by a second order adduct formation rate. Table 5-5 lists the available adduct formation rates for rat AAVal and GAVal based on in vitro or in vivo data. The most relevant rates are those derived from in vivo data.

The derivation of in vivo adduct formation rates requires three critical types of data from a single study: (1) the administered dose; (2) time course serum levels; and (3) time course adduct levels (including sufficient post dosing sample times to determine elimination rates) if longer than one day of exposure. The only studies meeting this requirement are those from Doerge et al. (2005, 224355) and Tareke et al. (2006, 224387). EPA has derived in vivo gender specific adduct formation rates based upon these single dose in vivo studies in male and female F344 rats. Table 5-8 lists the raw serum AUCs that were available in numerical tables from Doerge et al. (2005, 224355) and the levels of Hb that were taken from bar chart compilations in Tareke et al. (2006, 224387).

Table 5-8. Serum AUC data from Doerge et al. (2005, 224355) (Tables 1 and 3) and Hb adduct level data from Tareke et al. (2006, 224387) (Figure 3) for a 0.1 mg/kg single dose of AA in male and female F344 rats.

			Data extracted from Tareke et al. (2006, 224387) (Figure 3)		Data from Doerge et al. (2005, 224355) (Tables 1 and 3)	
Type of adduct	**Dosed compound**	**sex-route**	**Hb adduct level (pmole/g globin)**	**(SD)[a]**	**AUC (μM-hr)**	**(SD)[b]**
AAVal	AA	M-control	9	4	0	0
AAVal	AA	M-Diet	19.5	4	1.8	0.23
AAVal	AA	M-gavage	20	6	2.4	0.51
AAVal	AA	M-IV	46.5	4	4.1	0
AAVal	AA	F-control	12	4	0	0
AAVal	AA	F-Diet	23	4	1.5	0.15
AAVal	AA	F-gavage	29	14	4.5	0.31
AAVal	AA	F-IV	49.5	4	4.6	0
GAVal	AA	M-control	32.5	12	0	0
GAVal	AA	M-IV	36	4	0.58	0
GAVal	AA	M-gavage	64	20	1.3	0.2
GAVal	AA	M-Diet	98.5	20	1.9	0.35
GAVal	GA	M-IV	140	26	2.8	0
GAVal	GA	M-gavage	123	16	2.8	0.46
GAVal	AA	F-control	45	16	0	0
GAVal	AA	F-IV	48.5	16	0.6	0
GAVal	AA	F-Diet	102	18	1.5	0.2
GAVal	AA	F-gavage	131	66	4.4	0.46
GAVal	GA	F-IV	177	28	3.3	0
GAVal	GA	F-gavage	198	40	3.8	0.5

[a]Standard deviation is estimated from error bars in Tareke et al. (2006, 224387) (Figure 3).
[b]No standard deviations were reported for the IV AUC measurements.

A regression of the Hb adduct levels against the AUCs resulted in a slope that represents the in vivo second order adduct formation rate constant for AAVal or GAVal in units of pmoles per g globin per μM AA-hr or μM GA-hr, respectively. These units can be converted to (L/g globin/hr) $\times 10^{-6}$ with the values of the slopes listed in Table 5-9.

Table 5-9. Regression of Hb adduct levels to AUC to derive the slope of the regression line (i.e., the in vivo second-order rate constants for adduct formation)

Gender	AAVal Slope (SE) [L/g globin/hr]×10^{-6}	GAVal Slope (SE) [L/g globin/hr]×10^{-6}
Male	8.9 (2.2)	38.4 (3.9)
Female	5.9 (2.5)	30.6 (8.7)
Both	7.0 (1.5)	33.8 (4.9)

The second order AAVal adduct formation rate based on in vivo data for male, female, or both genders combined are 8.9, 5.9, and 7.0 (L/g globin/hr)×10^{-6}, respectively. The GAVal adduct formation rates for male, female, or both genders combined are 38.4, 30.6, and 33.8 (L/g globin/hr)×10^{-6}, respectively.

Tareke et al. (2006, 224387) do not report gender specific slopes, but they do report a slope of 7.5 for AAVal (both genders) and 34 for GAVal (both genders). The GAVal slope for both genders from Tareke et al. (2006, 224387) of 34 is the same as the rounded-up value derived by EPA. The higher AAVal formation rate of 7.5 from Tareke et al. (2006, 224387) for the combined male and female rat data compared with the value of 7.0 from EPA's analysis could have resulted from removal of outlier levels of AAVal in the female gavage-dose group. As seen in Table 5-8, the nearly threefold higher standard deviation for the AA Val adduct levels in the gavage female group compared to all other male and female dose groups suggests that there may have been an outlier. The relatively low AAVal mean level of 29 for the female gavage group, compared to the male value of 20, is also suspect considering that the female AUC is nearly twice the value of the males.

With little biological rationale to support gender differences in the rat for the formation of hemoglobin adducts, and the EPA analysis of the reported Doerge et al. (2005, 224355) and Tareke et al. (2006, 224387) data closely reproducing the rates for both genders reported by Tareke et al. (2006, 224387), EPA chose to use the AAVal adduct formation rate of 7.5 (L/g globin/hr)×10^{-6} and the GAVal adduct formation rate of 34.0 (L/g globin/hr)×10^{-6} in a model simulation that estimated the blood concentrations needed to generate the levels of Hb adducts reported by Tareke et al. (2006, 224387) from the rats exposed to AA in the drinking water in the Doerge et al. (2005, 224344) study. These estimates were then used to derive the AUC and the conversion factors for AA-AUC and GA-AUCs in male or female rats for a given external exposure to AA. Appendix E provides additional details on the model parameters, model code, and supporting data, as well as examples of the fits to the Hb adduct level data.

The estimated AUCs in blood for AA or GA were used in conjunction with the known external dose of 1 mg/kg-day to derive the following conversion factors for the internal AA-AUC or GA-AUC in rats per mg AA/kg-day from a drinking-water exposure (Tables 5-6 and 5-7).

AA-AUC in μM-hr AA per mg AA/kg-day
= 27.4 (for males) and 29.7 (for females) Equation-10

GA-AUC in μM-hr GA per mg AA/kg-day
= 16.2 (for males) and 29.2 (for females) Equation-11

The above values were used to estimate the rat internal AUC that would result from the $BMDL_5$ (i.e., as the POD) for neurotoxicity. The POD for neurotoxicity is a $BMDL_5$ of 0.27 mg AA/kg-day in male F344 rats from the Johnson et al. (1986, 061340) study. As discussed in the section on the MOA of AA induced neurotoxicity, there is uncertainty as to whether AA or GA or both are responsible for the observed effects, yet the current evidence tends to favor AA as the putative neurotoxin. Based on a choice of the parent AA as the putative neurotoxin, and using the male (i.e., gender specific) AA-AUC conversion factor of 27.4 μM-hr per mg AA/kg-day, the estimated F344 male rat $AA\text{-}AUC_{BMDL}$ (as the POD) from exposure to a $BMDL_5$ of 0.27 mg AA/kg-day is 7.4 μM-hr:

$$AA-AUC_{BMDL,\,male\ rat}\ (\mu M-hr) = BMDL_{5,\,male\ rat} \times AA-AUC\ conversion\ \ factor_{male\ rat}$$

$$= 0.27\ mg\ AA/kg\text{-day} \times \frac{27.4\ \mu M-hr_{male\ rat}}{mg\ AA/kg\text{-day}} = 7.4\ \mu M-hr \qquad \text{Equation-12}$$

5.1.3.2. ***Deriving the HED***

The HED is the administered dose in humans that would result in the same internal serum AA-AUC as produced in the male rat from the male rat $BMDL_5$ dose (i.e., an internal AA-AUC of 7.4 μM-hr). No direct measurement of human serum levels of AA are available to derive an $AA\text{-}AUC_{human}$/mg AA/kg-day conversion factor except from one study by Kopp and Dekant (2009, 224532) where human time course serum concentrations of AA were measured for 2 hours following a single oral dose of 20 μg AA/kg bw. As can be seen in Table 5-6, however, the resulting AA-AUC/mg AA/kg bw of 2.8 $\mu M\text{-}hr_{human}$ per mg AA/kg bw is grossly under the other estimates of from 140 to 246 $\mu M\text{-}hr_{human}$ per mg AA/kg bw, based on Hb adduct levels and various second order rate constants. Hb adduct levels were not measured by Kopp and Dekant (2009) to help resolve why there is such discordance with other estimates, and until resolved, the Kopp and Dekant (2009, 224532) AUC data are not considered sufficient for use in deriving the human AA-AUC conversion factor.

The other options for a human conversion factor listed in Table 5-6, however, are sufficient to estimate the AA-AUC per administered dose for use in this toxicological review. These values are based on the Fennell et al. (2005, 224299) in vivo data for human AAVal levels per administered dose divided by various second order rate constants derived from in vivo or in vitro data. These values span about twofold, from the lowest value of 140 to the highest of 246. A comparable spread of twofold is seen in the HED values based on these data. EPA

considers the most relevant and accurate adduct formation rate constant to be the one estimated from the linear regression of in vivo adduct levels versus AA-AUCs for all male and female mice and rats combined from the single dose studies of Doerge et el. (2005, 224348; 2005, 224355) and Tareke et al. (2006, 224387). This analysis estimated the in vivo AAVal formation rate to be 7.5×10^{-6} L/g globin/hour (see Table 5-5 and further discussion in Appendix E). The choice of a non-gender specific in vivo formation rate for humans is supported by the epidemiology results of Hartmann et al. (2008, 224480), who did not observe a gender-related difference in internal exposure and metabolism of AA in a study of a nonsmoking general population especially designed for an even distribution of age and gender.

The human AA-AUC conversion factor is calculated by dividing the measured human AAVal adduct level of 74.7 nmol of AAVal/g globin/mmol AA/kg bw (Fennell et al., 2005, 224299) by the in vivo AAVal adduct formation rate of 7.5×10^{-6} L/g globin/hr resulting in an AA-AUC conversion factor of 140.1 µM AA-hour$_{human}$ per mg AA/kg-day (Tables 5-5 and 5-6).

$$AA-AUC\ conversion\ factor_{human} = AAVal\ \ level \div AAVal\ \ formation\ rate \qquad \text{Equation-13}$$

$$AA-AUC\ conversion\ factor_{human} = \frac{74.7\ nmol\ AAVal}{(g\ globin)(mmoles\ AA/\ kg\ \text{-}\ \text{day})} \div \frac{7.5x10^{-6}\ L}{(g\ \ globin)(h)}$$

$$= \frac{9.96\ mM\ \ AA-hr}{mmoles\ AA/\ kg\ \text{-}\ \text{day}} \qquad \text{Equation-14}$$

The above human serum AA-AUC of 9.96 mM AA-hr/mmoles of AA/kg bw is converted to 140.1 µM AA-hr per mg AA/kg-day by converting mmoles of AA as reported in Fennell et al. (2005, 224299) to mg AA (by dividing by the molecular weight of AA [71.08]), and by converting mM to µM as follows:

$$AA-AUC\ conversion\ factor_{human} = \frac{9.96 mM\ AA-hr}{mmoles\ \ AA/\ kg\ \text{-}\ \text{day}}\ x\ \frac{1\ mmole\ AA}{71.08\ mg\ \ AA}$$

$$= \frac{0.1401\ mM\ \ AA-hr}{mg\ AA/\ kg\ \text{-}\ \text{day}}\ x\ \frac{1000\ \mu M}{1\ mM} = \frac{140.1\ \mu M\ \ AA-hr_{human}}{mg\ AA/\ kg\ \text{-}\ \text{day}} \qquad \text{Equation-15}$$

The HED based on this human AA-AUC conversion factor of 140.1 μM AA-hr/mg AA/kg-day and the rat AA-AUC_{BMDL5} of 7.4 μM-hr is 0.053 mg AA/kg-day.

$$HED = AA - AUC_{BMDL,male\,rat} \div AUC\ conversion\ \ factor_{human} \quad \text{Equation-16}$$

$$HED\ (mg\ AA/kg\text{-day}) = 7.4\ \mu M - hr \div \frac{140.1\ \mu M - hr_{human}}{mg\ AA/kg\text{-day}}$$

$$= 0.053\ mg\ AA/kg\text{-day} \quad \text{Equation-17}$$

5.1.3.3. ***Derivation of the RfD***

The HED (i.e., the human POD) as a daily dose of 0.053 mg AA/kg bw is divided by a total UF of 30 to derive the RfD: 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

$= 3\ (UF_{A\text{-}TD}) \times 1\ (UF_{A\text{-}TK}) \times 10\ (UF_H) \times 1\ (UF_S) \times 1\ (UF_L) \times 1\ (UF_D)$ Equation-18

A 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 provides support for the $UF_{A\text{-}TD}$ of 3. The equivalent AUC method was used to account for intraspecies toxicokinetic differences, and thus, the $UF_{A\text{-}TK}$ = 1 instead of the default value of 3.16 ($10^{1/2}$).

A 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 selected.

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

A UF to account for the extrapolation from a LOAEL to a NOAEL (UF_L) was not applied because the current approach is to address this extrapolation as one of the considerations in selecting a BMR for BMD modeling (i.e., UF_L =1). In this case, EPA concluded that a 5%

increase in response, is appropriate for use in deriving the RfD under the assumption that it represents a minimal biologically significant change.

A 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 doses comparable to those inducing degenerative nerve lesions with chronic oral exposure. 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. As discussed in Section 4.4, the magnitude of response at low doses, and the shape of the low dose-response curve for potentially serious heritable germ cell effects, are also research needs. Some of these data needs are currently being addressed.

The RfD for AA was calculated as follows:

$$\begin{aligned} \text{RfD} &= \text{HED} \div \text{UF} \\ &= 0.053 \text{ mg/kg-day} \div 30 \\ &= 0.002 \text{ mg/kg-day (rounded to one significant digit)} \end{aligned} \qquad \text{Equation-19}$$

5.1.4. Previous RfD Assessment

This RfD replaces the previous RfD for AA 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, 061311). The RfD was derived by dividing the NOAEL by a UF of 1,000: 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 (Friedman et al., 1995, 224307; Johnson et al., 1986,

061340) , as well as current methodology for characterizing the dose-response curve, for determining the POD (i.e., the BMDL), and for deriving the HED.

5.2. INHALATION REFERENCE CONCENTRATION

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

As discussed in Section 4.1, 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 Hb 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, 202900) or increased incidences in self-reported symptoms of neurological impairment and eye and respiratory irritation (Hagmar et al., 2001, 224453). These studies, however, provide limited 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 Hb) is not specific for AA alone (i.e., NMA can form the same adduct). Although these data are limited, EPA did derive an RfC from the Calleman et al. (1994, 202900) data for comparison purposes (Appendix F). The preferred derivation for the RfC, however, is based on a route-to-route extrapolation directly from the oral exposure POD.

The justification for deriving an RfC directly from the oral exposure POD used as the basis for the RfD includes: (1) a well characterized dose-response and identification of the most sensitive noncancer endpoint from an adequate database of oral exposure studies; (2) considerable evidence from occupational experience that dermal and inhalation exposures to AA induce peripheral neuropathies, including development of the types of degenerative lesions observed in nerves of rats exposed via drinking water; (3) evidence of rapid, nearly complete absorption from the oral route and rapid distribution throughout the body (Kadry et al., 1999, 224596; Miller et al., 1982, 061351); (4) evidence that the elimination kinetics of radioactivity from oral or i.v. administration of radiolabeled AA in rats is similar (Miller et al., 1982, 061351); (5) similar flux of AA through metabolic pathways following either single dose oral or single 6 hr inhalation exposures in rats (Sumner et al., 2003, 224347); (6) some route-to-route differences in the relative amounts of AA to GA, however, the differences are within twofold of each other; and (7) lack of support for portal of entry effects.

In the only animal inhalation kinetic study (i.e., no human inhalation kinetic information is available) Sumner et al. (2003, 224347) report statistically significantly larger percentages of urinary metabolites associated with GA formation following an inhalation exposure compared with an i.p. and gavage exposure. GAVal levels are also higher and AAVal levels lower (as indicators of serum AUCs), following the single 6 hr inhalation exposures versus the single gavage dose in rats, however, statistical significance was not reported for the adduct level differences, and the numbers are within twofold of each other. Doerge et al. (2005, 224348; 2005, 224355) report an increased percentage of GA formation observed in mice and F344 rats from a gavage or dietary exposure compared to an i.v. exposure that, in conjunction with the Sumner et al. (2003, 224347) results, indicate that there is first pass metabolism in the lungs following an inhalation exposure similar to the first pass metabolism in the liver from an oral exposure, but apparently the lungs may have a larger percent of oxidative metabolism of AA to GA.

Although in this only available inhalation kinetic study by Sumner et al. (2003, 224347) there do appear to be some route-to-route differences in the relative amounts of AA to GA, the differences are within twofold of each other, and the metabolic paths and total disposition are similar, supporting the derivation of the RfC based upon the oral POD used as the basis for the RfD.

The level of AA in the air that would result in a comparable intake to the oral exposure POD (i.e., the HEC, the human equivalent air concentration) is based on a 70 kg person who breathes 20 m^3 of air-day. The BMR predicted to affect 5% of the population was selected for the POD. A BMR of 5% extra risk was selected for the following reasons: (1) this effect level is considered to be a minimal biologically significant change given the critical effect of degenerative nerve changes; (2) the $BMDL_5$ remained near the range of observation; and (3) the 5% extra risk level is supportable given the relatively large number of animals used in the principal studies.

5.2.2. Methods of Analysis— Including Models (BMD, Equivalent AUCs, In Vivo Rate Constants, Etc.)

See Section 5.1 for derivation of the chronic oral RfD for AA, Section 3.5 for a discussion of the use of Hb adducts and AUCs to derive an HED, and Appendix E for details on the model used to estimate of in vivo second order rate constants for the formation of Hb adducts.

5.2.3. RfC Derivation—Including Application of Uncertainty Factors

The $BMDL_5$ for degenerative nerve lesions in male rats exposed to AA in drinking water for 2 years is taken as the POD for deriving the RfC. The internal dose metric remains the AA-AUC in male rat blood, and the HEC is calculated as the intake required to produce that same AUC value in human blood from an inhalation exposure as would an oral dose of

0.053 mg/kg-day (i.e., the oral HED_{BMDL}). The air concentration that would provide a 70 kg person who breathes 20 m^3 of air (U.S. EPA, 1994, 006488) that amount of daily intake is 0.18 mg/m^3 as follows[14]:

$$Air\ HEC_{BMDL} = Oral\ HED_{BMDL} \times 70\,kg \div (\frac{day}{20\,m^3})$$

$$= (0.053\,mg/kg\text{-day}) \times 70\,kg \div (\frac{day}{20\,m^3}) = 0.18\,mg/m^3$$

Equation-20

The HEC_{BMDL} of 0.18 mg/m^3 is the point of departure for a continuous inhalation exposure, and is divided by a total UF of 30 to derive the RfC: 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

$= 3\ (UF_{A\text{-}TD}) \times 1\ (UF_{A\text{-}TK}) \times 10\ (UF_H) \times 1\ (UF_S) \times 1\ (UF_L) \times 1\ (UF_D)$ Equation-21

A 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 the $UF_{A\text{-}TD}$ of 3. The equivalent AUC method was used to account for intraspecies toxicokinetic differences, and thus the $UF_{A\text{-}TK} = 1$ instead of the default value of 3.16 ($10^{1/2}$).

A 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 selected.

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

[14] Numerical values used in the derivation of the HEC_{BMDL} (0.18 mg/m^3) were rounded for presentation purposes in Equations 12 -17. Derivation of the HEC_{BMDL} results in an actual value of 0.184818 mg/m^3.

A UF to account for the extrapolation from a LOAEL to a NOAEL (UF_L) was not applied because the current approach is to address this extrapolation as one of the considerations in selecting a BMR for BMD modeling (i.e., UF_L =1). In this case, EPA concluded a 5% increase in response, is appropriate for use in deriving the RfD under the assumption that it represents a minimal biologically significant change.

A UF to account for database deficiency is not necessary for this derivation (i.e., UF_D = 1) because an AUC equivalence method was used to conduct the route-to-route extrapolation based on 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 AA is calculated as follows:

$$\begin{aligned} RfC &= HEC_{BMDL} \div UF \\ &= 0.18\ mg/m^3 \div 30 \\ &= 0.006\ mg/m^3 \text{ (rounded to one significant digit)} \end{aligned} \qquad \text{Equation-22}$$

5.2.4. Previous RfC Assessment

The previous IRIS assessment did not derive an RfC for AA.

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 AA. 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 POD; (4) the use of the oral POD to derive the RfC (i.e., the route-to-route extrapolation; and (5) the uncertainties in the derivation of the HED based on human Hb adduct data and second order rate constants.

U.S. EPA has developed default UFs to account for uncertainties in an RfD or RfC due to missing or inadequate data (U.S. EPA, 1994, 006488; U.S. EPA, 2002, 088824). The default UFs 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. UFs are used in the derivation of the RfD and RfC to adjust the POD downward and thus reduce the potential risk of adverse effects to public health.

The specific UFs used in deriving the AA RfD and RfC were previously discussed in Sections 5.1.3 and 5.2.3, respectively. A methodology to extrapolate an internal AUC was available to account for interspecies toxicokinetic differences. Default UFs 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 UFs used in the derivation of the RfD and RfC. Key points in the discussion are summarized in Table 5-10.

Table 5-10. Summary of uncertainty in the AA 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 increase the estimated risk in humans from exposure to AA.	The available AA 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. Although the human data are limited, they clearly demonstrate neurotoxicity as the predominant observable noncancer adverse effect. Although animal studies for inhalation exposures are limited, kinetic studies in animals and humans indicate no critical route specific endpoints. Heritable germ cell effects have been reported in animal studies at much higher levels of exposure (i.p. or dermal, 40–125 mg/kg), and further research is warranted to evaluate the potential for these effects at lower doses.
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 increase 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 (i.p. or dermal, 40–125 mg/kg), and further research is warranted to evaluate the potential for these effects at lower doses. Other reproductive effects have been observed in animals, but at levels three- to fivefold 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 increase or decrease the estimated risks to humans.	A BMD analysis with multiple model choices resulted in adequate fits to the AA dose-response data and provided valid estimates of the POD.	A number of BMD models provided reasonable fits to the AA 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 high degree of confidence in the BMD approach.
Use of the equivalent AUC method to estimate an oral exposure HED to derive the RfD	An alternate approach (e.g., using default UFs) could either increase or decrease the estimated risks to humans.	The AUC method used to estimate the human equivalent dose used in the derivation of the RfD.	The development of an HED based on estimates of the internal AUC/mg AA/kg bw provides a better estimate of interspecies toxicokinetic differences than UFs, and is more scientifically supportable. The choice of a nongender-specific in vivo formation rate for humans is supported by the epidemiology results of Hartmann et al. (2008, 224480) who did not observe a gender-related difference in internal exposure and metabolism of AA in a study of a nonsmoking general population especially designed for an even distribution of age and gender. Additional human serum data and in vivo adduct formation rate data, however, are needed to reduce uncertainty in the estimate of human AUC per intake of AA using the equivalent AUC method, or to develop a PBPK model that would provide additional capability to evaluate different dose metrics or dosage regimens.

Consideration/ approach	Impact on noncancer risk estimate	Decision	Justification
Estimate of 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 increase or decrease the estimated risks to humans.	Use an HEC for the inhalation exposure comparable to the daily intake level of the HED derived using the AUC method for an oral exposure.	Justification for deriving an RfC from the oral RfD is based on animal kinetic data suggesting some differences in relative levels of GA and AA between the inhalation and oral route, but sufficient similarities in metabolic pathways and internal disposition to support the extrapolation based on the oral POD. Additional animal or human inhalation kinetic data are needed to verify the limited available data, and to reduce uncertainty in the route-to-route extrapolation, as well as to develop a PBPK model that would provide additional capability to evaluate different dose metrics or dosage regimens. The alternate RfC based on the Calleman et al. (1994, 202900) data is comparable to the RfC based on the route-to-route extrapolation, and provides some additional scientific support for this value.
Default UF 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 UF for interspecies differences in toxicodynamics could increase or decrease the estimated risks to humans.	The default toxicodynamic UF was used in conjunction with the AUC method for deriving an HED/HEC in the derivation of the RfD and RfC.	The default UF for toxicodynamic differences is used in the absence of adequate chemical or species specific data to support a more informed extrapolation.
Default UF used to account for intrahuman variability: $UF_H = 10$	The magnitude of possible over- or underestimation in the default factor for intrahuman differences could increase or decrease the estimated risks to humans.	The default UF 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.

5.3.1. Areas of Uncertainty

5.3.1.1. *Completeness of the Database*

The human data for potential noncancer adverse effects from exposure to AA 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-Hb adduct levels and measures of neurological impairment in AA workers (Calleman et al., 1994, 202900; Hagmar et al., 2001, 224453), and one kinetic study in 24 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, 224299). No human studies were identified on the potential for

adverse reproductive or developmental effects from exposure to AA 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, 1953, 224514; Hazleton Laboratories, 1954, 061399) 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 AA is rapidly absorbed and distributed when it enters the body from either an oral or inhalation exposure (Sumner et al., 2003, 224347). 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 (Chapter 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 AA (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 AA. 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.

Although a database deficiency UF does not appear to be warranted in the derivation of the RfD and RfC ($UF_{DB} = 1$), 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 doses lower than those identified in currently available animal studies. As discussed in Section 4.4, single or repeated i.p. doses of AA or GA ranged from 40 to 125 mg/kg and one study employed five daily dermal applications of AA at 50 mg/kg. Heritable translocations appeared at high frequency at the lowest doses tested, which indicates that lower doses may have also elicited heritable translocations. Well-designed animal studies are needed to assess oral exposure dose-response relationships for AA- and GA-induced heritable germ cell effects,

particularly in the low dose region. Any future studies of possible associations between AA exposure and sperm characteristics in humans should adjust for smoking history and alcohol consumption, especially due to the growing evidence of associations between cigarette smoking and altered sperm endpoints.

5.3.1.2. *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 three to fivefold higher (i.e., a less sensitive response) than observed for neurotoxicity. Germ cell effects (e.g., heritable translocations or mutations, dominant lethals) have also been reported in animal studies, and are a potentially more serious adverse event than neurotoxicity, because heritable germ cell effects can occur not only in the exposed individual, but also in their offspring and subsequent generations. Heritable germ cells effects, however, have only been observed at relatively high levels of AA exposure in animal studies (40–125 mg/kg, i.p. or dermal) and there are no oral or inhalation exposure studies examining heritable germ cell effects.

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 AA. 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 FDA 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. In addition, the use of a relatively insensitive measure of neurotoxicity in the chronic principal studies (peripheral axonopathy detected by light microscopy, as opposed to ultrastructural changes detected by electron microscopy), raises concern for the possibility that, in looking at axonal degeneration, preceding terminal degeneration may have been missed, particularly at lower doses.

5.3.1.3. *Dose-Response Modeling and Determination of the POD*

BMD modeling was used to estimate the POD for the AA 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 BMDS (version 1.3.1) (U.S. EPA, 2002, 088824) 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, 224307; Johnson et al., 1986, 061340). The BMR predicted to affect 5% of the population, BMR5 was selected for the POD rather than the more commonly chosen BMR of 10% for the following reasons (1) the 95% lower bound of the 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, 005992).

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 the 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 AA, 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 5-11, all of the final POD estimates are within twofold 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 AA 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 5-11. 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 AA in drinking water

Model	BMD (ED_5)	BMDL (LED_5)
Johnson et al. (1986, 061340)		
Male		
Log-logistic	0.58	**0.27**
Female		
Probit	0.67	0.49
Friedman et al. (1995, 224307)		
Male		
Quantal quadratic	0.77	0.57
Female		
Gamma[a]	2.25	0.46

[a]Restrict power ≥ 1.

5.3.1.4. *Uncertainty in the Animal-to-Human Extrapolation AUC Method Used to Estimate the Oral Human Equivalent Dose*

The AUC methodology used to estimate the oral human equivalent dose (HED) (i.e., extrapolate the animal dose-response relationship to humans) to derive the RfD is dependent upon the accuracy of the measured or estimated conversion factors for estimating the rat and human AUCs/mg AA/kg bw. Currently there is a lack of sufficient in vivo data to accurately estimate human in vivo rate constants for the formation of Hb adducts. The range of HED values for the human AA-AUC, using a variety of alternate rate constants, are reasonably concordant with a range of values only twofold different from the lowest to the highest estimate. There is a fivefold range (and thus greater uncertainty) in estimated HEDs based on the human GA-AUC because of a wider range in the values of the available rate constants and conversion factors. Additional data are clearly needed to improve the accuracy and precision of these critical rate constants and conversion factors, not only for the derivation of the current reference values, but also to further improve estimates of the daily intake level in the general public based on Hb adduct levels as a biomarker of exposure.

5.3.1.5. *Uncertainty in the Route-to-Route Extrapolation to Derive the RfC*

A route-to-route extrapolation (oral-to-inhalation) of the dose-response relationship was performed to derive the RfC using the HED as the POD. Justification for deriving an RfC based on the oral POD comes from sufficient similarities in the metabolic pathways and internal disposition of GA and AA observed in animal kinetic studies using either the inhalation or oral route of exposure. More specifically, there is: (1) considerable evidence from occupational experience that dermal and inhalation exposures to AA induce peripheral neuropathies, including

development of the types of degenerative lesions observed in nerves of rats exposed via drinking water; (2) evidence of rapid, nearly complete absorption from the oral route and rapid distribution throughout the body (Kadry et al., 1999, 224596; Miller et al., 1982, 061351); (3) evidence that the elimination kinetics of radioactivity from oral or i.v. administration of radiolabeled AA in rats is similar (Miller et al., 1982, 061351); (4) similar flux of AA through metabolic pathways following either single dose oral or single 6 hr inhalation exposures in rats (Sumner et al., 2003, 224347); (5) some route differences in relative GA and AA serum levels, but with numbers that are within twofold of each other; and (6) lack of support for portal of entry effects. Additional animal or human inhalation kinetic data are needed to reduce the uncertainty in quantitating the internal disposition of AA or GA following different routes of exposure.

The alternate RfC based on the Calleman et al. (1994, 202900) data is comparable to the RfC based on the route-to-route extrapolation, and provides some additional scientific support for this value.

Since there are no credible default methods to estimate a safe daily inhaled intake level in the absence of inhalation study data or relevant animal and human kinetic data, the level of uncertainty in the RfC based on sufficient similarities in the disposition of AA and GA regardless of exposure route must be compared to the complete uncertainty of having no RfC. Additional animal or human inhalation kinetic data are needed to reduce the uncertainty in quantitating potential route differences in disposition.

5.3.1.6. *Use of Default Factors for the Interspecies Differences in Toxicodynamics in Conjunction with the Equivalent AUC Method to Derive the HED*

The equivalent AUC method to estimate the HED replaced the default factor for interspecies toxicokinetic differences of 3 ($UF_{A\text{-}TK}$ = 3.16 as a default; $UF_{A\text{-}TK}$ = 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)[15]. Thus the overall default factor for interspecies differences using the model was 3 (UF_A = 3 = $UF_{A\text{-}TK}$ of 1×$UF_{A\text{-}TD}$ of 3). This compares to a default factor of 10 without the use of the AUC method (UF_A = 10 = $UF_{A\text{-}TK}$ of 3.16×$UF_{A\text{-}TD}$ of 3.16). In the case of AA, using the default approach to derive the RfD[16] would result in a value 0.003 mg/kg-day as the RfD. One interpretation of this similarity is that the interspecies differences for parent AA toxicokinetics might scale roughly to the ratio of body weight to the ¾ power which for extrapolating between an average rat (250–350 g) and human (70kg) is approximately a fourfold reduction in dose on a mg/kg basis.

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[15] 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, 3.16 is rounded down to 3.

[16] 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.

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

5.3.1.7. *Intrahuman Variability*

Heterogeneity among humans is another source of uncertainty. In the absence of AA-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.

5.3.1.8. *Subchronic-to-Chronic Exposure Extrapolation*

Chronic oral toxicity studies for AA 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., $UF_S = 1$).

5.4. CANCER ASSESSMENT

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

As summarized in Section 4.8.1, AA 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 (Friedman et al., 1995, 224307; Johnson et al., 1986, 061340); 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., 1984, 202896); and increased incidences of lung tumors in strain A/J mice following i.p. injection of AA (Bull et al., 1984, 202897). In addition, one of the F344 rat chronic drinking water bioassays also found increased incidences of adrenal pheochromocytomas in males and CNS tumors of glial origin and oral cavity tumors in females (Johnson et al., 1986, 061340).

Human studies provide limited evidence of AA carcinogenicity (as discussed in Sections 4.1, 4.8.1, and 4.8.2). No statistically significant increased risks for cancer-related deaths were consistently found in the cohort mortality studies of AA workers (Marsh et al., 2007, 224578; Swaen et al., 2007, 224357). In most case-control studies and prospective studies, 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, pharyngeal, esophageal, laryngeal, ovarian, or prostate cancer. One case-control study reported a slightly increased risk of breast cancer later in life associated with the consumption of French fries during preschool (Michels et al., 2006, 224586), but there is considerable uncertainty in the accuracy of the exposure assessment methods. Increased risks of postmenopausal endometrial and ovarian cancer (Hogervorst et al., 2007, 224520) and renal cell

cancer (Hogervorst et al., 2008, 224521) with increasing dietary AA intake were reported in prospective studies of a Dutch population, but estimations of dietary AA levels in foods on the market at baseline in 1986 were based on food samples analyzed since 2001 and questionnaires did not include details regarding specifics of food preparation. Olesen et al. (2008, 224303) reported a significant positive association between AA-Hb adduct levels in red blood cells and ER+ breast cancer after adjusting for smoking, but this study is limited by the relatively small number of subjects (374 cases and 374 controls) and uncertainty regarding extrapolation to lifetime exposure from AA exposure as assessed by a few months of AA-Hb adduct measurements.

The mechanisms by which AA induces cancer in animals are not fully understood, however, the weight of the scientific evidence strongly supports a mutagenic MOA, as discussed in Section 4.8.3.1. 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, however, the available evidence in support of these hypotheses is judged to be inadequate. Therefore, the cancer dose-response relationships for tumors with statistically significantly elevated incidences in both of the available rat bioassays (thyroid tumors in both sexes, mammary gland tumors in females and TVMs in males) are the best available basis for deriving an oral cancer slope factor and IUR for AA.

The two chronic bioassays with F344 rats provide appropriate data to describe dose-response relationships for tumors induced by chronic oral exposure to AA. Strengths in both assays include sufficient numbers of animals in control and multiple exposure groups for statistical analysis of dose-response relationships, histological examinations of most tissues, and sufficient reporting of experimental details and results. Johnson et al. (1986, 061340) reported increased tumor incidences at sites in females (CNS, oral cavity, uterus, and pituitary) and males (adrenals), which were reported to not be elevated in the Friedman et al. (1995, 224307) bioassay. However, the Johnson et al. (1986, 061340) 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, 224307) study was designed to include different dose spacings to support better characterization of dose-response relationships in the low-dose region and substantially larger control (n = 204) and 0.1 mg/kg-day male rat (n = 204) groups to increase the statistical power in the study to detect significantly increased tumor incidence. Although glial tumors of brain and spinal cord were reported by Friedman et al. (1995, 224307) 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 excluded from the Friedman et al. (1995, 224307) analysis of the data. In addition incidences of oral cavity tumors, clitoral gland adenomas and uterine adenomas were reported not to be increased, but the number of these tumors was not reported.

Dose-response data from both bioassays were therefore analyzed for potential PODs in the derivation of an oral slope factor (Sections 5.4.2, 5.4.3, and 5.4.4).

5.4.2. Dose-Response Data

As discussed in the previous section, incidence data for tumors in both studies were evaluated to determine the best basis for the oral slope factor. These data included thyroid tumors in male and female rats, TVMs in male rats, and mammary gland tumors in females from the Friedman et al. (1995, 224307) bioassay; and thyroid tumors in male and female rats, TVMs, and adrenal pheochromocytomas in male rats, and mammary gland tumors, CNS tumors of glial origin, and oral cavity tumors in female rats from the Johnson et al. (1985, 067932) bioassay. Incidences of tumors with statistically significant increases in the 2-year bioassays with F344 rats exposed to AA in drinking water are shown in Table 5-12.

Table 5-12. Incidence of tumors with statistically significant increases in 2-year bioassays with F344 rats exposed to AA in drinking water

Reference/tumor type	Dose (mg/kg-day)							
	0	0	0.01	0.1	0.5	1.0	2.0	3.0
Johnson et al., (1986, 061340); males								
Follicular cell adenoma	1/60	–	0/58	2/59	1/59	–	7/59[e]	–
TVM	3/60	–	0/60	7/60	11/60[e]	–	10/60[e]	–
Adrenal pheochromocytoma	3/60	–	7/59	7/60	5/60	–	10/60 [e]	–
Johnson et al., (1986, 061340); females								
Follicular cell adenoma/carcinoma	1/58	–	0/59	1/59	1/58	–	5/60[f]	–
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[e]	–
CNS tumors of glial origin	1/60	–	2/59	1/60	1/60	–	9/61[e]	–
Oral cavity malignant + benign	0/60	–	3/60	2/60	3/60	–	8/60[e]	–
Uterus adenocarcinoma	1/60	–	2/60	1/60	0/59	–	5/60[f]	–
Clitoral adenoma, benign	0/2	–	1/3	3/4	2/4	–	5/5[f]	–
Pituitary gland adenoma	25/59	–	30/60	32/60	27/60	–	32/60[f]	–
Friedman et al., (1995, 224307); males[b]								
Follicular cell adenoma/carcinoma	3/100	2/102[d]	–	12/203	5/101	–	17/75[e]	–
TVM[c]	4/102	4/102	–	9/204	8/102	–	13/75[e]	–
Friedman et al., (1995, 224307); 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, 224307) and not those reported in the reevaluation study by Iatropoulos et al. (1998, 224628).
[d]The data reported in Table 4 in Friedman et al. (1995, 224307) lists one follicular cell adenoma in the second control group; however, the raw data obtained in the Tegeris Laboratories (1989, 224400) 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 significantly ($p < 0.05$) different from control, Fisher's exact test.
[f]Statistically significantly ($p < 0.05$) different from control, after Mantel-Haenszel mortality adjustment.

Sources: Friedman et al. (1995, 224307); Johnson et al. (1986, 061340).

5.4.3. Dose Adjustments and Extrapolation Method(s)

The current EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005, 086237) 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, 086237). In the case of AA, 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 AA exposure.

5.4.3.1. *Modeling of Tumor Incidence Data from the Friedman et al. (1995, 224307) Bioassay*

Data for both the individual incidence and the incidence of tumor bearing animals in the Friedman et al. (1995, 224307) drinking water bioassays were modeled to derive potential points of departure for an oral slope factor and IUR. For males, the tumor types were TVMs 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 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, 224307) study showed early mortalities (75 versus 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 TVMs and thyroid follicular cell adenoma and carcinoma, using the licensed software MULTI-WEIB (Howe and Crump, 1985, 402270). 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, 224400) 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 5-13. In addition, the results for considering female rats with either tumor are also presented in Table 5-13. The rat slope factors corresponding to mammary tumors and to follicular cell thyroid tumors in female F344 rats were very similar, 0.13 versus 0.11 $(mg/kg\text{-}day)^{-1}$. The BMR was selected so as to use a low BMR level as a POD 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, 086237) 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 risk estimates derived from different tumor sites. Both approaches were considered in this assessment. 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, 006424) *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 an approach that added 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-4 for the summed risk of mammary or thyroid tumors in female F344 rats). The method used to derive the summed risk is as follows:

(1) The central tendency or maximum likelihood estimates (MLEs) of unit potency (i.e., risk per unit of exposure) are estimated by R/BMD_R, and the upper confidence limit (UCL) on the unit risk estimated by $R/BMDL_R$.

(2) The central tendency or MLEs of unit potency (i.e., risk per unit of exposure estimated as R/BMD_R), are summed across the multiple sites.

(3) An estimate of the 95% upper bound on the summed unit risk is 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% UCL, according to the formula:

$$95\%\ UCL = MLE + (1.645 \times SD) \qquad \text{Equation-23}$$

where 1.645 is the t-statistic corresponding to a one-sided 95% CI and >120 degrees of freedom, and the SD is the square root of the variance of the MLE. The variances are summed across tumor sites to obtain the variance of the sum of the MLE. The 95% UCL on the sum of the individual MLEs is then calculated from the variance of the sum of the MLE.

The approach that used a POD for the combined incidence data 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 $(mg/kg\text{-}day)^{-1}$, or 0.23 $(mg/kg\text{-}day)^{-1}$, approximately twofold 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 $(mg/kg\text{-}day)^{-1}$.

Table 5-13. Points of departure and oral slope factors derived from Friedman et al. (1995, 224307) tumor incidence data for female rats exposed to AA in drinking water

Incidence modeled	BMD_R[a] (mg/kg-day)	$BMDL_R$[a] (mg/kg-day)	Oral Slope Factor based on rat BMDL [risk level/BMDL] $(mg/kg\text{-}day)^{-1}$
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 5-12 for incidences). For summed risk, EPA used an approach that was consistent with the NRC (1994, 006424) recommendation, resulting in a rat slope factor 0.21 (Appendix D).

Data Source: Friedman et al. (1995, 224307). See Appendix D for derivation of BMDs and BMDLs.

Because of mortality issues in the male rat data, time-to-tumor modeling was used (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 5-14. 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-6 for the summed risk of TVMs 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 $(mg/kg\text{-}day)^{-1}$.

Table 5-14. PODs derived from Friedman et al. (1995, 224307) tumors incidence data for male F344 rats exposed to AA in drinking water

Incidence modeled	BMD_R[a] (mg/kg-day)	$BMDL_R$[a] (mg/kg-day)	Oral slope factor based on rat BMDL (risk level/BMDL) (per mg/kg-day)
TVM	1.2	0.75	0.13
Follicular cell thyroid tumors	0.71	0.45	0.22
TVM or thyroid tumors[b]	0.70	0.30	0.33

[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 5-12 for incidences). For summed risk, EPA used an approach that was consistent with the NRC (1994, 006424) recommendation, resulting in a rat slope factor 0.32 (Appendix D).

Source: Friedman et al. (1995, 224307). See Appendix D for derivation of BMDs and BMDLs.

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-day)$^{-1}$ and 2.5-fold higher than for testicular tumors, 0.13 (mg/kg-day)$^{-1}$ (see Appendix D for more details).

5.4.3.2. *Modeling of Tumor Incidence Data from the Johnson et al. (1986, 061340) Bioassay*

Incidence data for tumors showing statistically significant elevations in the Johnson et al. (1985, 067932) drinking water bioassays were modeled to derive potential PODs for an oral slope factor and IUR. For males, the tumor types were TVM, thyroid follicular cell (adenoma/carcinoma), and adrenal pheochromocytomas. For females, the tumor types were mammary gland tumors (malignant and benign combined), thyroid follicular cell (adenoma/carcinoma), CNS tumors of glial origin, and oral cavity tumors (malignant and benign combined). The data for uterine adenocarcinomas and pituitary gland adenomas were not analyzed because the statistical significance of the elevated incidences in the high-dose group was only demonstrated after Mantel-Haenszel mortality adjustment and there is no clear evidence for a trend for increasing risk with increasing exposure level for the incidence data for these tumor sites shown in Table 5-12. The data for clitoral adenomas were not analyzed because the number of tissues examined in each group was very small (n = ≤ 5, Table 5-12).

Details of the modeling are described in Appendix D. The tumor data for each sex and tumor site were fit with the multistage model to estimate the BMD and the BMDL. During the last 4 months of the Johnson et al. (1986, 061340) study, there were increased mortalities in high-dose males and females, compared with controls, but no adjustment (e.g., excluding from incidence denominators animals that died before the time of first appearance of tumors) or special modeling was done for early mortalities because individual animal data for the time of death were not available.

The POD results (and oral slope factors) for separately modeling the female mammary, thyroid, CNS, and oral cavity tumor incidence data, and for the summed risks for multiple tumor types are presented in Table 5-15. The summed risks were calculated using a method consistent with the NRC (1994, 006424) recommended approach, and discussed in detail in Appendix D. Risks for tumors in different organs were summed to allow for the possibility that different tumor types can have different dose-response relationships. Consequently, the modeling for each of the tumor types was used as a basis for estimating a statistically appropriate upper bound on the summed risk. This estimate of overall risk describes the risk of developing any combination of the tumor types considered, not just the risk of developing all tumor types simultaneously.

Table 5-15. PODs and oral slope factors derived from Johnson et al. (1986, 061340) tumor incidence data for female F344 rats exposed to AA in drinking water

Incidence data modeled	BMD_R[a] (mg/kg-day)	$BMDL_R$[a] (mg/kg-day)	Oral slope factor based on rat BMDL (risk level/BMDL) (per mg/kg-day)
Mammary tumors	0.44	0.30	0.34
Follicular cell thyroid tumors	2.93	1.47	0.068
CNS tumors of glial origin	1.80	1.03	0.10
Oral cavity, malignant or benign	1.80	0.99	0.10
Mammary or thyroid tumors[b]			0.38
Mammary, thyroid or CNS tumors[b]			0.44
Mammary, thyroid, CNS or oral cavity tumors[b]			0.50

[a]R = 10% extra risk for mammary, thyroid, CNS, and oral cavity tumors.
[b]Summed risk were derived with a method that is consistent with the NRC (1994, 006424) recommendation. See Appendix D for a detailed discussion.

Source: Johnson et al. (1986, 061340). See Appendix D for derivation of BMDs, BMDLs, and slope factors.

The POD results (and oral slope factors) for modeling of the male tunica vaginalis, thyroid, and adrenal tumor incidence data separately and for summed risks are presented in Table 5-16.

Table 5-16. PODs and oral slope factors derived from Johnson et al. (1986, 061340) tumor incidence data for male F344 rats exposed to AA in drinking water

Incidence data modeled	BMD_R[a] (mg/kg-day)	$BMDL_R$[a] (mg/kg-day)	Oral slope factor based on rat BMDL (risk level/BMDL) (per mg/kg-day)
TVM	0.27	0.16	0.61
Follicular cell thyroid tumors	2.04	1.12	0.089
Adrenal pheochromocytomas	2.55	1.08	0.093
TVM or thyroid tumors[b]			0.67
TVM, thyroid or adrenal tumors[b]			0.71

[a]R = 10% extra risk for mammary, thyroid, and adrenal tumors.
[b]Summed risk were derived with a method that is consistent with the NRC (1994, 006424) recommendation. See Appendix D for a detailed discussion.

Source: Johnson et al. (1986, 061340). See Appendix D for derivation of BMDs and BMDLs.

5.4.3.3. *Comparison of Modeling Results*

The results of summing the risks for tumors observed in the two F344 rat bioassays are similar as shown in the comparison of rat cancer slope factors listed in Table 5-17.

Table 5-17. Comparison of oral slope factors based on summed risks for tumors at several sites in two bioassays of F344 rats exposed to AA in drinking water

Bioassay/sex/tumor sites	Oral slope factor based on rat BMDL (risk level/BMDL) (per mg/kg-day)
Friedman/female/mammary or thyroid	0.21
Johnson/female/mammary or thyroid	0.38
Johnson/female/mammary, thyroid, or CNS	0.44
Johnson/female/mammary, thyroid, CNS, or oral cavity	0.50
Friedman/male/TVM or thyroid	0.32
Johnson/male/TVM or thyroid	0.67
Johnson/male/TVM, thyroid, or adrenal	0.71

Note: Oral slope factors (= risk level/BMDL) are used to compare summed risks because the BMDLs in the summed risk analysis did not all have the same BMR (i.e., it is difficult to readily rank order BMDLs with different BMRs).

For tumors showing significantly elevated incidences in both bioassays (mammary or thyroid tumors in females and TVM and thyroid tumors in males), the oral slope factors based on the rat BMDLs are within a twofold range for each sex. The oral slope factor of 6.7×10^{-1} $(mg/kg\text{-}day)^{-1}$ derived from the Johnson et al. (1986, 061340) male rat data for the summed incidence of thyroid tumors or TVMs was selected as the best estimate of cancer risks as this estimate represented effects seen in the most sensitive species and sex, and was based on summed risks for tumors that were reproducibly observed in both assays.

The rat oral slope factor of 6.7×10^{-1} $(mg/kg\text{-}day)^{-1}$ is the upper bound on the summed risk, and can be used to derive a POD analogous to the $BMDL_{10}$ as follows:

$BMDL_R$ = BMR Level/Rat Oral Slope Factor (i.e., the upper bound on the summed risk)

$$BMDL_{10} = 0.1/6.7\times10^{-1}\ (mg/kg\text{-}day)^{-1}$$
$$= 1.5\times10^{-1}\ mg\ AA/kg\text{-}day \qquad \text{Equation-24}$$

This $BMDL_{10}$ of 1.5×10^{-1} mg AA/kg-day is the POD used as the basis for the HED used to derive the human oral slope factor, as discussed in the next sections (Sections 5.4.4 and 5.4.5).

The corresponding BMD_{10} of 2.38×10^{-1} mg AA/kg-day is calculated from the BMR divided by the sum of the central tendency for risks of thyroid or TVM tumors in male rats (4.2×10^{-1} $[mg/kg\text{-}day]^{-1}$; see Appendix D, Table D-10) as follows:

$$BMD_R = \text{BMR Level/Sum of the central tendency for risks}$$

$$BMD_{10} = 0.1/4.2\times10^{-1}\ (\text{mg/kg-day})^{-1}$$
$$= 2.38\times10^{-1}\ \text{mg AA/kg-day} \qquad \text{Equation-25}$$

5.4.4. HED—based on equivalent AUC for serum AA or GA.

Sections 3.5 and 5.3.1 previously demonstrated how a rat internal dose (e.g., AA-AUC) can be estimated following an external exposure based on the relationships among Hb adducts, serum levels, and administered dose as reported in studies by Doerge et al. (2005, 224344; 2005, 224348; 2005, 224355), and Tareke et al. (2006, 224387). The administered dose in humans that would produce a level of the AA-AUC or GA-AUC equivalent to the level in the rat (i.e., the HED) can also be estimated from the observed relationship between human Hb adduct levels and administered dose as reported by Fennell et al. (2005, 224299), and human Hb adduct formation rates.

The dose metric used to estimate the HED for the cancer risk estimates is the GA-AUC, since GA is considered to be the putative mutagenic carcinogen. The male GA-AUC conversion factor of 16.2 µM GA-hr/mg AA/kg bw (Table 5-7) was chosen as the best factor to estimate the internal GA-AUC following exposure to a BMD_{10} of 2.38×10^{-1} mg AA/kg-day or a $BMDL_{10}$ of 1.5×10^{-1} mg AA/kg-day. This conversion factor was based on Tareke et al. (2006, 224387) adduct data from the Doerge et al. (2005, 224344) 49-day drinking water study, and the nongender in vivo derived rate constants (i.e., based on the pooled male and female rat data) from Tareke et al. (2006, 224387) and Doerge et al. (2005, 224355). The equations used to derive a $GA\text{-}AUC_{BMD}$ of 3.86 µM-hr, and a $GA\text{-}AUC_{BMDL}$ of 2.43 µM-hr (as the POD) are as follows:

$$GA-AUC_{BMD,male\ rat}\ (\mu M-hr) = BMD_{male\ rat} \times AUC\ conversion\ \ factor_{male\ rat}$$
$$= 2.38\,x10^{-1} mg\ AA/kg\text{-day} \times \frac{16.2\,\mu M-hr_{male\ rat}}{mg\ AA/kg\text{-day}}$$
$$= 3.86\,\mu M-hr \qquad \text{Equation-26}$$

and

$$GA-AUC_{BMDL,male\ rat}\ (\mu M-hr) = BMDL_{male\ rat} \times AUC\ conversion\ \ factor_{male\ rat}$$
$$= 1.5\,x10^{-1} mg\ AA/kg\text{-day} \times \frac{16.2\,\mu M-hr_{male\ rat}}{mg\ AA/kg\text{-day}}$$
$$= 2.43\,\mu M-hr \qquad \text{Equation-27}$$

5.4.4.1. *Deriving the HED*

Similar to the HED derived for the AA-AUC dose metric discussed in Section 5.3.1, the HED derivation here for the GA-AUC dose metric requires a human GA-AUC conversion factor. The conversion factor is the measured human GAVal adduct level of 28.9 nmol/g globin/mmol

AA/kg bw from Fennell et al. (2005, 224299) divided by the in vivo GAVal adduct formation rate of 32.5×10^{-6} GAVal L/g globin/hr (Table 5-5) resulting in a conversion factor of 12.5 µM GA-hr_{human} per mg AA/kg-day (i.e., as a daily dose, seeTable 5-7) as follows:

$$GA-AUC\ conversion\ factor_{human} = GAVal\ \ level \div GAVal\ \ formation\ rate$$

$$GA-AUC\ conversion\ factor_{human} = \frac{28.9\ nmol\ GAVal}{(g\ globin)(mmoles\ AA/kg\text{-}\mathrm{day})} \div \frac{32.5\ x10^{-6}\ GAVal\ L}{(g\ globin)(h)}$$

$$= \frac{0.8892\ mM\ GA-hr}{mmoles\ AA/kg\text{-}\mathrm{day}} \qquad \text{Equation-28}$$

The GA-AUC of 0.8892 mM GA-hr/mmole AA/kg-day is converted to 12.5 µM GA-hr/mg AA/kg-day by converting mmoles to mg (molecular weight of AA = 71.08) and mM to µM as follows:

$$GA-AUC\ conversion factor_{human} = \frac{0.8892\ mM\ \ GA-hr}{mmole\ AA/kg\text{-}\mathrm{day}}\ x\ \frac{1 mmole\ AA}{71.08 mg\ \ AA} = \frac{0.0125 mM\ \ GA-hr}{mg\ AA/kg\text{-}\mathrm{day}}\ x\ \frac{1000\mu M}{1 mM}$$

$$= \frac{12.5\ \mu M\ GA-hr_{human}}{mg\ AA/kg\text{-}\mathrm{day}} \qquad \text{Equation-29}$$

There is a fivefold range of values in Table 5-7 for the human GA-AUC conversion factor (from 12.5 to 60.4), considerably greater than the range of values for AA-AUC conversion (twofold), due to the wider difference in the in vitro and in vivo based second order rate constants for GAVal adduct formation. As in the derivation of the RfD, the more scientifically supportable adduct formation rate constant is the in vivo rate of 32.5×10^{-6} GAVal L/g globin/hr derived from all of the single dose male and female mice and rat data reported by Doerge et el. (2005, 224348; 2005, 224355) and Tareke et al. (2006, 224387).

HEDs of 3.08×10^{-1} mg AA/kg-day and 1.94×10^{-1} mg AA/kg-day can be calculated for the male rat GA-AUC_{BMD} of 3.86 µM-hr, and the GA-AUC_{BMDL} of 2.43 µM-hr (as the POD for the oral slope factor), respectively, by applying the human GA-AUC conversion factor of 12.5 µM GA-hr/mg AA/kg-day, as follows:

$$HED_{BMD} = GA-AUC_{BMD,male\ rat} \div human \text{ GA - AUC conversion factor} \qquad \text{Equation-30}$$

$$HED_{BMD} = 3.86\,\mu M - hr \div \frac{12.5\,\mu M\ GA-hr_{human}}{mg\ AA/kg\text{ - day}} = 3.08\,x10^{-1}\ mg\ AA/kg\text{ - day} \qquad \text{Equation-31}$$

and

$$HED_{BMDL} = GA-AUC_{BMDL,male\ rat} \div human \text{ GA - AUC conversion factor} \qquad \text{Equation-32}$$

$$HED_{BMDL} = 2.43\,\mu M\ GA-hr \div \frac{12.5\,\mu M\ GA-hr_{human}}{mg\,AA/kg\text{ - day}} = 1.94x10^{-1}\ mg\,AA/kg\text{ - day} \qquad \text{Equation-33}$$

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; and (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 daily intakes of AA used to derive HEDs are the BMD of 2.38×10^{-1} mg AA/kg-day, and the BMDL of 1.50×10^{-1} mg AA/kg-day from the Johnson et al. (1986, 061340) data for summed risk of thyroid tumors or TVMs in male F344 rats. The equivalent AUC method was used to derive an HED_{BMD} of 3.08×10^{-1} mg AA/kg-day and HED_{BMDL} of 1.94×10^{-1} mg AA/kg-day.

The human oral slope factor 0.51 $[mg/kg\text{-}day]^{-1}$ is derived by linear extrapolation from the HED_{BMDL} (as the POD) of 1.94×10^{-1} mg AA/kg-day to the origin, corrected for background. The slope factor is calculated by dividing the benchmark response rate (10^{-1}) by the HED_{BMDL} (human oral slope factor = $0.1/[1.94\times10^{-1}$ mg AA/kg-day] = 0.51 $[mg/kg\text{-}day]^{-1}$).

With rounding to one significant figure, the human oral slope factor based on the HED_{BMDL} for a BMR of 10^{-1} is 0.5 per mg/kg-day.

The human oral slope factor for AA should not be used with exposures exceeding 0.31 mg AA/kg-day (an approximate estimate of the HED_{BMD10} for the summed risk), because above this level the fitted dose-response model better characterizes what is known about the carcinogenicity of AA. ADAFs are to be applied to the oral slope factors when assessing cancer risks for subpopulations <16 years of age or for lifetime exposures that begin in early life (U.S. EPA, 2005, 088823) (Section 5.4.6). The most current information on the application of ADAFs for cancer risk assessment can be found at www.epa.gov/cancerguidelines/.

5.4.5.2. *Inhalation Unit Risk*

No human or animal inhalation cancer dose-response data were available for AA to directly derive an IUR. The IUR was thus derived in a route-to-route extrapolation of the dose-response relationship (oral-to-inhalation exposure) by converting the HED_{BMDL} to a human equivalent air concentration (i.e., the HEC_{BMDL}) as the POD for the IUR.

Support for deriving an IUR value based on oral exposure data comes from: (1) dose-response data and identification of tumor types and incidence from two chronic oral bioassays; (2) evidence of rapid, nearly complete absorption from the oral route and rapid distribution throughout the body (Kadry et al., 1999, 224596; Miller et al., 1982, 061351); (3) evidence that the elimination kinetics of radioactivity from oral or i.v. administration of radiolabeled AA in rats is similar (Miller et al., 1982, 061351); (4) similar flux of AA through metabolic pathways following either single dose oral or single 6 hr inhalation exposures in rats (Sumner et al., 2003, 224347); (5) some route-to-route differences in the relative amounts of AA to GA, however, the differences are within twofold of each other; and (6) lack of support for portal of entry effects.

No human inhalation kinetic data are available. In the only available animal inhalation kinetic study (Sumner et al., (2003, 224347), the authors report statistically significant larger percentages of urinary metabolites associated with GA formation following an inhalation exposure compared with an i.p. and gavage exposure. GAVal levels are higher and AAVal levels lower following a single 6 hr inhalation exposure compared to levels from the single gavage dose in rats. Statistical significance was not reported for the adduct level differences, and the numbers were within twofold of each other. Doerge et al. (2005, 224348; 2005, 224355) report an increased percentage of GA formation observed in mice and F344 rats from a gavage or dietary exposure compared to an i.v. exposure that, in conjunction with the Sumner et al. (2003, 224347) results, indicate that there is first pass metabolism in the lungs following an inhalation

exposure similar to the first pass metabolism in the liver from an oral exposure, with possibly more lung metabolic conversion of AA to GA. Although in this single study with inhalation kinetic data, there do appear to be some route-to-route differences in the relative amounts of AA to GA, the differences are within twofold of each other, and the metabolic paths and total disposition are similar, supporting the calculation of an HEC_{BMDL} based on the HED_{BMDL} as the POD for deriving an IUR. The IUR is the upper bound risk estimate for a continuous lifetime inhalation exposure without consideration of increased early life susceptibility due to AA's mutagenic MOA.

The oral HED_{BMDL} of 1.94×10^{-1} mg AA/kg-day is based on the rat BMDL of 2.43 mg AA/kg-day for summed risk of thyroid tumors or TVMs in male F344 rats (Johnson et al., (1986, 061340). The HED_{BMD} is 3.08×10^{-1} mg AA/kg-day (i.e., the central estimate). The HEC_{BMDL} (as the POD for the IUR) is calculated as the air concentration that will provide a daily intake comparable to the oral HED_{BMDL}, and assumes a continuous 24-hour inhalation exposure for a 70 kg person who breathes 20 m^3-day air. The HEC_{BMD} of 1.1 mg/m^3, and HEC_{BMDL} of 6.8×10^{-1} mg/m^3 are calculated as follows:

$$HEC_{BMD} = Oral\ HED_{BMD} \times 70\ kg \div \left(\frac{day}{20\ m^3}\right)$$

$$= 3.08\,x10^{-1}\ mg/kg\text{-day} \times 70\,kg \div \left(\frac{day}{20\,m^3}\right) = 1.1\ mg/m^3 \qquad \text{Equation-34}$$

$$HEC_{BMDL} = Oral\ HED_{BMDL} \times 70\ kg \div \left(\frac{day}{20\ m^3}\right)$$

$$= 1.94\,x10^{-1}\ mg/kg\text{-day} \times 70\,kg \div \left(\frac{day}{20\,m^3}\right) = 6.8\,x10^{-1} mg/m^3 \qquad \text{Equation-35}$$

The IUR of 1.47×10^{-4} $(\mu g/m^3)^{-1}$ is calculated by dividing the BMR of 10^{-1} by the HEC_{BMDL} as follows:

$$IUR = \frac{BMR}{HEC_{BMDL}}$$

$$= \frac{0.1}{0.68\ mg/m^3} x \frac{1\,mg}{1000\ \mu g} = 1.47\times10^{-4}\ (\mu g/m^3)^{-1} \qquad \text{Equation-36}$$

With rounding to one significant figure, the IUR is 1×10^{-4} per µg/m^3.

As noted in the discussion on the oral slope factor, ADAFs are to be applied to the slope factors when assessing cancer risks for subpopulations <16 years of age or for lifetime exposures that begin in early life (U.S. EPA, 2005, 088823) (Section 5.4.6).

5.4.6. Application of ADAFs

Because a mutagenic MOA for AA carcinogenicity is sufficiently supported in laboratory animals and relevant to humans (Section 4.8.3), and in the absence of chemical-specific data to evaluate differences in susceptibility between adults and children, increased early-life susceptibility is assumed and the age-dependent adjustment factors (ADAFs) should be applied, as appropriate, along with specific exposure data in accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005, 088823). Specifically, ADAFs should be applied to the slope factor when assessing cancer risks to individuals <16 years of age or for lifetime exposures that begin early in life. The oral slope factor of 0.5 per mg/kg-day and the IUR of 1×10^{-4} per µg/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 (U.S. EPA, 2005, 088823). The 10- 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.

5.4.6.1. *Oral exposure.*

To calculate a lifetime-risk estimate for continuous oral exposure from birth with a life expectancy of 70 years, the ADAFs are first applied to obtain risk estimates for continuous exposure over the three age groups:

$$\text{Risk for birth through <2 yr} = 5\times10^{-4} \text{ per µg/kg-day}\times10\times2 \text{ yr}/70 \text{ yr}$$
$$= 1.4\times10^{-4} \text{ per µg/kg-day} \qquad \text{Equation-37}$$

$$\text{Risk for ages 2 through <16} = 5\times10^{-4} \text{ per µg/kg-day}\times3\times14 \text{ yr}/70 \text{ yr}$$
$$= 3.0\times10^{-4} \text{ per µg/kg-day} \qquad \text{Equation-38}$$

$$\text{Risk for ages 16 until 70} = 5\times10^{-4} \text{ per µg/kg-day}\times1\times54 \text{ yr}/70 \text{ yr}$$
$$= 3.9\times10^{-4} \text{ per µg/kg-day} \qquad \text{Equation-39}$$

To calculate the lifetime-risk estimate for continuous exposure from birth for a population with default life expectancy of 70 years, the risk associated with each of the three relevant time periods is summed:

$$\text{Risk} = 1.4 \times 10^{-4} + 3.0 \times 10^{-4} + 3.9 \times 10^{-4} = 8.3 \times 10^{-4} \text{ per } \mu\text{g/kg-day} \qquad \text{Equation-40}$$

Using the above full lifetime unit risk estimate of 8.3×10^{-4} per μg/kg-day for continuous exposure from birth to 70 years, the lifetime chronic oral exposure level of acrylamide corresponding to an extra risk of 1×10^{-6} can be estimated as follows:

$$1 \times 10^{-6} \div 8.3 \times 10^{-4} \text{ per } \mu\text{g/kg-day} = 0.001 \ \mu\text{g/kg-day} \qquad \text{Equation-41}$$

5.4.6.2. ***Inhalation exposure.***

To calculate a lifetime risk estimate for continuous inhalation exposure from birth with a life expectancy of 70 years, the ADAFs are first applied to obtain risk estimates for continuous exposure over the three age groups:

$$\text{Risk for birth through } <2 \text{ yr} = 1 \times 10^{-4} \text{ per } \mu\text{g/m}^3 \times 10 \times 2 \text{ yr}/70 \text{ yr}$$
$$= 2.9 \times 10^{-5} \text{ per } \mu\text{g/m}^3 \qquad \text{Equation-42}$$

$$\text{Risk for ages 2 through } <16 = 1 \times 10^{-4} \text{ per } \mu\text{g/m}^3 \times 3 \times 14 \text{ yr}/70 \text{ yr}$$
$$= 6.0 \times 10^{-5} \text{ per } \mu\text{g/m}^3 \qquad \text{Equation-43}$$

$$\text{Risk for ages 16 until 70} = 1 \times 10^{-4} \text{ per } \mu\text{g/m}^3 \times 1 \times 54 \text{ yr}/70 \text{ yr}$$
$$= 7.7 \times 10^{-4} \text{ per } \mu\text{g/m}^3 \qquad \text{Equation-44}$$

To calculate the lifetime-risk estimate for continuous exposure from birth for a population with default life expectancy of 70 years, the risk associated with each of the three relevant time periods is summed:

$$\text{Risk} = 2.9 \times 10^{-5} + 6.0 \times 10^{-5} + 7.7 \times 10^{-4} = 1.7 \times 10^{-4} \text{ per } \mu\text{g/m}^3 \qquad \text{Equation-45}$$

Using the above full lifetime-unit risk estimate of 1.7×10^{-4} per $\mu g/m^3$ for continuous exposure from birth to 70 years, the lifetime chronic inhalation exposure level of acrylamide corresponding to an extra risk of 1×10^{-6} can be estimated as follows:

$$1 \times 10^{-6} \div 1.7 \times 10^{-4} \text{ per } \mu\text{g/m}^3 = 0.006 \ \mu\text{g/m}^3 \qquad \text{Equation 46}$$

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 (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 AA, specifically the cancer oral slope factor and the IUR. These uncertainties arise either from incomplete knowledge about AA's toxic effects and MOA 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 use of the AUC method in deriving the oral slope factor; (5) derivation of the IUR based on the oral POD (i.e., the route-to-route extrapolation); and (6) the choice of the low-dose linear method of extrapolation from the POD to estimate the oral cancer slope factor and IUR.

In the case of AA, the uncertainties in the underlying data and methods used to derive the oral cancer slope factor 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 oral cancer slope factor and IUR values. The discussion is accompanied by a summary of the main points in Table 5-18.

Table 5-18. Summary of uncertainty in the AA cancer risk assessment

Consideration/ Approach	Impact on cancer risk estimate	Decision	Justification
Completeness of the database	New data could increase or decrease the estimate of risks for AA-induced cancer in humans.	Based on the currently available data, EPA classified AA as "likely to be carcinogenic to humans" (U.S. EPA, 2005, 086237).	The available human epidemiology studies as of 2009 provide limited to inadequate support for definitive statements. Animal bioassays, however, clearly demonstrate multisite carcinogenicity, and provide good support for AA being classified as likely to be carcinogenic to humans.
Selection of bioassay	Analysis based on alternative bioassays or human data could increase or decrease the estimated risks of AA related cancer in humans.	The Friedman et al. (1995, 224307) and Johnson et al. (1986, 061340) studies were chosen for use in the derivation of the oral cancer slope factor and IUR.	In the absence of direct human data, the Friedman et al. (1995, 224307) and the Johnson et al. (1986, 061340) 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, 224307) study was also an author for the Johnson et al. (1986, 061340) study). Ongoing NTP studies will add considerable new chronic bioassay data on tumor types in rats and mice for both AA and GA (U.S. FDA, 2009, 224492).
Selection of tumor types and relevance to humans	A different selection of tumor types from the Johnson et al. (1986, 061340) study could increase or decrease the estimated risks of AA related cancer in humans.	Tumor types used in the derivation of the oral cancer slope factor and IUR included reproducible and statistically significant increases in thyroid and testicular tumors in male rats, and thyroid, mammary gland, and CNS 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 carcinogenicity 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 increase or decrease the estimated risks of AA related cancer in humans.	A BMD analysis was used to fit to the AA 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, 1995, 005992; U.S. EPA, 2005, 086237). 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. The BMD to BMDL ratios reflect a relatively low level of uncertainty in the model results for these data sets. EPA cancer guidelines (U.S. EPA, 2005, 086237) were followed to calculate risks for multiple tumor sites.

Consideration/ Approach	Impact on cancer risk estimate	Decision	Justification
Use of the equivalent AUC method in the derivation of the oral cancer slope factor	Alternative methods and additional kinetic data could increase or decrease the estimate of risks to humans.	A method to estimate AUCs normalized to administered dose was used to estimate the oral HED in the derivation of the oral cancer slope factor.	The equivalent AUC method was especially important to estimating the oral HED in the oral cancer slope factor derivation because the putative toxin was the AA metabolite, GA. The default UF for interspecies toxicokinetic differences would not account for differences in the internal levels of GA, while the AUC method did. The choice of a nongender-specific in vivo formation rate for humans is supported by the epidemiology results of Hartmann et al. (2008, 224480) who did not observe a gender-related difference in internal exposure and metabolism of AA in a study of a nonsmoking general population especially designed for an even distribution of age and gender. Additional human serum data and in vivo adduct formation rate data, however, are needed to reduce uncertainty in the estimate of human GA-AUC per intake of AA using the equivalent AUC method, or to develop a PBPK model that would provide additional capability to evaluate different dose metrics or dosage regimens.
Route-to-route extrapolation to derive the IUR	Additional animal or human inhalation kinetic data could increase or decrease the estimate of risks to humans.	The HEC_{BMDL} is calculated from the oral HED_{BMDL} as the POD to derive the IUR.	Justification for calculating an HEC_{BMDL} from HED_{BMDL} as the POD to derive the IUR. is based on animal kinetic data suggesting some differences in relative levels of GA and AA between the inhalation and oral route, but sufficient similarities in metabolic pathways and internal disposition to support the route-to-route extrapolation. Additional animal or human inhalation kinetic data are needed to verify the limited available data, and to reduce uncertainty in the route-to-route extrapolation, as well as to develop a PBPK model that would provide additional capability to evaluate different dose metrics or dosage regimens.
Choice of low-dose extrapolation approach	An low-dose extrapolation that assumed a nonlinear dose-response relationship at lower doses would likely decrease 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, 2005, 086237), a mutagenic MOA prompts the use of a linear low-dose extrapolation from the POD. The MOA 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 increase or decrease the estimated risk for susceptible subpopulations.	ADAFs are to be applied to the slope factors when assessing cancer risks for sub-populations <16-years of age or for lifetime exposures that begin in early life. ADAF's should only be applied as appropriate and in conjunction with site specific exposure information.	Neither the extent of interindividual variability in AA metabolism nor human variability in response to AA 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 ADAFs to quantitatively account for some of the potential differences in age-dependent response to carcinogens with a mutagenic MOA (U.S. EPA, 2005, 088823).

5.4.7.1. *Areas of Uncertainty*

5.4.7.1.1. ***Completeness of the database.*** Uncertainty in the cancer 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 AA as "likely to be carcinogenic to humans" (U.S. EPA, 2005, 086237). The uncertainty in the database is being actively addressed in on-going studies sponsored by U.S. 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 AA induced cancer in humans.

5.4.7.1.2. ***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 AA exposure via the drinking water, both in the F344 rats (Friedman et al., 1995, 224307; Johnson et al., 1986, 061340). Strengths in both assays include sufficient numbers of animals in control and multiple exposure groups for statistical analysis of dose-response relationships, histological examinations of most tissues, and sufficient reporting of experimental details and results. 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, 224307) study was also an author for the Johnson et al. (1986, 061340)). Ongoing NTP studies at U.S. FDA research laboratories will add considerable new chronic bioassay data in rats

and mice for both AA and GA (U.S. FDA, 2009, 224492). The impact of these new data could be either to increase or decrease the estimate of risks of AA 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. Johnson et al. (1986, 061340) reported increased tumor incidences at sites in females (CNS, oral cavity, uterus, and pituitary) and males (adrenals), which were reported to be not elevated in the Friedman et al. (1995, 224307) bioassay. However, the Johnson et al. (1986, 061340) study had abnormally high CNS and oral cavity tumors in control males and possible confounding effects from a viral infection. Although glial tumors of brain and spinal cord were reported by Friedman et al. (1995, 224307) 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 excluded from the Friedman et al. (1995, 224307) analysis of the data. In addition incidences of oral cavity tumors, clitoral gland adenomas and uterine adenomas were reported not to be increased, but the number of these tumors was not reported. Rice (2005, 224393) raised the issue of under-reporting of CNS tumors in the Friedman et al. (1995, 224307) 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 AA induced cancer in humans.

The relevance of the tumor types observed in animals to humans based on a proposed MOA was considered in Section 4.8.3. The available limited human data do not provide any support for AA 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, 224427). 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, 224307) chronic rat bioassay, whereas GA-DNA adducts have been observed in a much wider array of tissues.

5.4.7.1.3. ***Methods for the dose-response modeling and estimate of cancer risks.*** For AA, there is a lack of knowledge about the underlying biology, but extensive guidance (U.S. EPA, 1995, 005992; U.S. EPA, 2005, 086237) 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 (TVMs 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, 224307) 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. During the last 4 months of the Johnson et al. (1986, 061340) study, there were increased mortalities in high-dose males and females, compared with controls, but no adjustment (e.g., excluding from incidence denominators animals that died before the time of first appearance of tumors) or special modeling was done for early mortalities because individual animal data for the time of death were not available. For the BMR as a POD for the cancer dose-response, the lowest BMR was selected consistent with a resulting BMD that remained close to the empirical data (U.S. EPA, 1995, 005992).

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% CI on the BMD. The multistage modeling of the Johnson et al. (1986, 061340) male rat data yielded a summed incidence (thyroid or TVM tumors) BMD_{10} of 0.238 mg/kg-day, and $BMDL_{10}$ of 0.150 mg/kg-day, an approximately 1.6-fold difference. The multistage modeling of the female rat data yielded a summed incidence (mammary or thyroid or CNS tumors) BMD_{10} of 0.44 mg/kg-day, and $BMDL_{10}$ of 0.32 mg/kg-day, an approximately 1.4-fold difference. The BMD to BMDL ratios for the Johnson et al. (1986, 061340) male female individual tumor types ranged from 1.5 to 2.4. 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, 2005, 086237) 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 risk estimates derived from different tumor sites. Both approaches were considered in this assessment. For the Friedman et al. (1995, 224307) 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 $(mg/kg\text{-}day)^{-1}$. The

summed risks based on the Johnson et al. (1986, 061340) male and female rat data yielded cancer slope factors ranging from 0.4 to 0.7 $(mg/kg\text{-}day)^{-1}$. The relatively similar results for the Friedman et al. (1995, 224307) analysis using different approaches to calculating risks, and the relatively narrow range of slope factors based on summed risk for various combinations of male or female tumor incidence in the Johnson et al. (1986, 061340) study 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.

5.4.7.1.4. ***Adequacy of the AUC method for use in deriving the cancer slope factor.*** The AUC methodology used to estimate the oral HED and derive the cancer slope factor is dependent upon the accuracy of the measured or estimated conversion factors used to estimate the rat and human AUCs/mg AA/kg bw. Currently there is a lack of sufficient data to accurately estimate human in vivo rate constants for the formation of Hb adducts. The estimated human AA-AUC based upon a variety of alternate rate constants (including in vivo constants for rats and in vitro constants for human) are reasonably concordant with a range of values only fourfold different from the lowest to the highest estimate. A wider range (and thus greater uncertainty), however, exist for the rate constants and conversion factors needed to estimate the human GA-AUC. Additional data are clearly needed for these critical rate constants and conversion factors not only for the derivation of reference standards, but for the considerable effort going into estimating daily intake levels based on Hb adduct concentrations in the general public.

5.4.7.1.5. ***Uncertainty in the route-to-route extrapolation to derive the IUR.*** A route-to-route extrapolation (oral-to-inhalation) of the dose-response relationship was performed to derive the IUR based upon the daily intake from the oral POD. Justification for deriving an IUR from the oral POD comes from animal kinetic studies that observed some differences in relative levels of GA and AA between the inhalation and oral route, but sufficient similarities in metabolic pathways and internal disposition to support the route-to-route extrapolation. More specifically, there is: (1) a well characterized dose-response and identification of the tumor type and incidence from two chronic oral bioassays; (2) evidence of rapid, nearly complete absorption from the oral route and rapid distribution throughout the body (Kadry et al., 1999, 224596; Miller et al., 1982, 061351); (3) evidence that the elimination kinetics of radioactivity from oral or i.v. administration of radiolabeled AA in rats is similar (Miller et al., 1982, 061351); (4) similar flux of AA through metabolic pathways following either a single dose oral or single 6 hr inhalation exposures in rats, and similar GAVal levels (an indicator of serum GA-AUC) following an oral or inhalation exposure, although AAVal levels were somewhat lower (statistical significance not reported) (Sumner et al., 2003, 224347); (5) some route differences in relative GA and AA serum levels, however the numbers are within twofold of each other; and (6) lack of support for portal of entry effects. Additional animal or human inhalation kinetic data are

needed to reduce the uncertainty in quantitating the internal disposition of AA or GA following different routes of exposure.

5.4.7.1.6. ***Choice of low-dose extrapolation approach.*** The MOA 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, 2005, 086237), 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.

5.4.7.1.7. ***Human population variability and sensitive subpopulations.*** Neither the extent of interindividual variability in AA metabolism nor human variability in response to AA has been well characterized. Factors that could contribute to a range of human response to AA include variations in CYP450, epoxide hydrolase, or GST levels (or activity) because of age-related, gender, or genetic differences or due to 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 AA or antioxidant protection systems. Incomplete understanding of the potential differences in metabolism and susceptibility across exposed human populations represents a 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 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 subpopulations <16 years of age or for lifetime exposures that begin in early life (U.S. EPA, 2005, 088823) (Section 5.4.6).

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 1-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, 061340), with the external AA exposure as the dose metric. The current derivation of the oral slope factor of 0.5 $(mg/kg\text{-}day)^{-1}$ is based on the summed risks for increased incidence of thyroid tumors and TVMs in male F344 rats exposed to AA in drinking water for 2 years (Johnson et al., 1986, 061340). The dose metric used in the current estimation of the HED is GA-AUC rather than the external AA exposure. GA is considered to be the putative toxin for the hypothesized mutagenic MOA leading to carcinogenicity, and is thus a better internal dose metric to correlate to response than the internal (or external) level of AA. Differences in the metabolism of GA between rats and humans had a considerable impact on the resulting slope factors.

The previous IUR 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, 224308). This assumption was supported by the data on the distribution of AA following oral or i.v. administration in rats (Miller et al., 1982, 061351). The current IUR of 1.0×10^{-4} $(\mu g/m^3)^{-1}$ is based on EPA's subsequent methodology for inhalation dosimetry (U.S. EPA, 1994, 006488), and an estimation of the HEC_{BMDL} from the oral HED_{BMDL} as the POD. Animal toxicokinetic data for AA and GA following different routes of exposure indicate sufficient similar internal disposition of GA or AA to support a route-to-route extrapolation.

5.5. QUANTITATING RISK FOR HERITABLE GERM CELL EFFECTS

U.S. EPA's *Guidelines for Mutagenicity Risk Assessment* (1986, 001466) 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 (Waters and Nolan, 1995, 224531), and included four case studies, one of which addressed AA's effects (Dearfield et al., 1995, 224315). AA 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, 224315) 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.

5.5.1.1. *Parallelogram Approach*

The parallelogram approach was originally formulated by F.H. Sobels (1977, 224286; 1982, 224291; 1989, 224293) 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; and 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 5-2. 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, 224531).

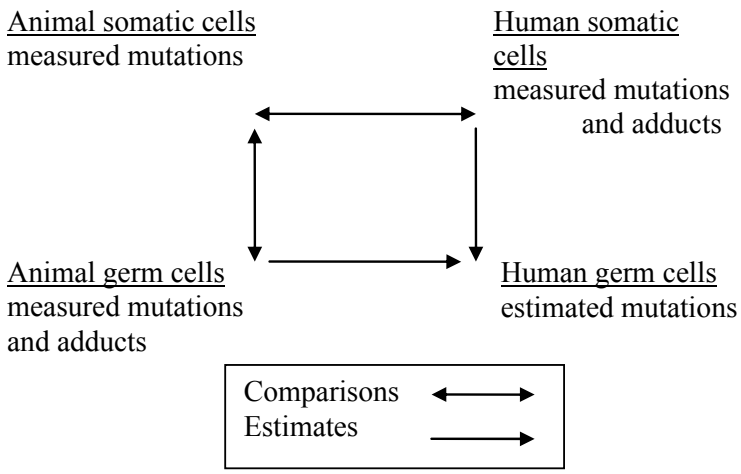

Figure 5-2. Original parallelogram approaches for estimating risk of heritable germ cell effects.

Dearfield et al. (1995, 224315) evaluated two modifications to the original parallelogram approach for use in quantitating the risk for AA, as presented in Figure 5-3. The first modification (Figure 5-3a) 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 5-3a. The EC/U.S. EPA workshop participants who evaluated this case study concluded, however, that the modified parallelogram approach in Figure 5-3a 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 the parallelogram approach in Figure 5-3a. 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-2009.

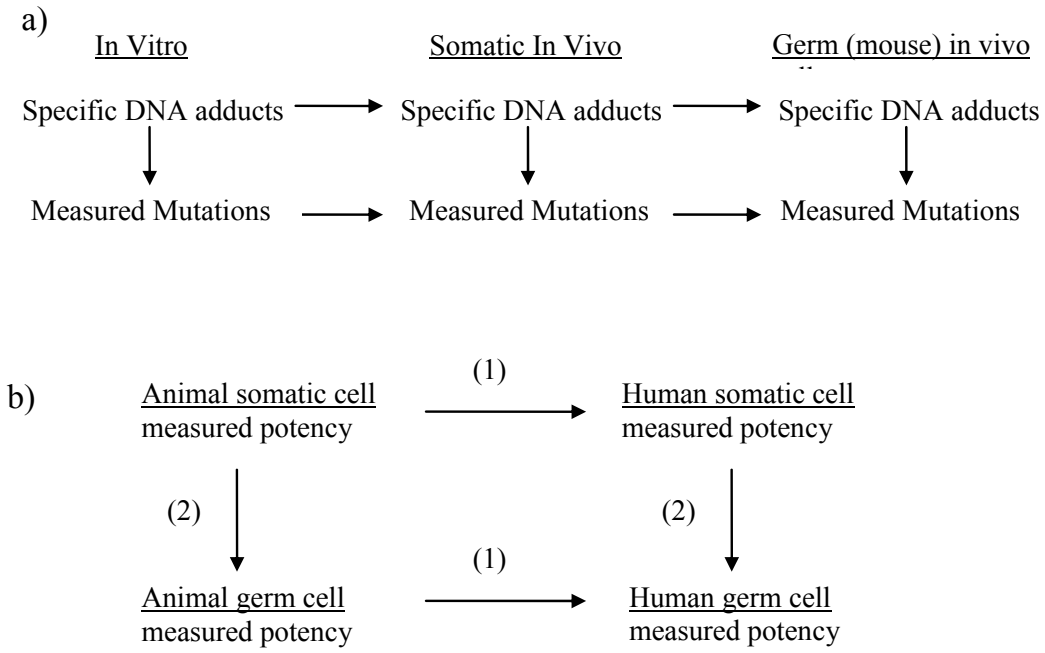

Figure 5-3. Two modifications in the parallelogram approach for estimating risk of heritable germ cell effects from exposure to AA.

A second parallelogram approach shown in Figure 5-3b 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 5-3a, however, the types of data needed to implement the approach in Figure 5-3b are not available for AA. Specifically, the only information on AA's effects in human somatic cells, is Hb adduction (Bergmark et al., 1993, 224424; Boettcher et al., 2005, 224446; Fennell et al., 2005, 224299), and GA induced UDS in human epithelial cells in vitro (Butterworth et al., 1992, 202898). Other deficiencies in the AA database that preclude implementation of the Figure 5-4b 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 EC/U.S. EPA 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.

5.5.1.2. *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, 224389) 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; and (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 5-3b) 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 AA, and to extrapolate risk from rodent (e.g., mice) experimental models to humans (ICPEMC, 1993, 224627; ICPEMC, 1993, 224624):

Table 5-19. Risk extrapolation factors

Parameter	REF[a] (for AA)	REF[a] (default)
Locus specificity	2	2–5
DNA repair variability	0.1	0.1
Metabolic variability	1	1
Dose rate variability	1	0.1
Exposure route	1	0.5
Germ cell stage specificity	1	1
Dose-response kinetics	1	1
Overall REF	0.2	

[a]An REF of 1 indicates equivalency between the animal and human.

Source: ICPEMC (1993, 224624)

There is considerable uncertainty in the above assumptions and risk extrapolation factors. The REFs for AA shown above are mostly in accordance with default values proposed by the ICEPMC Taskgroup, as described by Favor et al. (1994, 224290). The default REF for locus specificity (2–5) is based on information that, on average, human genes exhibit higher spontaneous mutability. The default REF for DNA-repair variability (0.1) is based on information that the overall human repair capability is 10–100 times more efficient than that of mice. The default REF for metabolic variability (1) was proposed because, in lieu of sufficient data linking metabolic and mutagenic heterogeneity in humans, or the lack of animal data, it is impossible to make an informed approximation of the magnitude of the default factor. The default REF for dose-rate variability (0.1) is based on information for several chemicals

indicating that at least an order of magnitude reduction in mutations occurred following split dose or chronic dosing. The default REF for exposure route (0.5) is based on the frequency of i.p. data for genetic studies and the presumption that the i.p. route is the most efficient route, but for other routes (or where routes are the same in mice and humans), a higher default REF was recommended (Favor et al., 1994, 224290). The default REF for germ-cell specificity (1) is based on information that spermatogenesis and spermiogenesis in mice and humans are similar and the protamines of mice and human spermatozoa have high homology. The default REF for dose-response kinetics (1) is recommended when the dose-response curve appears to be linear or if no data exist (Favor et al., 1994, 224290). Dearfield et al. (1995, 224315) did not clearly explain the basis for recommending deviations from the default REFs for dose-rate variability and exposure route for AA.

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 (Adler et al., 2000, 224322; Generoso et al., 1996, 224346) compared to AA, and is also the primary inducer of DNA-adducts in somatic cells ((Besaratinia and Pfeifer, 2005, 224433). The dominant lethal effect has been shown to require the conversion of AA to GA, using wild type and CYP2E1 knockout mice (Ghanayem et al., 2005, 224354; Ghanayem et al., 2005, 224351). The AA 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.

5.5.1.3. *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 1,000 for dominant single gene diseases, and 10 for dominant chromosomal diseases (i.e., this assumption represents another major source of uncertainty in these calculations). 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, 1993, 224624; ICPEMC, 1993, 224627):

$$\text{Number of new diseases in the offspring descendent from exposed parents} = REF \times M_{mouse} \times L_{human} \times D \times N \qquad \text{Equation-47}$$

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).

5.5.1.4. *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, 224315) 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, 224315) used numbers developed by UNSCEAR (1986, 224467) and Sankaranarayanan (1982, 224448) of 1.5×10^{-3} and 6.2×10^{-8}, respectively. These mutation frequencies in humans were used in the following equation (ICPEMC, 1993, 224624; ICPEMC, 1993, 224627) to derive the number of new diseases in the offspring descendent from exposed parents:

$$\text{Number of new diseases in the offspring} = REF \text{ X } Spon_{humans} \times D/DD \times N \qquad \text{Equation-48}$$

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, 224315) derived a doubling dose in mice based on four data sets (Adler, 1990, 224296; Adler et al., 1994, 224314; Ehling and Neuhäuser-Klaus, 1992, 224391; Shelby et al., 1987, 088819)using the following equation:

$$DD = \frac{\text{Spontaneous mutation rate}}{\text{Induced mutation rate/unit exposure}} \qquad \text{Equation-49}$$

as an example using data from Ehling and Neuhauser-Klaus (1992, 224391):

$$DD = \frac{22/248{,}413}{[(6/23{,}489) - (22/248{,}413)]/100 \text{ mg/kg}} = 53.1 \text{ mg/kg} \qquad \text{Equation-50}$$

The other estimates were 1.8 mg/kg, 3.3 mg/kg, and 0.39 mg/kg for the Shelby et al. (1987, 088819), Adler et al. (1994, 224314), and Adler (1990, 224296) 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 nonlinear response. Dearfield et al. (1995, 224315) 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, 224314) 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, 224314) 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 1,000 newborns (Lyon et al., 1983, 224617). Nevertheless, the accuracy of extrapolation of these high exposure rates to the expected human exposure scenarios presented in Table 5-20 is another major uncertainty in the calculations.

Table 5-20. Heritable genetic risk estimates for humans exposed to AA

				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
Chromoso-mal 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, 224391).
[b]Shelby et al. (1987, 088819).
[c]Adler et al. (1994, 224314).
[d]Adler (1990, 224296).

Source: Dearfield et al. (1995, 224315).

5.5.1.5. *Quantitative Assessment for Various Exposure Routes and Levels*

The results of the Dearfield et al. (1995, 224315) quantitative analysis for risk of heritable germ cell effects from different routes and levels of exposure are presented in Table 5-20. In these derivations, N is set at 1 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 (Adler, 1990, 224296; Adler et al., 1994, 224314; Ehling and Neuhäuser-Klaus, 1992, 224391; Shelby et al., 1987, 088819). 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 $7.3\times10^{-5}/10^6$ offspring for gene mutations leading to disease (using the doubling dose approach and the Ehling and Neuhauser-Klaus (1992, 224391) data) to $3\times10^{-2}/10^6$ for chromosomal alterations, using the

modified direct approach and the Shelby et al.(1987, 088819) data. The oral exposure level that Dearfield et al. (1995, 224315) used was derived from estimates of drinking water consumption and AA levels in drinking water. By using the Fennell et al. (2005, 224299) updated upper estimate of daily oral exposure to an average adult male based on background Hb adduct levels (i.e., 1.26 µg/kg-day instead of Dearfield et al.'s (1995, 224315) 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, 088819) data. Table 5-20 also presents risk for induced genetic disease in offspring from fathers exposed via inhalation or dermal exposures in occupational settings that are considerably higher.

5.5.1.6. *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, 224315) 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, 224315) 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, 224315) quantitative results for risk assessment purposes. An NTP expert panel (NTP/CERHR, 2004, 224300), charged with evaluating the evidence for AA's adverse reproductive and developmental effects, reviewed the Dearfield et al. (1995, 224315) 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, 224315) 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-day×5 days), it is likely that such effects would occur at lower dose levels" (NTP/CERHR, 2004, 224300). 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.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

AA (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. 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. AA forms during the high-temperature heating of starchy foods. AA 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

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Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS).

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, and the lower end of the range of animal oral doses associated with germ cell effects, particularly male-mediated implantation losses, is close to the lowest exposure levels associated with neurotoxicity in orally exposed animals. To date, associations between human exposure to AA and reproductive effects (including possible effects on sperm characteristics) have not been adequately studied.

Studies in mice exposed dermally or by i.p. injection show 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. As reviewed by LoPachin et al. (2007, 224566), there is potential for cumulative effects from exposure to AA and other type-2 alkenes (which can produce similar noncancer effects via common mechanisms of action), since human exposure can be related to environmental pollution (e.g., acrolein, acrylonitrile), contamination of food (e.g., AA, methyl acrylate), and endogenous generation (e.g., acrolein, 4-hydroxy-2-nonenal).

In accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005, 086237), 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. In addition, CNS tumors were

found in both of the chronic F344 rat bioassays. The elevation of the incidence for CNS tumors was significant in the first bioassay and of uncertain statistical significance in the second. There are no animal data on the carcinogenicity of chronic inhalation exposure to AA.

The available human studies on potential AA carcinogenicity are for either dietary exposures—numerous case-control studies and prospective studies—or occupational exposures from inhalation and/or dermal exposure—two cohort mortality studies with follow-up analyses. These studies are judged as providing limited or no evidence of carcinogenicity in humans.

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 CAs (i.e., translocations), formation of GA-DNA in mammalian somatic cells, the positive mouse lymphoma assay response, and mutagenicity of GA 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.

6.1.1. On-Going Studies at the U.S. FDA

The U.S. FDA's NCTR under the auspices of the NTP, has been conducting long-term carcinogenicity bioassays for AA and GA in male and female F344 rats and $B6C3F_1$ mice, as well as a developmental neurotoxicity study of AA in F344 rats. EPA will continue to evaluate the impact on the reference values of new results from these and other national (e.g., NTP) and international studies as they become available.

6.1.2. Suggestions for Additional Studies

To further resolve if there is dose-concordance and a 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 mutations. An example of a study that would help resolve the difference between AA and GA mutagenicity leading to tumors would be to breed wild type lacI mice with knockouts for CypIIE1, and then evaluate mutations in the target tissue.

Additional studies are needed that identify the types of mutations in oncogenes or tumor suppressor genes for the tumors induced in rodents by AA (or GA). 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, as well as 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 in orally exposed animals and to examine possible associations between AA exposure and sperm characteristics in humans (adjusting for smoking history and alcohol consumption). Such studies should reduce uncertainty in the interspecies extrapolation 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.

Because of the direct correlation of Hb adducts to administered dose as a biomarker of exposure, there is considerable potential to develop more accurate measures of exposure of AA to the general population, and to develop accurate estimates of the internal dose of both AA and GA for a given daily intake. These estimates, however, depend upon accurate values for the formation rate of Hb adducts in humans and test species, and currently there are limited data to derive in vivo formation rates. Additional studies are needed in humans and test species that measure all three of the following critical variables to derive in vivo formation rate constants – administered dose, serum and adduct levels over time following a single dose, and time course data for adduct levels at a sufficiently long time postdosing to calculate accurate adduct elimination rates.

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

An increased incidence of degenerative lesions of peripheral nerves was selected as the critical effect for derivation of the RfD for AA. 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. Two 2-year drinking water bioassays with F344 rats were selected as co-principal studies in the derivation of an RfD (Friedman et al., 1995, 224307; Johnson et al., 1986, 061340). The final quantitative RfD value is based on the dose-response data from the Johnson et al. (1986, 061340) 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 both studies. The 95% lower confidence limits of the estimated dose associated

with a 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, 061340) and Friedman et al. (1995, 224307) studies, respectively. The lowest of the BMDLs was from the Johnson et al. (1986, 061340) data with a value of 0.27 mg/kg-day for 5% extra risk for mild-to-moderate lesions. This value was chosen as the most sensitive response, and was selected as the POD for deriving the RfD. An internal dose metric of AA-AUC in the blood from an oral exposure in rat was estimated from in vivo rat data, and the administered dose in humans that would result in a comparable internal AA-AUC was calculated using conversion factors developed from human adduct data and second order adduct formation rate constants. The estimated AA-AUC in rat blood following exposure at a $BMDL_5$ was used to derive a HED of 0.53 mg AA/kg-day as the POD for the RfD. The POD was 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 RfD of 0.002 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. 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 AA 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 sensitive endpoint. Uncertainty exist as to whether behavioral or functional effects (that were not evaluated in these bioassays) would have lower LOAELs than the histological effects that were evaluated, and that were used to derive the RfD. There is also uncertainty as to the dose-response relationship for heritable germ cell effects. These two uncertainties lower the overall confidence in the RfD from high to medium-to-high. There are ongoing studies sponsored by the NTP and FDA that address some of these data needs.

6.2.2. Noncancer/Inhalation

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 limited in characterizing the dose-response relationships for inhalation exposure. The inhalation RfC for AA was therefore derived from the oral data, using the same value of estimated daily intake as was used for the oral POD (i.e., the HEC_{BMDL}), and assuming a continuous inhalation exposure over 24 hours.

The justification for deriving an RfC directly from the oral exposure POD used as the basis for the RfD includes: (1) a well characterized dose-response and identification of the most sensitive noncancer endpoint from an adequate database of oral exposure studies; (2) considerable evidence from occupational experience that dermal and inhalation exposures to AA

induce peripheral neuropathies, including development of the types of degenerative lesions observed in nerves of rats exposed via drinking water; (3) evidence of rapid, nearly complete absorption from the oral route and rapid distribution throughout the body (Kadry et al., 1999, 224596; Miller et al., 1982, 061351); (4) evidence that the elimination kinetics of radioactivity from oral or i.v. administration of radiolabeled AA in rats is similar (Miller et al., 1982, 061351); (5) similar flux of AA through metabolic pathways following either single dose oral exposure or single 6 hr inhalation exposures in rats (Sumner et al., 2003, 224347); (6) some route-to-route differences in the relative amounts of AA to GA, however, the differences are within twofold of each other; and (7) lack of support for portal of entry effects.

The HEC_{BMDL} was calculated from the HED_{BMDL}, assuming a continuous exposure to a 70 kg person who breathes 20 m^3-day air. The HEC_{BMDL} of 0.18 mg/m^3 was used as the POD for the RfC. The HEC_{BMDL} was 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 0.006 mg/m^3.

The overall confidence in the RfC study is similar to that for the RfD, with additional uncertainty as to the toxicokinetics for an inhalation exposure, specifically concerning different internal disposition of AA and GA due to qualitatively similar but possibly quantitatively different first pass effects in lung versus the liver. The similar RfC derived from the Calleman et al. (1994, 202900) data provide some additional confidence in RfC with an overall confidence of medium. Additional kinetic data (e.g., serum data) or improved estimates of the AA and GA-AUC from different exposure routes in humans or test animals based on Hb adduct levels would help improve the confidence in the RfC based on an HED derived from oral data. There is low to medium confidence in the database because inhalation studies are lacking. The overall confidence in the RfC is medium (i.e., less than the confidence in the RfD).

For comparison purposes, and to support an improved design of future studies, the limited human data from the Calleman et al. (1994, 202900) study were used to derive an RfC. The results of this derivation are presented in Appendix F.

6.2.3. Cancer/Oral

Two bioassays with F344 rats provide appropriate data to describe dose-response relationships for tumors induced by chronic oral exposure to AA. Dose-response data for tumors observed in both bioassays were used to derive HED_{BMDLs} (as the POD) for an oral slope factor based on linear extrapolation to the origin, corrected for background (Sections 5.4.2, 5.4.3, and 5.4.4). Support for a linear extrapolation comes from evidence of a mutagenic MOA for AA, including observations of: (1) strong evidence of mutagenicity from in vitro assays and somatic cells from in vivo assays; (2) male-mediated dominant lethal effects following subchronic oral exposure at dose levels (2.8–13.3 mg/kg-day) in the vicinity of chronic oral dose levels that induced carcinogenic effects in rats (0.5–3 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.

Oral slope factors were calculated based on summed risks for increased incidence of tumor types that were reproducibly observed in both of the F344 rat bioassays (mammary or thyroid tumors in females and TVM and thyroid tumors in males). The resulting slope factors were all within a fourfold range across studies, and within a twofold range within studies.

The male rat oral slope factor of 0.6 $(mg/kg\text{-}day)^{-1}$ derived from the Johnson et al. (1986, 061340) male rat BMDL for the summed risk of thyroid tumors or testicular tumors (TVMs) was selected for calculating a POD. The choice was based on the following: (1) there were reproducible thyroid and TVM tumors in both studies; and (2) this choice represented the most sensitive species, sex and tumor type among the other mammary, thyroid, CNS, and TVM tumor summations. The male rat $BMDL_{10}$ of 1.5×10^{-1} that was calculated from the summed risks for thyroid or TVM tumors was used as the POD for deriving a human oral slope factor.

The rat $BMDL_{10}$ was converted to a HED_{BMDL} based on comparable levels of GA-AUC in blood between the rat and human relative to their respective administered doses. GA 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 HED_{BMDL} (1.9×10^{-1} mg/kg-day) at the BMR of 0.1 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, and in the absence of chemical-specific data to evaluate differences in susceptibility, increased early-life susceptibility is assumed, and the ADAFs should be applied to the slope factor based on specific exposure data, as appropriate, in accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005, 088823).

The principal 2-year studies (Friedman et al., 1995, 224307; Johnson et al., 1986, 061340) provided corroborative results for most, but not all, tumor types. There remain some uncertainties concerning the differences between the two study tumor types and incidence data, in particular for the CNS tumors, and in the histopathological interpretation of the male TVMs. The database is also incomplete with only one animal species tested, and little human data to support AA's carcinogenic potential in humans. At this time, the preponderance of evidence supports a mutagenic MOA for AA-induced tumors observed in the F344 rat bioassays (thyroid, mammary gland, and TVMs). Although an alternate MOA has been proposed involving

hormonal pathway disruption for tumors specific to F344 rats, supporting data are limited or nonexistent. Additional MOA data would be useful in this regard.

6.2.4. Cancer/Inhalation

No animal or human cancer data were available to directly derive an IUR. An AA PBTK model was also not available to simulate both oral and inhalation first pass effects. Available studies, however, do support a route-to-route extrapolation of the oral dose-response relationship for an inhalation exposure. Support for the calculation of a HEC_{BMDL} (as the POD to derive the IUR) from the oral HED_{BMDL} comes from: (1) a characterized dose-response and identification of tumor types and incidence from two chronic oral bioassays; (2) evidence of rapid, nearly complete absorption from the oral route and rapid distribution throughout the body (Kadry et al., 1999, 224596; Miller et al., 1982, 061351); (3) evidence that the elimination kinetics of radioactivity from oral or i.v. administration of radiolabeled AA in rats is similar (Miller et al., 1982, 061351); (4) similar flux of AA through metabolic pathways following either single dose oral exposure or single 6 hr inhalation exposures in rats (Sumner et al., 2003, 224347); (5) some route-to-route differences in the relative amounts of AA to GA, however, the differences are within twofold of each other, and the metabolic paths and total disposition are similar (Sumner et al., 2003, 224347); and (6) lack of support for portal of entry effects.

The extrapolation assumes that an exposure (oral or inhalation) will yield a comparable internal dose using the internal dose metric of area under the time-concentration curve for GA in blood. As for the derivation of the oral slope factor, the AA metabolite, GA, is considered to be the putative mutagen and most directly related to AA's carcinogenicity.

The IUR is derived from the HEC_{BMDL10}, i.e., the 95% lower bound on the exposure associated with an 10^{-1} extra cancer risk, by dividing the risk (as a fraction) by the HEC_{BMDL}. The IUR 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 IUR for AA is based on the HEC that would produce a comparable daily intake to that resulting from exposure to the oral HED_{BMDL}. The oral HED_{BMDL} itself is an estimate of the oral dose of AA for a human that would result in a level of GA in blood comparable to what was observed in the male F344 rats following exposure to the rat oral $BMDL_{10}$. An HEC_{BMDL} of 6.8×10^{-1} mg/m^3 was calculated as being the concentration needed to achieve the same daily intake level as would be achieved with exposure to the oral HED_{BMDL} of 1.9×10^{-1} mg/kg-day. The conversion assumes a continuous 24-hour inhalation exposure for a 70 kg person who breathes 20 m^3 per day of air. The HEC_{BMDL} of 6.8×10^{-1} mg/m^3 is the POD used to derive the IUR of 1×10^{-4} $(\mu g/m^3)^{-1}$ based on a benchmark response level of 10^{-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), ADAFs should be applied to the IUR based on specific exposure data, as appropriate, in accordance with the

Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens (U.S. EPA, 2005, 088823).

7. REFERENCES

Abdelmagid HM; Tabatabai MA (1982). Decomposition of acrylamide in soils. J Environ Qual, 11: 701-704. 204385

Abernethy DJ; Boreiko CJ (1987). Acrylonitrile and acrylamide fail to transform C3H/10T1/2 cells. Environ Mutagen, 9 (suppl 8): 2. 224278

Abou-Donia MB; Ibrahim SM; Corcoran JJ; Lack L; Friedman MA; Lapadula DM (1993). Neurotoxicity of glycidamide, an acrylamide metabolite, following intraperitoneal injections in rats. J Toxicol Environ Health A Curr Iss, 39: 447 - 464. 224288

Adler I-D (1990). Clastogenic effects of acrylamide in different germ-cell stages of male mice. In Allen J; Bridges B; Lyon M (Ed.),Biology of mammalian germ cell mutagenesis, Banbury Report (pp. 115-131). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. 224296

Adler I-D; Baumgartner A; Gonda H; Friedman MA; Skerhut M.. (2000). 1-Aminobenzotriazole inhibits acrylamide-induced dominant lethal effects in spermatids of male mice. Mutagenesis, 15: 133-136. 224322

Adler I-D; Gonda H; Hrabé de Angelis M; Jentsch I; Otten IS; Speicher MR (2004). Heritable translocations induced by dermal exposure of male mice to acrylamide. Cytogenetic and Genome Research, 104: 271-276. 224343

Adler I-D; Ingwersen I; Kliesch U; El Tarras A (1988). Clastogenic effects of acrylamide in mouse bone marrow cells. Mutat Res Genet Toxicol Environ Mutagen, 206: 379-385. 224301

Adler I-D; Kliesch U; Jentsch I; Speicher MR (2002). Induction of chromosomal aberrations by decarbazine in somatic and germinal cells of mice. Mutagenesis, 17: 383-389. 224336

Adler I-D; Zouh R; Schmid E (1993). Perturbation of cell division by acrylamide in vitro and in vivo. Mutat Res, 301: 249-254. 224310

Adler ID; Reitmeir P; Schmöller R; Schriever-Schwemmer G (1994). Dose response for heritable translocations induced by acrylamide in spermatids of mice. Mutat Res, 309: 285-291. 224314

afssa (2003). Acrylamide: Point d'information N°2 . Agence Francaise de Securite Sanitaire des Aliments. Maisons-Alfort, France.http://www.afssa.fr/Documents/RCCP2002sa0300.pdf . 416795

Agrawal AK; Seth PK; Squibb RE; Tilson HA; Uphouse LL; Bondy SC (1981). Neurotransmitter receptors in brain regions of acrylamide- treated rats. I: Effects of a single exposure to acrylamide. Pharmacol Biochem Behav, 14: 527-531. 224361

Agrawal AK; Squibb RE (1981). Effects of acrylamide given during gestation on dopamine receptor binding in rat pups. Toxicol Lett, 7: 233-238. 061304

Agrawal AK; Squibb RE; Bondy SC (1981). The effects of acrylamide treatment upon the dopamine receptor. Toxicol Appl Pharmacol, 58: 89-99. 224356

Albert Einstein College of Medicine (1980). A fetal toxicity study of acrylamide in rats. Albert Einstein College of Medicine. Wayne, NJ. 224363

Aldous CN; Farr CH; Sharma RP (1983). Evaluation of acrylamide treatment on levels of major brain biogenic amines, their turnover rates, and metabolites. Toxicol Sci, 3: 182-186 . 224365

Ali SF (1983). Acrylamide-induced changes in the monoamines and their acid metabolites in different regions of the rat brain. Toxicol Lett, 17: 101-105. 224378

Ali SF; Hong J-S; Wilson WE; Uphouse LL; Bondy SC (1983). Effect of acrylamide on neurotransmitter metabolism and neuropeptide levels in several brain regions and upon circulating hormones. Arch Toxicol, 52: 35-43. 061319

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS).

Allen, B; Zeiger, E; Lawrence, G; Friedmand M; Shipp A (2005). Dose–response modeling of in vivo genotoxicity data for use in risk assessment: some approaches illustrated by an analysis of acrylamide. Regul Toxicol Pharmacol, 41: 6-27 . 224386

Amador A; Steger RW; Bartke A; Johns A; Siler-Khodr TM; Parker Jr CR; Shepherd AM (1985). Testicular LH receptors during aging in Fisher 344 rats. J Androl, 6: 61-64. 224392

Ao L; Liu SX; Yang MS; Fong C-C; An H; Cao J (2008). Acrylamide-induced molecular mutation spectra at HPRT locus in human promyelocytic leukaemia HL-60 and NB4 cell lines. Mutagenesis, 23: 309-315. 224395

Augustsson K; Skog K; Jägerstad M; Dickman PW; Steineck G (1999). Dietary heterocyclic amines and cancer of the colon, rectum, bladder, and kidney: a population-based study. Lancet, 353: 703-707. 224397

Auld RB; Bedwell SF (1967). Peripheral neuropathy with sympathetic overactivity from industrial contact with acrylamide. Can Med Assoc J, 96: 652-654. 061310

Bachmann M; Myers JE; Bezuidenhout BN (1992). Acrylamide monomer and peripheral neuropathy in chemical workers. Am J Ind Med, 21: 217 - 222. 224401

Backer LC; Dearfield KL; Erexson GL; Campbell JA; Westbrook-Collins B; Allen JW (1989). The effects of acrylamide on mouse germ-line and somatic cell chromosomes. Environ Mol Mutagen, 13: 218-226. 224404

BAG (2002). Acrylamide; New study available on the Internet FOPH. Bundesamt für Gesundheit (BAG, Federal Office of Public Health). Bern, Switzerland .http://www.bag.admin.ch/dokumentation/medieninformationen/01217/index.html?lang=fr&msg-id=4095. 416785

Bailey E; Farmer PB; Bird I; Lamb JH; Peal JA (1986). Monitoring exposure to acrylamide by the determination of S-(2-carboxyethyl)cysteine in hydrolyzed hemoglobin by gas chromatography-mass spectrometry. Anal Biochem, 157: 241-248. 224410

Banerjee S; Segal A (1986). In vitro transformation of C3H/10T1/2 and NIH/3T3 cells by acrylonitrile and acrylamide. Cancer Lett, 32: 293-304. 224412

Barber DS; Hunt JR; Ehrich MF; Lehning EJ; LoPachin RM (2001). Metabolism, toxicokinetics and hemoglobin adduct formation in rats following subacute and subchronic acrylamide dosing. Neurotoxicology, 22: 341-353. 224414

Barber DS; LoPachin RM (2004). Proteomic analysis of acrylamide-protein adduct formation in rat brain synaptosomes. Toxicol Appl Pharmacol, 201: 120-136. 224415

Barber DS; Stevens S; LoPachin RM (2007). Proteomic analysis of rat striatal synaptosomes during acrylamide intoxication at a low dose rate. Toxicol Sci, 100: 156-167. 224416

Barfknecht TR; Mecca DJ; Naismith RW (1988). The genotoxic activity of acrylamide. Environ Mol Mutagen, 11 (Suppl 11): 9. 224417

Bartke A; Sweeney CA; Johnson L; Castracane VD; Doherty PC (1985). Hyperprolactenemia inhibits development of Leydig cell tumors in aging Fischer rats. Exp Aging Res, 11: 123-127. 224418

Batiste-Alentorn M; Xamena N; Creus A; Marcos R; Aaron CS (1991). Genotoxicity studies with the unstable Zeste-White (UZ) system of Drosophila melanogaster: Results with ten carcinogenic compounds. Environ Mol Mutagen, 18: 120 - 125. 224419

Bergmark E (1997). Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers and nonsmokers. Chem Res Toxicol, 10: 78-84. 224422

Bergmark E; Calleman CJ; Costa LG (1991). Formation of hemoglobin adducts of acrylamide and its epoxide metabolite glycidamide in the rat. Toxicol Appl Pharmacol, 111: 352-363. 224423

Bergmark E; Calleman CJ; He F; Costa LG (1993). Determination of hemoglobin adducts in humans occupationally exposed to acrylamide. Toxicol Appl Pharmacol, 120: 45-54. 224424

Besaratinia A; Pfeifer GP (2004). Genotoxicity of acrylamide and glycidamide. J Natl Cancer Inst, 96: 1023-1029. 224427

Besaratinia A; Pfeifer GP (2005). DNA adduction and mutagenic properties of acrylamide. Mutat Res Genet Toxicol Environ Mutagen, 580: 31-40. 224433

Besaratinia A; Pfeifer GP (2007). A review of mechanisms of acrylamide carcinogenicity. Carcinogenesis, 28: 519-528. 224436

BfR (2003). Abschätzung der Acrylamid-Aufnahme durch hochbelastete Nahrungsmittel in Deutschland (Assessment of acrylamide intake from foods containing high acrylamide levels in Germany). Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment). Berlin, Germany.www.bfr.bund.de/.../abschaetzung_der_acrylamidaufnahme_durch__hochbelastete__nahrungsmittel__in__deutschland_kurzfassung.pdf. 224437

Bio/Dynamics Inc (1979). A fetal toxicity study of acrylamide in rats. U.S. Environmental Protection Agency. Research Triangle Park, NC. EPA878211679. 224439

Bishop JB; Morris RW; Seely JC; Hughes LA; Cain KT; Generoso WM (1997). Alterations in the reproductive patterns of female mice exposed to xenobiotics. Toxicol Sci, 40: 191-204. 024984

Bjellaas T; Janàk K; Lundanes E; Kronberg L; Becher G (2005). Determination and quantification of urinary metabolites after dietary exposure to acrylamide. Xenobiotica, 35: 1003-1018. 224440

Bjellaas T; Oleson PT; Frandsen H; Haugen M; Stølen LH; Paulsen JE; Alexander J; Lundanes E; Becher G (2007). Comparison of estimated dietary intake of acrylamide with hemoglobin adducts of acrylamide and glycidamide. Toxicol Sci, 98: 110-117. 224444

Bjellaas T; Stølen LH; Haugen M; Paulsen JE; Alexander J; Lundanes E; Becher G (2007). Urinary acrylamide metabolites as biomarkers for short-term dietary exposure to acrylamide. Food Chem Toxicol, 445: 1020-1026. 224443

Boettcher MI; Bolt HM; Angerer J (2006). Acrylamide exposure via the diet: influence of fasting on urinary mercapturic acid metabolite excretion in humans. Arch Toxicol, 80: 817-819. 224451

Boettcher MI; Bolt HM; Drexler H; Angerer J (2006). Excretion of mercapturic acids of acrylamide and glycidamide in human urine after single oral administration of deuterium-labelled acrylamide. Arch Toxicol, 80: 55-61. 224449

Boettcher MI; Schettgen T; Kutting B; Pischetsrieder M; Angerer J (2005). Mercapturic acids of acrylamide and glycidamide as biomarkers of the internal exposure to acrylamide in the general population. Mutat Res Genet Toxicol Environ Mutagen, 580: 167-176. 224446

Bonassi S; Lando C; Ceppi M; Landi S; Rossi AM; Barale R (2004). No association between increased levels of high-frequency sister chromatid exchange cells (HFCs) and the risk of cancer in healthy individuals. Environ Mol Mutagen, 43: 134-136. 224455

Bondy SC; Tilson HA; Agrawal AK (1981). Neurotransmitter receptors in brain regions of acrylamide- treated rats. II: Effects of extended exposure to acrylamide. Pharmacol Biochem Behav, 14: 533-537. 224468

Bowyer JF; Latendresse JR; Delongchamp RR; Muskhelishvili L; Warbritton AR; Thomas M; Tareke E; McDaniel LP; Doerge DR (2008). The effects of subchronic acrylamide exposure on gene expression, neurochemistry, hormones, and histopathology in the hypothalamus-pituitary-thyroid axis of male Fischer 344 rats. Toxicol Appl Pharmacol, 230: 208-215. 224470

Brady ST; Pfister KK; Bloom GS (1990). A monoclonal antibody against kinesin inhibits both anterograde and retrograde fast axonal transport in squid axoplasm. PNAS, 87: 1061–1065. 224473

Brat DJ; Brimijoin S (1993). Acrylamide and glycidamide impair neurite outgrowth in differentiating N1E.115 neuroblastoma without disturbing rapid bidirectional transport of organelles observed by video microscopy. J Neurochem, 60: 2145-2152. 224474

Brown L; Rhead MM; Bancroft KCC; Allen N (1980). Model studies of the degradation of acrylamide monomer. Water Res, 14: 775-778. 224478

Budavari S (2001). Acrylamide. In O'Neil MJ; Smith A; Heckelman PE; Budavari S (Ed.),The Merck index: An encyclopedia of chemicals, drugs, and biologicals (pp. 24). Whitehouse Station, NJ: Merck and Co. Inc. 224489

Bull RJ; Robinson M; Laurie RD; Stoner GD; Greisiger E; Meier JR; Stober J (1984). Carcinogenic effects of acrylamide in SENCAR and A/J mice. Cancer Res, 44: 107-111. 202896

Bull RJ; Robinson M; Stober JA (1984). Carcinogenic activity of acrylamide in the skin and lung of Swiss-ICR mice. Cancer Lett, 24: 209-212. 202897

Burek JD; Albee RR; Beyer JE; Bell TJ; Carreon RM; Morden DC; Wade CE; Hermann EA; Gorzinski SJ (1980). Subchronic toxicity of acrylamide administered to rats in the drinking water followed by up to 144 days of recovery. J Environ Pathol Toxicol Oncol, 4: 157-182. 061311

Butterworth BE; Eldridge SR; Sprankle CS; Working PK; Bentley KS; Hurtt ME (1992). Tissue-specific genotoxic effects of acrylamide and acrylonitrile. Environ Mol Mutagen, 20: 148-155. 202898

Calleman CJ (1996). The metabolism and pharmacokinetics of acrylamide: implications for mechanisms of toxicity and human risk estimation. Drug Metab Rev, 28: 527-590. 202899

Calleman CJ; Wu Y; He F; Tian G; Bergmark E; Zhang S; Deng H; Wang Y; Crofton KM; Fennell T; Costa LG (1994). Relationships between biomarkers of exposure and neurological effects in a group of workers exposed to acrylamide. Toxicol Appl Pharmacol, 126: 361-371. 202900

Carere A (2006). Genotoxicity and carcinogenicity of acrylamide: A critical review. Ann Ist Super Sanita, 42: 144-155. 224267

Cavanagh JB (1964). The significance of the "dying-back" process in experimental and human neurological disease. Int Rev Exp Pathol, 3: 219-267. 224266

CFR (2005). Acrylate-acrylamide resins. Code of Federal Regulations. Washington, DC. 21CFR173.5. http://www.gpoaccess.gov/cfr/index.html. 224268

Chapin RE; Fail PA; George JD; Grizzle TB; Heindel JJ; Harry GJ; Collins BJ; Teague J (1995). The reproductive and neuronal toxicities of acrylamide and three analogues in Swiss mice, evaluated using the continuous breeding protocol. Toxicol Sci, 27: 9-24. 224265

Chevolleau, S; Jacques, C; Canlet, C; Tulliez J; Debrauwer L (2007). Analysis of hemoglobin adducts of acrylamide and glycidamide by liquid chromatography-electrospray ionization tandem mass spectrometry, as exposure biomarkers in French population. J Chromatogr A, 1167: 125-134. 224269

Cihák R; Vontorková M (1988). Cytogenetic effects of acrylamide in the bone marrow of mice. Mutat Res, 209: 91-94. 224270

Cihák R; Vontorková M (1990). Activity of acrylamide in single-, double-, and triple-dose mouse bone marrow micronucleus assays. Mutat Res, 234: 125-127. 224271

CIR (1991). Final report on the safety assessment of polyacrylamide. Int J Toxicol, 10: 193-203. 224274

Clarke CH; Sickles DW (1996). Decreased GAP-43 accumulation in neurite tips of cultured hippocampal neurons by acrylamide. Neurotoxicology, 17: 397-406. 224275

Clegg ED; Cook JC; Chapin RE; Foster PMD; Daston GP (1997). Leydig cell hyperplasia and adenoma formation: Mechanisms and relevance to humans. Reprod Toxicol, 11: 107-121. 224277

Collins BW; Howard DR; Allen JW (1992). Kinetochore-staining of spermatid micronuclei: studies of mice treated with X-radiation or acrylamide. Mutat Res, 281: 287-294. 224280

Collins JJ; Swaen GMH; Marsh GM; Utidjian HMD; Caporossi JC; Lucas LJ (1989). Mortality patterns among workers exposed to acrylamide. J Occup Environ Med, 31: 614-617. 224284

Cook JC; Klinefelter GR; Hardisty JF; Sharpe RM; Foster PMD (1999). Rodent leydig cell tumorigenesis: A review of the physiology, pathology, mechanisms and relevance to humans. Crit Rev Toxicol, 29: 169-261. 001577

Costa LG; Deng H; Calleman CJ; Bergmark E (1995). Evaluation of the neurotoxicity of glycidamide, an epoxide metabolite of acrylamide: behavioral, neurochemical and morphological studies. Toxicology, 98: 151-161. 224287

Costa LG; Deng H; Gregotti C; Manzo L; Faustman EM; Bergmark E; Calleman CJ (1992). Comparative studies on the neuro- and reproductive toxicity of acrylamide and its epoxide metabolite glycidamide in the rat. Neurotoxicology, 13: 219-224. 224285

Crofton KM; Padilla S; Tilson HA; Anthony DC; Raymer JH; MacPhail RC (1996). The impact of dose rate on the neurotoxicity of acrylamide: the interaction of administered dose, target tissue concentrations, tissue damage, and functional effects. Toxicol Appl Pharmacol, 139: 163-176. 145493

Croll BT; Arkell GM; Hodge RPJ (1974). Residues of acrylamide in water. Water Res, 8: 989-993. 224297

Damjanov I; Friedman MA (1998). Mesotheliomas of tunica vaginalis testis of Fischer 344(F344) rats treated with acrylamide: a light and electron microscopy study. In Vivo, 12: 495-502. 089567

Davenport JG; Farrell DF; Sumi M (1976). 'Giant axonal neuropathy' caused by industrial chemicals: neurofilamentous axonal masses in man. Neurology, 26: 919-923. 224305

Dearfield KL; Abernathy CO; Ottley MS; Brantner JH; Hayes PF (1988). Acrylamide: its metabolism, developmental and reproductive effects, genotoxicity, and carcinogenicity. Mutat Res, 195: 45-77. 224308

Dearfield KL; Douglas GR; Ehling UH; Moore MM; Sega GA; Brusick DJ (1995). Acrylamide: a review of its genotoxicity and an assessment of heritable genetic risk. Mutat Res-Fundam Mol Mech Mutagen, 330: 71-99. 224315

DeGrandchamp RL; Lowndes HE (1990). Early degeneration and sprouting at the rat neuromuscular junction following acrylamide administration. Neuropathol Appl Neurobiol, 16: 239-254. 224320

DeGrandchamp RL; Reuhl KR; Lowndes HE (1990). Synaptic terminal degeneration and remodeling at the rat neuromuscular junction resulting from a single exposure to acrylamide. Toxicol Appl Pharmacol, 105: 422-433. 224321

Deng H; He F; Zhang S; Calleman CJ; Costa LG (1993). Quantitative measurements of vibration threshold in healthy adults and acrylamide workers. Int Arch Occup Environ Health, 65: 53-56. 224326

Diembeck W; Dusing HJ; Akhiana M (1998). Dermal absorption and penetration of acrylamide ([14C] acrylamide as tracer) in different cosmetic formulations and polyacrylamide-solution after topical application to excised pig skin. Beiersdorf. Hamburg, Germany. 224331

Dixit R; Husain R; Mukhtar H; Seth PK (1981). Effect of acrylamide on biogenic amine levels, monoamine oxidase, and cathepsin D activity of rat brain. Environ Res, 26: 168-173. 224335

Dixit R; Seth PK; Mukhtar H (1982). Metabolism of acrylamide into urinary mercapturic acid and cysteine conjugates in rats. Drug Metab Dispos, 10: 196-197. 061317

Dobrzynska M; Lenarczyk M; Gajewski AK (1990). Induction of dominant lethal mutations by combined X-ray-acrylamide treatment in male mice. Mutat Res, 232: 209-215. 224341

Dobrzynska MM (2007). Assessment of DNA damage in multiple organs from mice exposed to x-rays or acrylamide or a combination of both using the comet assay. In Vivo, 21: 657-662. 224338

Doerge DR; da Costa GG; McDaniel LP; Churchwell MI; Twaddle NC; Beland FA (2005). DNA adducts derived from administration of acrylamide and glycidamide to mice and rats. Mutat Res Genet Toxicol Environ Mutagen, 580: 131-141. 224344

Doerge DR; Twaddle NC; Boettcher MI; McDaniel LP; Angerer J (2007). Urinary excretion of acrylamide and metabolites in Fischer 344 rats and B6C3F1 mice administered a single dose of acrylamide. Toxicol Lett, 169: 34-42. 224359

Doerge DR; Young JF; Chen JJ (2008). Using dietary exposure and physiologically based pharmacokinetic/pharmacodynamic modeling in human risk extrapolations for acrylamide toxicity. J Agric Food Chem, 56: 6031-6038. 224362

Doerge DR; Young JF; McDaniel LP; Twaddle NC; Churchwell MI (2005). Toxicokinetics of acrylamide and glycidamide in B6C3F1 mice. Toxicol Appl Pharmacol, 202: 258-267. 224348

Doerge DR; Young JF; McDaniel LP; Twaddle NC; Churchwell MI (2005). Toxicokinetics of acrylamide and glycidamide in Fischer 344 rats. Toxicol Appl Pharmacol, 208: 199-209. 224355

Donovan JW; Pearson T (1987). Ingestion of acrylamide with severe encephalopathy, neurotoxicity and hepatotoxicity. Vet Hum Toxicol, 29: 462. 224425

Doroshyenko O; Fuhr U; Kunz D; Frank D; Kinzig M; Jetter A; Reith Y; Lazar A; Taubert D; Kirchheiner J; Baum M; Eisenbrand G; Berger FI; Bertow D; Berkessel A; Sörgel F; Schömig E; Tomalik-Scharte D (2009). In vivo role of cytochrome P450 2E1 and glutathione-S-transferase activity for acrylamide toxicokinetics in humans. Cancer Epidemiol Biomarkers Prev, 18: 433-443. 224430

Dourson M; Hertzberg R; Allen B; Lynne Habera, Ann Parkera, Oliver Kronera, Andy Maiera and Melissa Kohrman (2008). Evidence-based dose-response assessment for thyroid tumorigenesis from acrylamide. Regul Toxicol Pharmacol, 52: 264-289. 224432

Dow Chemical Company (1984). Appendix I to acrylamide monomer two-year chronic toxicity-oncogenicity study; histopathologic observations--male rats. 067922

Dumitru DL (1989). Occupational intoxication with acrylamide: discussion of causes with acute and chronic intoxication. Rev Ig Med Muncii Med Soc Bacteriol Virusol Parazitol Epidemiol Pneumoftiziol Pneumoftiziol, 35: 359-364. 356205

Dybing E; Farmer PB; Andersen M; Fennell TR; Lalljie SPD; Müller DJG; Olin S; Petersen BJ; Schlatter J; Scholz G; Scimeca JA; Slimani N; Törnqvist M; Tuijtelaars S; Verger P (2005). Human exposure and internal dose assessments of acrylamide in food. Food Chem Toxicol, 43: 365-410. 224377

Dybing E; Sanner T (2003). Risk assessment of acrylamide in foods. Toxicol Sci, 75: 7-15. 224380

EC (2002). Opinion of the scientific committee on food on new findings regarding the presence of acrylamide in food. Scientific Committee on Food; Health & Consumer Protection Directorate-General;European Commission. Brussels, Belgium. SCF/CS/CNTM/CONT/4 Final. http://ec.europa.eu/food/fs/sc/scf/out131_en.pdf. 224396

Edwards PM (1975). The distribution and metabolism of acrylamide and its neurotoxic analogues in rats. Biochem Pharmacol, 24: 1277-1282. 224382

Edwards PM (1976). The insensitivity of the developing rat foetus to the toxic effects of acrylamide. Chem Biol Interact, 12: 13-18. 224385

Ehling UH (1988). Quantification of the genetic risk of environmental mutagens. Risk Anal, 8: 45-57. 224389

Ehling UH; Neuhäuser-Klaus A (1992). Reevaluation of the induction of specific-locus mutations in spermatogonia of the mouse by acrylamide. Mutat Res, 283: 185-191. 224391

EIC (2002). Toxicological review of acrylamide (CAS No. 79-06-01). Environ International Corporation. Ruston, LA. 224394

Eskin TA; Lapham LW; Maurissen JP; Merigan WH (1985). Acrylamide effects on the macaque visual system II Retinogeniculate morphology. Invest Ophthalmol Vis Sci, 26: 317-329. 224376

Exxon JH (2006). A review of the toxicology of acrylamide. J Toxicol Environ Health B Crit Rev, 9: 397-412. 224398

FAO/WHO (2002). Health implications of acrylamide in food : report of a joint FAO/WHO consultation, WHO Headquarters, Geneva, Switzerland, 25-27 June 2002. Geneva, Switzerland: World Health Organization in collaboration with the Food and Agriculture Organization of the United Nations. 324865

FAO/WHO (2005). Summary and Conclusion of the sixty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives. Presented at Meeting of the Joint FAO/WHO Expert Committee on Food Additives, 2/8-17/2005, Rome, Italy. 224279

FAO/WHO (2009). The FAO/WHO acrylamide in food network. Retrieved 15-SEP-09, from http://www.foodrisk.org/acrylamide/index.cfm. 224281

Favor J; Layton D; Sega G; Wassom J; Burkhart J; Douglas G; Dearfield K; Brusick D (1994). Genetic risk extrapolation from animal data to human disease: A taskgroup report of the International Commission for Protection Against Environmental Mutagens and Carcinogens . Mutat Res, 330: 23-34. 224290

Favor J; Shelby MD (2005). Transmitted mutational events induced in mouse germ cells following acrylamide or glycidamide exposure. Mutat Res, 580: 21-30. 224283

Feng Y; Forgac M (1992). Cysteine 254 of the 73kDa A subunit is responsible for inhibition of the coated vesicle (H+)-ATPase upon modification by sulfhydryl reagents. J Biol Chem, 267: 5817-5822. 224292

Fennell TR; Snyder RW; Krol WL; Sumner SC (2003). Comparison of the hemoglobin adducts formed by administration of N-methylolacrylamide and acrylamide to rats. Toxicol Sci, 71: 164-175. 224295

Fennell TR; Sumner SC; Snyder RW; Burgess J; Spicer R; Bridson WE; Friedman MA (2005). Metabolism and hemoglobin adduct formation of acrylamide in humans. Toxicol Sci, 85: 447-459. 224299

Field EA; Price CJ; Sleet RB; Marr MC; Schwetz BA; Morrissey RE (1990). Developmental toxicity evaluation of acrylamide in rats and mice. Fundam Appl Toxicol, 14: 502-512. 224302

Frantz SW; Dryzga MD; Fresheur NL (1995). In vitro/in vivo determination of cutaneous penetration of residual acrylamide monomer from polyacrylamide water solutions. Dow Chemical Toxicology Research Laboratory. Midland, Michigan. 224304

Friedman MA; Dulak LH; Keefe RT (1999). Effect of acrylamide on rat hormone levels in a 28-day drinking water study. Unknown. Unknown. 224381

Friedman MA; Dulak LH; Stedham MA (1995). A lifetime oncogenicity study in rats with acrylamide. Fundam Appl Toxicol, 27: 95-105. 224307

Friedman MA; Tyl RW; Marr MC; Myers CB; Gerling FS; Ross WP (1999). Effects of lactational administration of acrylamide on rat dams and offspring. Reprod Toxicol, 13: 511-520. 224311

Fuhr U; Boettcher MI; Kinzig-Schippers M; Weyer A; Jetter A; Lazar A; Taubert D; Tomalik-Scharte D; Pournara P; Jakob V; Harlfinger S; Klaassen T; Berkessel A; Angerer J; Sörgel F; Schömig E (2006). Toxicokinetics of acrylamide in humans after ingestion of a defined dose in a test meal to improve risk assessment for acrylamide carcinogenicity. Cancer Epidemiol Biomarkers Prev, 15: 266-271. 224319

Fullerton PM (1969). Electrophysiological and histological observations on peripheral nerves in acrylamide poisoning in man. J Neurol Neurosurg Psychiatry, 32: 186-192. 224325

Fullerton PM; Barnes JM (1966). Peripheral neuropathy in rats produced by acrylamide. Br J Ind Med, 23: 210-221. 061323

Gamboa da Costa G; Churchwell MI; Hamilton LP; Von Tungeln LS; Beland FA; Marques MM; Doerge DR (2003). DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal mice. Chem Res Toxicol, 16: 1328-1337. 194572

Garey J; Paule MG (2007). Effects of chronic low-dose acrylamide exposure on progressive ratio performance in adolescent rats. Neurotoxicology, 28: 998-1002. 224337

Garland TO; Patterson MWH (1967). Six cases of acrylamide poisoning. Br Med J, 4: 134-138. 061324

Generoso WM; Sega GA; Lockhart AM; Hughes LA; Cain KT; Cacheiro NL; Shelby MD (1996). Dominant lethal mutations, heritable translocations, and unscheduled DNA synthesis induced in male mouse germ cells by glycidamide, a metabolite of acrylamide. Mutat Res, 371: 175-183. 224346

Ghanayem BI; Witt K; Kissling GE; Tice RR; Recio L (2005). Absence of acrylamide-induced genotoxicity in CYP2E1-null mice: Evidence consistent with a glycidamide-mediated effect. Mutat Res, 578: 284-297. 224354

Ghanayem BI; Witt KL; El-Hadri L; Hoffler U; Kissling GE; Shelby MD; Bishop JB (2005). Comparison of germ cell mutagenicity in male CYP2E1-null and wild-type mice treated with acrylamide: Evidence supporting a glycidamide-mediated effect. Biol Reprod, 72: 157-163. 224351

Gho M; McDonald K; Ganetzky B; Saxton WM (1992). Effects of kinesin mutations on neuronal functions. Science, 258: 313-316. 224358

Gilbert SG; Maurissen JPJ (1982). Assessment of the effects of acrylamide, methylmercury, and 2,5-hexanedione on motor functions in mice. J Toxicol Environ Health, 10: 31-41. 061325

Gjerløff T; Elsborg H; Bonde JP (2001). Svaer kronisk acrylamid-intoksikation [Severe chronic acrylamide intoxication]. Ugeskr Laeger, 163: 4204-4205. 224367

Goodman DG; Ward JM; Squire RA; Chu KC; Linhart MS (1979). Neoplastic and nonneoplastic lesions in aging F344 rats. Toxicol Appl Pharmacol, 48: 237-248. 224373

Gould RM; Brady ST (2004). Neuropathology: Many paths lead to hereditary spastic paraplegia. Curr Biol, 14: R903-R904. 224407

Granath F; Ehrenberg L; Törnqvist M (1992). Degree of alkylation of macromolecules in vivo from variable. Mutat Res, 284: 297-306. 224411

Gutierrez-Espeleta GA; Hughes LA; Piegorsch WW; (1992). Acrylamide: Dermal exposure produces genetic damage in male mouse germ cells. Fundam Appl Toxicol, 18: 189-192. 224413

Haber LT; Maier A; Kroner OL; Kohrman MJ (2009). Evaluation of human relevance and mode of action for túnica vaginalis mesotheliomas resulting from oral exposure to acrylamide. Regul Toxicol Pharmacol, 53: 134-149. 224431

Hagmar L; Bonassi S; Strömberg U; Mikoczy Z; Lando C; Hansteen IL; Montagud AH; Knudsen L; Norppa H; Reuterwall C; Tinnerberg H; Brogger A; Forni A; Högstedt B; Lambert B; Mitelman F; Nordenson I; Salomaa S; Skerfving S (1998). Cancer predictive value of cytogenetic markers used in occupational health surveillance programs: A report from an ongoing study by the European Study Group on Cytogenetic Biomarkers and Health. Mutat Res, 405: 171-178. 224438

Hagmar L; Törnqvist M; Nordander C; Rosén I; Bruze M; Kautiainen A; Magnusson A-L; Malmberg B; Aprea P; Granath F; Axmon A (2001). Health effects of occupational exposure to acrylamide using hemoglobin adducts as biomarkers of internal dose. Scand J Work Environ Health, 27: 219-226. 224453

Hagmar L; Wirfalt E; Paulsson B; Tornqvist M (2005). Differences in hemoglobin adduct levels of acrylamide in the general population with respect to dietary intake, smoking habits and gender. Mutat Res, 580: 157-165. 224457

Hanioka N; Tanaka-Kagawa T; Miyata Y; Matsushima E; Makino Y; Ohno A; Yoda R; Jinno H; Ando M (2003). Functional characterization of three human cytochrome p450 2E1 variants with amino acid substitutions. Xenobiotica, 33: 575-586. 224460

Hansch C; Leo A; Hoekman D (1995). Exploring QSAR. Hydrophobic, electronic, and steric constants. In SR Heller (Ed.),ACS professional reference book (pp. 6). Washington, DC: American Chemical Society. 224462

Harris CH; Gulati AK; Friedman MA; Sickles DW (1994). Toxic neurofilamentous axonopathies and fast axonal transport. V. Reduced bidirectional vesical transport in cultured neurons by acrylamide and glycidamide. J Toxicol Environ Health, 42: 343-356. 224464

Harry GJ (1992). Acrylamide-induced alterations in axonal transport. Mol Neurobiol, 6: 203-216. 224479

Hartmann EC; Boettcher MI; Schettgen T; Fromme H; Drexler H; Angerer J (2008). Hemoglobin adducts and mercapturic acid excretion of acrylamide and glycidamide in one study population. J Agric Food Chem, 56: 6061-6068. 224480

Hashimoto K; Aldridge WN (1970). Biochemical studies on acrylamide, a neurotoxic agent. Biochem Pharmacol, 19: 2591-2604. 063972

Hashimoto K; Sakamoto J; Tanii H (1981). Neurotoxicity of acrylamide and related compounds and their effects on male gonads in mice. Arch Toxicol, 47: 179-189. 061328

Hashimoto K; Tanii H (1985). Mutagenicity of acrylamide and its analogues in Salmonella typhimurium. Mutat Res, 158: 129-133. 224504

Hays SM; Aylward LL (2008). Biomonitoring Equivalents (BE) dossier for acrylamide (AA) (CAS No. 79-06-1). Regul Toxicol Pharmacol, 51: S57-S67. 224510

Hays SM; Aylward LL (2008). Using Biomonitoring Equivalents to interpret human biomonitoring data in a public health risk context. J Appl Toxicol, 29: 275-288. 224507

Hazleton Laboratories (1953). Inhalation toxicity supplement to reports dated May 2, 1951 and August 13, 1952. Hazleton Laboratories. Princeton, NJ. 224514

Hazleton Laboratories (1954). Chronic inhalation exposure - acrylamide. Hazleton Laboratories . Princeton, NJ . 061399

He F; Zhang S; Wang H; Li G; Zhang ZM; Li FL; Dong XM; Hu FR (1989). Neurological and electroneuromyographic assessment of the adverse effects of acrylamide on occupationally exposed workers. Scand J Work Environ Health, 15: 125-129. 061330

Hersch MI; McLeod JG; Satchell PM; Early RG; Sullivan CE (1989). Breathing pattern, lung inflation reflex and airway tone in acrylamide neuropathy. Respir Physiol, 76: 257-276. 061331

Heudorf U; Hartmann E; Angerer J (2009). Acrylamide in children - exposure assessment via urinary acrylamide metabolites as biomarkers. Int J Hyg Environ Health, 212: 135-141. 224517

Hinson JA; Roberts DW (1992). Role of covalent and noncovalent interactions in cell toxicity: Effects on proteins. Annu Rev Pharmacol Toxicol, 32: 471-510. 224518

Ho WH; Wang SM; Yin HS (2002). Acrylamide disturbs the subcellular distribution of GABAa receptor in brain neurons. J Cell Biochem, 85: 561-571. 224519

Hogervorst JG; Schouten LJ; Konings EJ; Goldbohm RA; van den Brandt PA (2007). A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. Cancer Epidemiol Biomarkers Prev, 16: 2304-2313. 224520

Hogervorst JG; Schouten LJ; Konings EJ; Goldbohm RA; van den Brandt PA (2008). Dietary acrylamide intake and the risk of renal cell, bladder, and prostate cancer. Am J Clin Nutr, 87: 1428-1438. 224521

Hogervorst JG; Schouten LJ; Konings EJ; Goldbohm RA; van den Brandt PA (2008). Dietary acrylamide intake is not associated with gastrointestinal cancer risk. J Nutr, 138: 2229-2236. 224522

Holland N; Ahlborn T; Turteltaub K; Markee C; Moore D 2nd; Wyrobek AJ; Smith MT (1999). Acrylamide causes preimplantation abnormalities in embryos and induces chromatinadducts in male germ cells of mice. Reprod Toxicol, 13: 167-178. 224523

Hoorn AJW; Custer LL; Myhr BC; Brusick D; Gossen J; Vijg J (1993). Detection of chemical mutagens using Muta® mouse: A transgenic mouse model. Mutagenesis, 8: 7-10. 224524

Howe RB; Crump KS (1985). MULTI-WEIB: A Computer Program to Extrapolate Time to Tumor Animal Toxicity Data to Low Doses. 1.0. Ruston, LA: Clement Associates; KS Crump Division 402270

HSDB (2005). Acrylamide. Retrieved 15-SEP-09, from http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~NMTupu:1. 224272

Hurd DD; Saxton WM (1996). Kinesin mutations cause motor neuron disease phenotypes by disrupting fast axonal transport in Drosophila. Genetics, 144: 1075-1085. 224273

Husain R; Dixit R; Das M; Seth PK (1987). Neurotoxicity of acrylamide in developing rat brain: Changes in the levels of brain biogenic amines and activities of monoamine oxidase and acetylcholine esterase. Ind Health, 25: 19-28. 061336

IARC (1994). Ethylene oxide. In IARC Monographs on the Evaluation of Carcinogenic Risks to HumansVolume 60: Some Industrial Chemicals (pp. 73-159). Lyon, France: International Agency for Research on Cancer, World Health Organization. 018474

IARC (1994). IARC monographs on some industrial chemicals - summary of data reported and evaluation: Acrylamide. IARC. Geneva, Switzerland. 224633

IARC (1999). IARC monographs on the evaluation of carcinogenic risks to humans, Part One: Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide - Acrylonitrile. IARC. Geneva, Switzerland. 224635

IARC (2007). 1,3-Butadiene. In IARC monographs on the evaluation of carcinogenic risks to humans (pp. 45-184). Geneva, Switzerland: International Agency for Research on Cancer. 224637

IARC (2007). Ethylene oxide. In IARC monographs on the evaluation of carcinogenic risks to humans (pp. 185-309). Geneva, Switzerland: International Agency for Research on Cancer. 224636

Iatropoulos M; Lebish I; Wang CX; Williams GM (1998). Microscopic evaluation of proliferative mesothelial lesions diagnosed previously as mesotheliomas of the tunica vaginalis testis. Cytec Industries. West Paterson, NJ. 224628

ICPEMC (1993). Genetic risk extrapolation from animal data to human disease, Final report. U.S. Environmental Protection Agency. Oak Ridge National Laboratory. 224624

ICPEMC (1993). Use of in vivo genetic toxicology data to construct human risk assessments, Final report. Submitted to Department of Health. Canada. 224627

Igisu H; Goto I; Kawamura Y; Kato M; Izumi K; Kuroiwa Y (1975). Acrylamide encephaloneuropathy due to well water pollution. J Neurol Neurosurg Psychiatry, 38: 581-584. 061355

Igisu H; Matsuoka M (2002). Acrylamide encephalopathy. J Occup Health, 44: 63-68. 224318

Ikeda GJ; Miller E; Sapienza PP; Michel TC; King MT; Sager AO (1985). Maternal-foetal distribution studies in late pregnancy. II. Distribution of [1-14C]acrylamide in tissues of beagle dogs and miniature pigs. Food Chem Toxicol, 23: 757-761. 224352

Ikeda GJ; Miller E; Sapienza PP; Michel TC; King MT; Turner VA; Blumenthal H; Jackson WE III; Levin S (1983). Distribution of 14C-labelled acrylamide and betaine in foetuses of rats, rabbits, beagle dogs and miniature pigs. Food Chem Toxicol, 21: 49-58. 224332

IRMM (2006). Acrylamide monitoring database. Retrieved 04-FEB-10, from http://irmm.jrc.ec.europa.eu/html/activities/acrylamide/database.htm . 399218

IRRM (2004). Monitoring database on acrylamide levels in food maintained by the Institute for Reference Materials and Measurements together with Health and Consumer Protection DG. Retrieved 15-SEP-09, from http://irmm.jrc.ec.europa.eu/html/activities/acrylamide/database.htm. 224383

Jiang L; Cao J; An Y; Geng C; Qu S; Jiang L; Zhong L (2007). Genotoxicity of acrylamide in human hepatoma G2 (HepG2) cells. Toxicol In Vitro, 21: 1486-1492. 224388

JIFSAN (2002). Acrylamide in food workshop: scientific issues, uncertainties, and research strategies. Retrieved 15-SEP-09, from http://jifsan.umd.edu/news/event_record.php?id=21. 224402

JIFSAN (2004). Food Industry Coalition/JIFSAN Workshop. Update: Scientific Issues, Uncertainties, and Research Strategies on Acrylamide in Food. Retrieved 15-SEP-09, from http://jifsan.umd.edu/news/event_record.php?id=19. 224420

Jin L; Chico-Galdo V; Massart C; Gervy C; De Maertelaere V; Friedman M; Van Sande J (2008). Acrylamide does not induce tumorigenesis or major defects in mice in vivo. J Endocrinol, 198: 301-307. 224426

Johnson KA; Beyer JE; Bell TJ; Schuetz DJ; Gorzinski SJ (1985). Acrylamide: a two-year drinking water chronic toxicity-oncogenicity study in Fischer 344 rats: electron microscopy portion. submitted under TSCA. Dow Chemical. 067932

Johnson KA; Gorzinski SJ; Bodner KM; Campbell RA (1984). Acrylamide: a 2-year drinking water chronic toxicity- oncogenicity study in Fischer 344 rats; final report. Dow Chemical. Midland, MI. NTIS No. OTS0507273. 067926

Johnson KA; Gorzinski SJ; Bodner KM; Campbell RA; Wolf CH; Friedman MA; Mast RW (1986). Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. Toxicol Appl Pharmacol, 85: 154-168. 061340

Johnsrud EK; Koukouritaki SB; Divakaran K; Brunengraber LL; Hines RN;p McCarver DG (2003). Human hepatic CYP2E1 expression during development. J Pharmacol Exp Ther, 307: 402-407. 224616

Jung R; Engelhart G; Herbolt B; Jäckh R; Müller W (1992). Collaborative study of mutagenicity with Salmonella typhimurium TA102. Mutat Res, 278: 265-270. 224612

Kadry AM; Friedman MA; Abdel-Rahman MS (1999). Pharmacokinetics of acrylamide after oral administration in male rats. Environ Toxicol Pharmacol, 7: 127-133. 224596

Kaplan ML; Murphy SD; Gilles FH (1973). Modification of acrylamide neuropathy in rats by selected factors. Toxicol Appl Pharmacol, 24: 564-579. 224594

Kaster J; Kamendulis L; Friedman M; Klaunig J (1998). Syrian hamster embryo (SHE) cell transformation by acrylamide and hormones. Toxicologist, 42: A375. 224588

Kellert M; Scholz K; Wagner S; Dekant W; Völkel W (2006). Quantitation of mercapturic acids from acrylamide and glycidamide in human urine using a column switching tool with two trap columns and electrospray tandem mass spectrometry. J Chromatogr A, 1131: 58-66. 224571

Kesson CM; Baird AW; Lawson DH (1977). Acrylamide poisoning. Postgrad Med, 53: 16-17. 224568

Khan MA; Davis CA; Foley GL; Friedman MA; Hansen LG (1999). Changes in thyroid gland morphology after acute acrylamide exposure. Toxicol Sci, 47: 151-157. 224565

Kirman CR; Gargas ML; Deskin R; Tonner-Navarro L; Andersen ME (2003). A physiologically based pharmacokinetic model for acrylamide and its metabolite, glycidamide, in the rat. J Toxicol Environ Health A Curr Iss, 66: 253-274. 087778

Klaunig J (2000). Cellular effects of acrylamide, as cited in EIC 2002, 224394. Indiana University School of Medicine. Indianapolis, IN. Unpublished data. 594245

Klaunig JE (2008). Acrylamide carcinogenicity. J Agric Food Chem, 56: 5984-5988. 224554

Klaunig JE; Kamendulis LM (2005). Mechanisms of acrylamide induced rodent carcinogenesis. Adv Exp Med Biol, 561: 49-62. 224550

Kligerman AD; Atwater AL; Bryant MF; Erexson GL; Kwanyuen P; Dearfield KL (1991). Cytogenetic studies of ethyl acrylate using C57BL/6 mice. Mutagenesis, 6: 137-141. 006236

Knaap AG; Kramers PG; Voogd CE; Bergkamp WG; Groot MG; Langebroek PG; Mout HC; van der Stel JJ; Verharen HW (1988). Mutagenic activity of acrylamide in eukaryotic systems but not in bacteria. Mutagenesis, 3: 263-268. 224547

Ko MH; Chen WP; Lin-Shiau SY; Hsieh ST (1999). Age-dependent acrylamide neurotoxicity in mice: morphology, physiology, and function. Exp Neurol, 158: 37-46. 224540

Konings EJM; Baars AJ; van Klaveren JD; Spanjer MC; Rensen PM; Hiemstra M; van Kooij JA; Peters PWJ (2003). Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. Food Chem Toxicol, 41: 1569-1579. 224538

Koo LC; Ho JH-C; Saw D (1983). Active and passive smoking among female lung cancer patients and controls in Hong Kong. J Exp Clin Canc Res, 2: 367-375. 024400

Kopp EK; Dekant W (2009). Toxicokinetics of acrylamide in rats and humans following single oral administration of low doses. Toxicol Appl Pharmacol, 235: 135-142. 224532

Krebs O; Favor J (1997). Somatic and germ cell mutagenesis in lambda lacZ transgenic mice treated with acrylamide or ethylnitrosourea. Mutat Res, 388: 239-248. 224526

Krishna G; Theiss JC (1995). Concurrent analysis of cytogenetic damage in vivo: a multiple endpoint-multiple tissue approach. Environ Mol Mutagen, 25: 314-320. 224525

KS Crump Group (1999). Consideration of the potency classification of acrylamide based on the incidence of tunica vaginalis mesotheliomas (TVMs) in male F344 rats. Unpublished report prepared for the Acrylamide Monomer Producers Association. Frankfort, KY. 224622

KS Crump Group (1999). Mechanism of acrylamide induction of benign mammary fibroadenomas in the aging female F344 rat: relevance to human health risk assessment. Unpublished report prepared for the Acrylamide Monomer Producers Association. Frankfort, KY. 224623

Lähdetie J; Suutari A; Sjöblom T (1994). The spermatid micronucleus test with the dissection technique detects the germ cell mutagenicity of acrylamide in rat meiotic cells. Mutat Res, 309: 255-262. 224500

Lande SS; Bosch SJ; Howard PH (1979). Degradation and leaching of acrylamide in soil. J Environ Qual, 8: 133-137. 224498

Lapadula DM; Bowe M; Carrington CD; Dulak L; Friedman M; Abou-Donia MB (1989). In vitro binding of [14C]acrylamide to neurofilament and microtubule proteins of rats. Brain Res, 481: 157-161. 224486

Larsson SC; Akesson A; Bergkvist L; Wolk A (2009). Dietary acrylamide intake and risk of colorectal cancer in a prospective cohort of men. Eur J Cancer, 45: 513-516. 224466

Larsson SC; Akesson A; Wolk A (2009). Dietary acrylamide intake and prostate cancer risk in a prospective cohort of Swedish men. Cancer Epidemiol Biomarkers Prev, 18: 1939-1941. 224461

Larsson SC; Akesson A; Wolk A (2009). Long-term dietary acrylamide intake and breast cancer risk in a prospective cohort of Swedish women. Am J Epidemiol, 169: 376-381. 224463

Larsson SC; Akesson A; Wolk A (2009). Long-term dietary acrylamide intake and risk of epithelial ovarian cancer in a prospective cohort of Swedish women. Cancer Epidemiol Biomarkers Prev, 18: 994-997. 224483

Larsson SC; Håkansson N; Akesson A; Wolk A (2009). Long-term dietary acrylamide intake and risk of endometrial cancer in a prospective cohort of Swedish women. Int J Cancer, 124: 1196-1199. 224484

Lehning EJ; Balaban CD; Ross JF; LoPachin RM (2003). Acrylamide neuropathy III. Spatiotemporal characteristics of nerve cell damage in forebrain. Neurotoxicology, 24: 125-136. 224549

Lehning EJ; Balaban CD; Ross JF; LoPachin RM (2003). Acrylamide neuropathy. II. Spatiotemporal characteristics of nerve cell damage in brainstem and spinal cord. Neurotoxicology, 24: 109-123. 224548

Lehning EJ; Persaud A; Dyer KR; Jortner BS; LoPachin RM (1998). Biochemical and morphologic characterization of acrylamide peripheral neuropathy. Toxicol Appl Pharmacol, 151: 211-221. 224454

Lewis RJ (ed.) (1997). Hawley's condensed chemical dictionary. New York, NY: John Wiley & Sons, Inc. 224609

Lide DR (2000). CRC handbook of chemistry and physics. 81st edition. Boca Raton, FL: CRC Press LLC. 196090

Lijinsky W; Andrews AW (1980). Mutagenicity of vinyl compounds in Salmonella typhimurium. Birth Defects Res B Dev Reprod Toxicol, 1: 259-267. 017361

Lipscomb JC (2004). Evaluating the relationship between variance in enzyme expression and toxicant concentration in health risk assessment. Hum Ecol Risk Assess, 10: 39-55. 224551

Lipscomb JC; Teuschler LK; Swartout J; Popken D; Cox T; Kedderis GL (2003). The impact of cytochrome P450 2E1-dependent metabolic variance on a risk-relevant pharmacokinetic outcome in humans. Risk Anal, 23: 1221-1238. 192847

LoPachin RM; Barber DS (2006). Synaptic cysteine sulfhydryl groups as targets of electrophilic neurotoxicants. Toxicol Sci, 94: 240-255. 224552

LoPachin RM; Barber DS; Gavin T (2008). Molecular mechanisms of the conjugated alpha,beta-unsaturated carbonyl derivatives: Relevance to neurotoxicity and neurodegenerative diseases. Toxicol Sci, 104: 235-249. 224560

LoPachin RM; Barber DS; Geohagen BC; Gavin T; He D; Das S (2007). Structure-toxicity analysis of type-2 alkenes: In vitro neurotoxicity. Toxicol Sci, 95: 136-146. 224566

LoPachin RM; Barber DS; He D; Das S (2006). Acrylamide inhibits dopamine uptake in rat striatal synaptic vesicles. Toxicol Sci, 89: 224-234. 224567

LoPachin RM; DeCaprio AP (2005). Protein adduct formation as a molecular mechanism in neurotoxicity. Toxicol Sci, 86: 214-225. 224556

LoPachin RM; Gavin T; Geohagen BC; Das S (2007). Neurotoxic mechanisms of electrophilic type-2 alkenes: Soft soft interactions described by quantum mechanical parameters. Toxicol Sci, 98: 561-570. 224569

LoPachin RM; Geohagen BC; Gavin T (2009). Synaptosomal toxicity and nucleophilic targets of 4-hydroxy-2-nonenal. Toxicol Sci, 107: 171-181. 224561

LoPachin RM; Ross JF; Lehning EJ (2002). Nerve terminals as the primary site of acrylamide action: A hypothesis. Neurotoxicology, 23: 43-59. 224562

LoPachin RM; Ross JF; Reid ML; Das S; Mansukhani S; Lehning EJ (2002). Neurological evaluation of toxic axonopathies in rats: acrylamide and 2,5-hexanedione. Neurotoxicology, 23: 95-110. 224564

LoPachin RM; Schwarcz AI; Gaughan CL; Mansukhani S; Das S (2004). In vivo and in vitro effects of acrylamide on synaptosomal neurotransmitter uptake and release. Neurotoxicology, 25: 349-363. 224570

LoPachin RM Jr; Lehning EJ (1994). Acrylamide-induced distal axon degeneration: a proposed mechanism of action. Neurotoxicology, 15: 247-260. 224557

Lyon MF; Adler ID; Bridges BA; Ehrenberg L; Golberg L; Kilian DJ Kondo S; Moustacchi E; Putrament A; Sankaranarayanan K; Sobels FH; Sram RJ; Streisinger G; Sundaram K (1983). Estimation of genetic risks and increased incidence of genetic disease due to environmental mutagens. Mutat Res, 115: 255-291. 224617

Manjanatha MG; Aidoo A; Shelton SD; Bishop ME; McDaniel LP; Lyn-Cook LE; Doerge DR (2006). Genotoxicity of acrylamide and its metabolite glycidamide administered in drinking water to male and female Big Blue mice. Environ Mol Mutagen, 47: 6-17. 224572

Mapp C; Mazzotta M; Bartolucci GB; Fabbri L (1977). Nervous system disease caused by acrylamide: 1st cases in Italy. Med Lav, 68: 1-12. 224611

Marchetti F; Bishop J; Lowe X; Wyrobek AJ (2009). Chromosomal mosaicism in mouse two-cell embryos after paternal exposure to acrylamide. Toxicol Sci, 107: 194-205. 224574

Marchetti F; Lowe X; Bishop J; Wyrobek AJ (1997). Induction of chromosomal aberrations in mouse zygotes by acrylamide treatment of male germ cells and their correlation with dominant lethality and heritable translocations. Environ Mol Mutagen, 30: 410-417. 224573

Marlowe C; Clark MJ; Mast RW; Friedman MA; Waddell WJ (1986). The distribution of [14C]acrylamide in male and pregnant Swiss-Webster mice studied by whole-body autoradiography. Toxicol Appl Pharmacol, 86: 457-465. 224576

Marsh GM; Lucas LJ; Youk AO; Schall LC (1999). Mortality patterns among workers exposed to acrylamide: 1994 follow up. Occup Environ Med, 56: 181-190. 224577

Marsh GM; Youk AO; Buchanich JM; Kant IJ; Swaen G (2007). Mortality patterns among workers exposed to acrylamide: Updated follow up. J Occup Environ Med, 49: 82-95. 224578

Martins C; Oliveira NG; Pingarilho M; Gamboa da Costa G; Martins V; Marques MM; Beland FA; Churchwell MI; Doerge DR; Rueff J; Gaspar JF (2007). Cytogenic damage induced by acrylamide and glycidamide in mammalian cells: Correlation with specific glycidamide-DNA adducts. Toxicol Sci, 95: 383-390. 224580

Marty JP; Vincent CM (1998). In vitro percutaneous absorption of acrylamide across human skin. Research Unit in Dermopharmacology and Cosmetology, University of Paris. Paris, France. 224582

Maurissen JPJ; Weiss B; Davis HT (1983). Somatosensory thresholds in monkeys exposed to acrylamide. Toxicol Appl Pharmacol, 71: 266-279. 061346

Mayerhofer A; Hemmings HC Jr; Snyder GL; Greengard P; Boddien S; Berg U; Brucker C (1999). Functional dopamine-1 receptors and DARPP-32 are expressed in human ovary and granulosa luteal cells in vitro. J Clin Endocrinol Metab, 84: 257-264. 224583

McCollister DD; Oyen F; Rowe VK (1964). Toxicology of acrylamide. Toxicol Appl Pharmacol, 6: 172-181. 061347

McConnell RF; Western HH; Ulland BM (1992). Proliferative lesions of the testes in rats with selected examples from mice. In Guides for Toxicologic Pathology (pp. 1-32). Washington, DC: STP/ARP/AFIP. 224613

Mei N; Hu J; Churchwell MI; Guo L; Moore MM; Doerge DR; Chen T (2008). Genotoxic effects of acrylamide and glycidamide in mouse lymphoma cells. Food Chem Toxicol, 46: 628-636. 224585

Michels KB; Rosner BA; Chumlea WC; Colditz GA; Willett WC (2006). Preschool diet and adult risk of breast cancer. Int J Cancer, 118: 749-754. 224586

Miller MJ; Carter DE; Sipes IG (1982). Pharmacokinetics of acrylamide in Fischer-334 rats. Toxicol Appl Pharmacol, 63: 36-44. 061351

Moore MM; Amtower A; Doerr C; Brock KH; Dearfield KL (1987). Mutagenicity and clastogenicity of acrylamide in L5178Y mouse lymphoma cells. Environ Mutagen, 9: 261-267. 224589

Mori H; Arakawa S; Ohkawa T; Ohkawa R; Takada S; Morita T; Okinaga S (1994). The involvement of dopamine in the regulation of steroidogenesis in rat ovarian cells. Horm Res, 41: 36-40. 224591

Moser VC; Anthony DC; Sette WF; MacPhail RC (1992). Comparison of subchronic neurotoxicity of 2-hydroxyethyl acrylate and acrylamide in rats. Fundam Appl Toxicol, 18: 343-352. 224592

Mottram DS; Wedzicha BL; Dodson AT (2002). Acrylamide is formed in the Maillard reaction. Nature, 19: 448-449. 224595

Mucci LA; Adami HO; Wolk A (2006). Prospective study of dietary acrylamide and risk of colorectal cancer among women. Int J Cancer, 118: 169-173. 224601

Mucci LA; Dickman PW; Steineck G; Adami HO; Augustsson K (2003). Dietary acrylamide and cancer of the large bowel, kidney, and bladder: Absence of an association in a population-based study in Sweden. Br J Cancer, 88: 84-89. 224597

Mucci LA; Lindblad P; Steineck G; Adami HO (2004). Dietary acrylamide and risk of renal cell cancer. Int J Cancer, 109: 774-776. 224598

Mucci LA; Sandin S; Bälter K; Adami HO; Magnusson C; Weiderpass E (2005). Acrylamide intake and breast cancer risk in Swedish women. JAMA, 293: 1326-1327. 224600

Müller W; Engelhart G; Herbold B; Jäckh R; Jung R (1993). Evaluation of mutagenicity testing with Salmonella typhimurium TA102 in three different laboratories. Environ Health Perspect, 101: 33-36. 224602

Mulloy KB (1996). Two case reports of neurological disease in coal mine preparation plant workers. Am J Ind Med, 30: 56-61. 224604

Myers JE; Macun I (1991). Acrylamide neuropathy in a South African factory: An epidemiologic investigation. Am J Ind Med, 19: 487-493. 224605

Nagao T (1994). Developmental abnormalities due to exposure of mouse paternal germ cells, preimplantation embryos, and organogenic embryos to acrylamide. Congenital Anomalies, 34: 35-46. 224606

Neuhäuser-Klaus A; Schmahl W (1989). Mutagenic and teratogenic effects of acrylamide in the mammalian spot test. Mutat Res, 6: 157-162. 224607

Neumann F (1991). Early indicators for carcinogenesis in sex-hormone-sensitive organs. Mutat Res, 248: 341-356. 224608

NRC (1983). Risk assessment in the federal government: Managing the process. National Research Council. Washington, DC. 194806

NRC (1994). Science and judgment in risk assessment. National Research Council. Washington, DC. 006424

NSF/ANSI (2009). Standard 60: Drinking water treatment chemicals - health effects . Ann Arbor, MI: National Sanitation Foundation; American National Standards Institute. 399318

NTP (1989). Toxicology and carcinogenesis studies of N-methylolacrylamide (CAS No. 924-42-5) in F344/N rats and B6C3F1 mice (gavage studies). National Toxicology Program. Research Triangle Park, NC. NIH Publication No. 89-2807. NTP TR 352. . 224294

NTP (1993). Final report on the reproductive toxicity of acrylamide (ACRL) (CAS No. 79-06-1) in CD-1 (trade name) swiss mice. National Toxicology Program. Research Triangle Park, NC. 224289

NTP/CERHR (2004). Expert panel report on reproductive and developmental toxicity of acrylamide. National Toxicology Program Center for the Evaluation of Risks to Human Reproduction. Research Triangle Park, NC.http://cerhr.niehs.nih.gov/. 224300

Ogawa M; Oyama T; Isse T; Yamaguchi T; Murakami T; Endo Y; Kawamoto T (2006). Hemoglobin adducts as a marker of exposure to chemical substances, especially PRTR class I designated chemical substances. J Occup Health, 48: 314-328. 597196

Olesen PT; Olsen A; Frandsen H (2008). Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish diet, cancer and health study. Int J Cancer, 122: 2094-2100. 224303

Ølstørn HB; Paulsen JE; Alexander J (2007). Effects of perinatal exposure to acrylamide and glycidamide on intestinal tumorigenesis in Min/+ mice and their wild-type litter mates . Anticancer Res, 27: 3855-3864. 224309

Osterman-Golkar S; Ehrenberg L; Segerbäck D; Hällström I (1976). Evaluation of genetic risks of alkylating agents. II. Haemoglobin as a dose monitor. Mutat Res, 34: 1-10. 224312

Pacchierotti F; Tiveron C; D'Archivio M; Bassani B; Cordelli E; Leter G; Spanò M (1994). Acrylamide-induced chromosomal damage in male mouse germ cells detected by cytogenetic analysis of one-cell zygotes. Mutat Res, 309: 273-284. 224316

Padilla S; Atkinson MB; Breuer AC (1993). Direct measurement of fast axonal organelle transport in the sciatic nerve of rats treated with acrylamide. J Toxicol Environ Health, 39: 429-445. 224323

Park J; Kamendulis LM; Friedman MA; Klaunig JE (2002). Acrylamide-induced cellular transformation. Toxicol Sci, 65: 177-183. 224330

Paulsson B; Athanassiadis I; Rydberg P; Törnqvist M (2003). Hemoglobin adducts from glycidamide: Acetonization of hydrophilic groups for reproducible gas chromatography/tandem mass spectrometric analysis. Rapid Commun Mass Spectrom, 17: 1859-1865. 224345

Paulsson B; Grawé J; Törnqvist M (2002). Hemoglobin adducts and micronucleus frequencies in mouse and rat after acrylamide or N-methylolacrylamide treatment . Mutat Res, 516: 101-111. 224334

Paulsson B; Kotova N; Grawé J; Henderson A; Granath F; Golding B; Törnqvist M (2003). Induction of micronuclei in mouse and rat by glycidamide, genotoxic metabolite of acrylamide. Mutat Res, 535: 15-24. 224340

Paulsson B; Rannug A; Henderson AP; Golding BT; Törnqvist M; Warholm M (2005). In vitro studies of the influence of glutathione transferases and epoxide hydrolase on the detoxification of acrylamide and glycidamide in blood. Mutat Res, 580: 53-59. 224349

Pearson RG; Songstad J (1967). Application of the principle of hard and soft acids and bases to organic chemistry. J Am Chem Soc, 89: 1827-1836. 224360

Pelucchi C; Galeone C; Dal Maso L; Talamini R; Montella M; Ramazzotti V; Negri E; Franceschi S; La Vecchia C; (2007). Dietary acrylamide and renal cell cancer. Int J Cancer, 120: 1376-13777. 224369

Pelucchi C; Galeone C; Levi F; Negri E; Franceschi S; Talamini R; Bosetti C; Giacosa A; La Vecchia C (2006). Dietary acrylamide and human cancer. Int J Cancer, 118: 467-471. 224364

Perreault SD (2003). Distinguishing between fertilization failure and early pregnancy loss when identifying male-mediated adverse pregnancy outcomes. Adv Exp Med Biol, 518: 189-198. 224370

Petersen DW; Kleinow KM; Kraska RC; Lech JJ (1985). Uptake, disposition and elimination of acrylamide in rainbow trout. Toxicol Appl Pharmacol, 80: 58-65. 224371

Post EJ; McLeod JG (1977). Acrylamide autonomic neuropathy in the cat. Part 1. Neurophysiological and histological studies. Journal of Neurological Sciences, 33: 353-374. 224374

Prentice DE; Meikle AW (1995). A review of drug-induced Leydig cell hyperplasia and neoplasia in the rat and some comparisons with man. Hum Exp Toxicol, 14: 562-572. 085738

Rafales LS; Lasley SM; Greenland RD; Mandybur T (1983). Effects of acrylamide on locomotion and central monoamine function in the rat. Pharmacol Biochem Behav, 19: 635-644. 061363

Ramsey JC; Young JD; Gorzinski SJ (1984). Acrylamide: toxicodynamics in rats. Dow Chemical USA. Midland, MI. 87-8213943. 067937

Raymer JH; Sparacino CM; Velez GR; Padilla S; MacPhail RC; Crofton KM (1993). Determination of acrylamide in rat serum and sciatic nerve by gas chromatography-electron-capture detection. Journal of Chromatography B: Biomedical Sciences and Applications, 619: 223-234. 224375

Reid E (2003). Science in motion: Common molecular pathological themes emerge in the hereditary spastic paraplegias. J Med Genet, 40: 81-86. 224379

Reid E; Kloos M; Ashley-Koch A; Hughes L; Bevan S; Svenson IK; Lennon Graham F; Gaskell PC; Dearlove A; Pericak-Vance MA; Rubinsztein DC; Marchuk DA (2002). A kinesin heavy chain (KIF5A) mutation in heredity spastic paraplegia (SPG 10). Am J Hum Genet, 71: 1189-1194. 224390

Rice JM (2005). The carcinogenicity of acrylamide. Mutat Res, 580: 3-20. 224393

Roach JA; Andrzejewski D; Gay ML; Nortrup D; Musser SM (2003). Rugged LC-MS/MS survey analysis for acrylamide in foods. J Agric Food Chem, 51: 7547-7554. 224399

Robinson M; Bull RJ; Knutsen GL; Shields RP; Stober J (1986). A combined carcinogen bioassay utilizing both the lung adenoma and skin papilloma protocols. Environ Health Perspect, 68: 141-145. 224403

Russell LB; Hunsicker PR; Cacheiro NL; Generoso WM (1991). Induction of specific-locus mutations in male germ cells of the mouse by acrylamide monomer. Mutat Res, 262: 101-107. 224406

Russo A; Gabbani G; Simoncini B (1994). Weak genotoxicity of acrylamide on premeiotic and somatic cells of the mouse. Mutat Res, 309: 263-272. 224409

Rutledge JC; Generoso WM; Shourbaji A; Cain KT; Gans M; Oliva J (1992). Developmental anomalies derived from exposure of zygotes and first-cleavage embryos to mutagens. Mutat Res, 296: 167-177. 224429

Sabri MI; Spencer PS (1990). Acrylamide impairs fast and slow axonal transport in rat optic system. Neurochem Res, 15: 603-608. 224434

Sakamoto J; Hashimoto K (1986). Reproductive toxicity of acrylamide and related compounds in mice- effects on fertility and sperm morphology. Arch Toxicol, 59: 201-205. 224442

Sakamoto J; Kurosaka Y; Hashimoto K (1988). Histological changes of acrylamide-induced testicular lesions in mice. Exp Mol Pathol, 48: 324-334. 061365

Sankaranarayanan K (1982). Genetic effects of ionizing radiation in multicellular eukaryotes and the assessment of genetic radiation hazards in man. Amsterdam: Elsevier. 224448

Satchell PM; McLeod JG (1981). Megaoesophagus due to acrylamide neuropathy. J Neurol Neurosurg Psychiatry, 44: 906-913. 061368

Schettgen T; Weiss T; Drexler H; Angerer J (2003). A first approach to estimate the internal exposure to acrylamide in smoking and non-smoking adults from Germany. Int J Hyg Environ Health, 206: 9-14. 224452

Schmid TE; Wang X; Adler ID (1999). Detection of aneuploidy by multicolor FISH in mouse sperm after in vivo treatment with acrylamide, colchicine, diazepam, or thiabenzole. Mutagenesis, 14: 173-179. 224458

Sega GA; Alcota RP; Tancongco CP; Brimer PA (1989). Acrylamide binding to the DNA and protamine of spermiogenic stages in the mouse and its relationship to genetic damage. Mutat Res, 216: 221-230. 224477

Sega GA; Generoso EE (1990). Measurement of DNA breakage in specific germ-cell stages of male mice exposed to acrylamide, using an alkaline-elution procedure. Mutat Res, 242: 79-87. 224465

Sega GA; Generoso EE; Brimer PA; Malling HV (1990). Acrylamide exposure induces a delayed unscheduled dna synthesis in germ cells of male mice that is correlated with the temporal pattern of adduct formation in testis DNA. Environ Mol Mutagen, 16: 137-142. 224482

Segerbäck D; Calleman CJ; Schroeder JL; Costa LG; Faustman EM (1995). Formation of N-7-(2-carbamoyl-2-hydroxyethyl)guanine in DNA of the mouse and the rat following intraperitoneal administration of [14C]acrylamide . Carcinogenesis, 16: 1161-1165. 224485

Shelby MD; Cain KT; Cornett CV; Generoso WM (1987). Acrylamide: Induction of heritable translocation in male mice. Environ Mutagen, 9: 363-368. 088819

Shelby MD; Cain KT; Hughes LA; Braden PW; Generoso WM (1986). Dominant lethal effects of acrylamide in male mice. DNA Repair (Amst), 173: 35-40. 094659

Shipp A; Lawrence G; Gentry R; McDonald T; Bartow H; Bounds J; Macdonald N; Clewell H; Allen B; Van Landingham C (2006). Acrylamide: Review of toxicity data and dose-response analyses for cancer and noncancer effects. Crit Rev Toxicol, 36: 481-608. 224488

Shiraishi Y (1978). Chromosome aberrations induced by monomeric acrylamide in bone marrow and germ cells of mice. Mutat Res, 57: 313-324. 224490

Sickles DW (1989). Toxic neurofilamentous axonopathies and fast anterograde axonal transport. I. The effects of single doses of acrylamide on the rate and capacity of transport. Neurotoxicology, 10: 91-102. 224491

Sickles DW (1989). Toxic neurofilamentous axonopathies and fast anterograde axonal transport. II. The effects of single doses of neurotoxic and non-neurotoxic diketones and beta, beta'-iminodipropionitrile (IDPN) on the rate and capacity of transport. Neurotoxicology, 10: 103-112. 224493

Sickles DW (1991). Toxic neurofilamentous axonopathies and fast anterograde axonal transport. III. Recovery from single injections and multiple dosing effects of acrylamide and 2,5-hexanedione. Toxicol Appl Pharmacol, 108: 390-396. 224494

Sickles DW (1992). Toxic neurofilamentous axonopathies and fast anterograde axonal transport. IV. In vitro analysis of transport following acrylamide and 2,5-hexanedione. Toxicol Lett, 61: 199-204. 224495

Sickles DW; Brady ST; Testino A; Friedman MA; Wrenn RW (1996). Direct effect of the neurotoxicant acrylamide on kinesin-based microtubule motility. J Neurosci, 46: 7-17. 224496

Sickles DW; Stone JD; Friedman MA (2002). Fast axonal transport: a site of acrylamide neurotoxicity? Neurotoxicology, 23: 223-251. 224497

Smith BL (1991). Fibroadenomas. In Breast Diseases (pp. 34-37). Philadelphia: JP Lippincott Company. 224499

Smith CJ; Perfetti TA; Rumple MA; Rodgman A; Doolittle DJ (2000). "IARC group 2A Carcinogens" reported in cigarette mainstream smoke. Food Chem Toxicol, 38: 371-383. 224502

Smith MK; Zenick H; Preston RJ; George EL; Long RE (1986). Dominant lethal effects of subchronic acrylamide administration in the male Long-Evans rat. Mutat Res, 173: 273-277. 224276

SNFA (2002). Analytical methodology and survey results for acrylamide in foods; Press Release, 26 April 2002. Retrieved 15-SEP-09, from http://www.slv.se/en-gb/group1/Food-Safety/Acrylamide/Analytical-methodology-and-survey-results-for-acrylamide-in-foods/. 224282

SNT (2002). Risk assessment of acrylamide intake from foods with special emphasis on cancer risk; Report from the ScientificCommittee of the Norwegian Food Control Authority, 6 June2002. Norwegian Food Safety Authority. Oslo, Norway.http://www.mattilsynet.no/english/food_safety. 418763

Sobel W; Bond GG; Parsons TW; Brenner FE (1986). Acrylamide cohort mortality study. Br J Ind Med, 43: 785-788. 067940

Sobels FH (1977). Some problems associated with the testing for environmental mutagens and a perspective for studies in "comparative mutagenesis". Mutat Res, 46: 245-260. 224286

Sobels FH (1982). The parallelogram: An indirect approach for the assessment of genetic risks from chemical mutagens. In Bora KC; Douglas GR; Nestman ER (Ed.),Progress in mutation research (pp. 323-327). Amsterdam: Elsevier. 224291

Sobels FH (1989). Models and assumptions underlying genetic risk assessment. Mutat Res, 212: 77-89. 224293

Solleveld HA; Haseman JK; McConnell EE (1984). Natural history of body weight gain, survival, and neoplasia in the F344 rat. J Natl Cancer Inst, 72: 929-940. 224298

Solomon JJ; Fedyk J; Mukai F; Segal A (1985). Direct alkylation of 2'-deoxynucleosides and DNA following in vitro reaction with acrylamide. Cancer Res, 45: 3465-3470. 224306

Spencer PS; Sabri MI; Schaumburg HH; Moore CL (1979). Does a defect of energy metabolism in the nerve fiber underlie axonal degeneration in polyneuropathies? Ann Neurol, 5: 501-507. 224317

Spencer PS; Schaumburg HH (1974). A review of acrylamide neurotoxicity. Part II. Experimental animal neurotoxicity and pathologic mechanisms. Can J Neurol Sci, 1: 152-169. 061376

Spencer PS; Schaumburg HH (1977). Ultrastructural studies of the dying-back process. IV. Differential vulnerability of PNS and CNS fibers in experimental central-peripheral distal axonopathies. J Neuropathol Exp Neurol, 36: 300-320. 224313

Stadler RH; Blank I; Varga N; Robert F; Hau J; Guy PA; Robert M-C; Riediker S (2002). Acrylamide from Maillard reaction products. Nature, 419: 449. 224324

Stamler JS; Lamas S; Fang FC (2001). Nitrosylation. the prototypic redox-based signaling mechanism. Cell, 106: 675-683. 224327

Stone JD; Peterson AP; Eyer J; Oblak TG; Sickles DW (1999). Axonal neurofilaments are nonessential elements of toxicant-induced reductions in fast axonal transport: video-enhanced differential interference microscopy in peripheral nervous system axons. Toxicol Appl Pharmacol, 161: 50-58. 224328

Stone JD; Peterson AP; Eyer J; Sickles DW (2000). Neurofilaments are non-essential elements of toxicant-induced reductions in fast axonal transport: Pulse labeling in CNS neurons. Neurotoxicology, 21: 447-457. 224333

Sublet VH; Zenick H; Smith MK (1989). Factors associated with reduced fertility and implantation rates in females mated to acrylamide-treated rats. Toxicology, 55: 53-67. 061380

Sumner SC; Fennell TR; Moore TA; Chanas B; Gonzalez F; Ghanayem BI (1999). Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. Chem Res Toxicol, 12: 1110-1116. 224342

Sumner SC; MacNeela JP; Fennell TR (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. 224339

Sumner SC; Williams CC; Snyder RW; Krol WL; Asgharian B; Fennell TR (2003). Acrylamide: A comparison of metabolism and hemoglobin adducts in rodents following dermal, intraperitoneal, oral, or inhalation exposure. Toxicol Sci, 75: 260-270. 224347

Suzuki K; Pfaff LD (1973). Acrylamide neuropathy in rats. An electron microscopic study of degeneration and regeneration. Acta Neuropathol, 24: 197-213. 224350

Svensson K; Abramsson L; Becker W; Glynn A; Hellenäs KE; Lind Y; Rosén J (2003). Dietary intake of acrylamide in Sweden. Food Chem Toxicol, 41: 1581-1586. 224353

Swaen GM; Haidar S; Burns CJ; Bodner K; Parsons T; Collins JJ; Baase C (2007). Mortality study update of acrylamide workers. Occup Environ Med, 64: 396-401. 224357

Takahashi M; Ohara T; Hashimoto K (1971). Electrophysiological study of nerve injuries in workers handling acrylamide. Int Arch Arbeitsmed , 28: 1-11. 061400

Tanigawa H; Onodera H; Maekawa A (1987). Spontaneous mesotheliomas in Fischer rats-a histological and electron microscopic study. Toxicol Pathol, 15: 157-163. 224366

Tareke E; Rydberg P; Karlsson P; Eriksson S; Törnqvist M (2000). Acrylamide: A cooking carcinogen? Chem Res Toxicol, 13: 517-522. 224368

Tareke E; Rydberg P; Karlsson P; Eriksson S; Törnqvist M (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J Agric Food Chem, 50: 4998-5006. 224384

Tareke E; Twaddle NC; McDaniel LP; Churchwell MI; Young JF; Doerge DR (2006). Relationships between biomarkers of exposure and toxicokinetics in Fischer 344 rats and B6C3F1 mice administered single doses of acrylamide and glycidamide and multiple doses of acrylamide. Toxicol Appl Pharmacol, 217: 63-75. 224387

Tegeris Laboratories (1989). Initial submission: A lifetime oncogenicity study in rats with acrylamide (final report) with cover letter dated 043092. U.S. Environmental Protection Agency. Washington, DC. 88-920002367. http://www.ntis.gov/search/product.aspx?ABBR=OTS0536419. 224400

Thier R; Lewalter J; Selinski S; Bolt HM (2002). Possible impact of human CYP2E1 polymorphisms on the metabolism of acrylonitrile. Toxicol Lett, 128: 249-255. 224405

Tilson HA (1981). The neurotoxicity of acrylamide: An overview. Neurobehav Toxicol Teratol, 3: 445-461. 061388

Tilson HA; Cabe PA (1979). The effects of acrylamide given acutely or in repeated doses on fore- and hindlimb function of rats. Toxicol Appl Pharmacol, 47: 253-260. 224408

Tong GC; Cornwell WK; Means GE (2004). Reactions of acrylamide with glutathione and serum albumin. Toxicol Lett, 147: 127-131. 224421

Törnqvist M; Fred C; Haglund J; Helleberg H; Paulsson B; Rydberg P (2002). Protein adducts: quantitative and qualitative aspects of their formation, analysis and applications. J Chromatogr B Analyt Technol Biomed Life Sci, 778: 279-308. 597198

Tornqvist M; Osterman-Golkar S; Paulsson B (2005). Acrylamide: Genetic Toxicity and Exposure Assessment. Mutat Res Genet Toxicol Environ Mutagen, 580: 1-178. 224638

Törnqvist M; Paulsson B; Vikström AC; Granath F (2008). Approach for cancer risk estimation of acrylamide in food on the basis of animal cancer tests and in vivo dosimetry. J Agric Food Chem, 56: 6004-6012. 224428

Tripathy NK; Patnaik KK; Nabi MJ (1991). Acrylamide is genotoxic to the somatic and germ cells of Drosophila melanogaster. Mutat Res, 259: 21-27. 224435

Tsuda H; Shimizu CS; Taketomi MK; Hasegawa MM; Hamada A; Kawata KM; Inui N (1993). Acrylamide; Induction of DNA damage, chromosomal aberrations and cell transformation without gene mutations. Mutagenesis, 8: 23-29. 224441

Turek FW; Desjardins C (1979). Development of Leydig cell tumors and onset of changes in the reproductive and endocrine systems of aging F344 rats. J Natl Cancer Inst, 63: 969-975. 224445

Twaddle NC; McDaniel LP; Gamboa da Costa G; Churchwell MI; Beland FA; Doerge DR (2004). Determination of acrylamide and glycidamide serum toxicokinetics in B6C3F1 mice using LC-ES/MS/MS. Cancer Lett, 207: 9-17. 224447

Tyl RW, Friedman MA, Losco PE, Fisher LC, Johnson KA, Strother DE, Wolf CH (2000). Rat two-generation reproduction and dominant lethal study of acrylamide in drinking water. Reprod Toxicol, 14: 385-401. 224456

Tyl RW; Friedman MA (2003). Effects of acrylamide on rodent reproductive performance. Reprod Toxicol, 17: 1-13. 224450

Tyl RW; Marr MC; Myers CB; Ross WP; Friedman MA (2000). Relationship between acrylamide reproductive and neurotoxicity in male rats. Reprod Toxicol, 14: 147-157. 224459

UNSCEAR (1986). Genetic and somatic effects of ionizing radiation, report to the General Assembly. United Nations Scientific Committee on the Effects of Atomic Radiation. New York. 224467

Uphouse L; Russell M (1981). Rapid effects of acrylamide on spiroperidol and serotonin binding in neural tissue. Neurobehav Toxicol Teratol, 3: 281-284. 224469

Uphouse LL; Nemeroff CB; Mason G; Prange AJ; Bondy SC (1982). Interactions between "handling" and acrylamide on endocrine responses in rats. Neurotoxicology, 3: 121-125. 224472

Urban M; Kavvadias D; Riedel K; Scherer G; Tricker AR (2006). Urinary mercapturic acids and a hemoglobin adduct for the dosimetry of acrylamide exposure in smokers and nonsmokers. Inhal Toxicol, 18: 831-839. 224476

U.S. EPA (1986). Guidelines for mutagenicity risk assessment (EPA/630/R-98/003). Fed Regist, 51: 34006-34012. 001466

U.S. EPA (1986). Guidelines for the health risk assessment of chemical mixtures (EPA/630/R-98/002). Fed Regist, 51: 34014-34025. 001468

U.S. EPA (1988). Recommendations for and documentation of biological values for use in risk assessment. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency. Cincinnati, OH. EPA/600/6-87/008. 064560

U.S. EPA (1991). Guidelines for developmental toxicity risk assessment. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, DC. EPA/600/FR-91/001. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=23162. 202859

U.S. EPA (1994). Interim policy for particle size and limit concentration issues in inhalation toxicity studies. U.S. Environmental Protection Agency, Health Effects Division. Washington, DC.http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=186068. 076133

U.S. EPA (1994). Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency. Research Triangle Park, NC. EPA/600/8-90/066F . http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=71993. 006488

U.S. EPA (1995). The use of the benchmark dose approach in health risk assessment. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, DC. EPA/630/R-94/007. http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=42601. 005992

U.S. EPA (1996). Guidelines for reproductive toxicity risk assessment. Office of Research and Development. Washington, DC. 030019

U.S. EPA (1998). Assessment of thyroid follicular cell tumors. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, D.C.. EPA/630/R-97/002. 030018

U.S. EPA (1998). Guidelines for neurotoxicity risk assessment. National Center for Environmental Assessment; Office of Research and Development; U.S. Environmental Protection Agency. Washington, DC. EPA/630/R-95/001Fa. http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=4555 . 030021

U.S. EPA (2000). Benchmark dose technical guidance document [external review draft]. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, DC. EPA/630/R-00/001. http://www.epa.gov/raf/publications/benchmark-dose-doc-draft.htm . 052150

U.S. EPA (2000). Science policy council handbook: Risk characterization. Office of Science Policy, Office of Research and Development, U.S. Environmental Protection Agency. Washington, D.C.. EPA 100-B-00-002. www.epa.gov/spc/pdfs/rchandbk.pdf. 052149

U.S. EPA (2000). Supplementary guidance for conducting health risk assessment of chemical mixtures. Risk Assessment Forum. Washington, DC. 004421

U.S. EPA (2002). A review of the reference dose and reference concentration processes. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, DC. EPA/630/P-02/0002F. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=55365. 088824

U.S. EPA (2002). Benchmark dose software (BMDS). 1.3.1. Washington, DC: U.S. Environmental Protection Agency 537121

U.S. EPA (2003). National primary drinking water regulations: Organic chemicals. U.S. Environmental Protection Agency. Washington, DC.http://www.epa.gov/safewater/mcl.html. 224471

U.S. EPA (2005). Guidelines for carcinogen risk assessment, Final Report. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, DC. EPA/630/P-03/001F. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=116283. 086237

U.S. EPA (2005). Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. U.S. Environmental Protection Agency Risk Assessment Forum. Washington, DC. EPA/630/R-03/003F. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=116283. 088823

U.S. EPA (2006). A framework for assessing health risk of environmental exposures to children. National Center for Environmental Assessment. Washington, DC. EPA/600/R-05/093F. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=158363. 194567

U.S. EPA (2006). Peer review handbook (EPA/100/B-06/002). Science Policy Council, U.S. Environmental Protection Agency. Washington, DC. EPA/100/B-06/002. http://www.epa.gov/iris/backgr-d.htm. 194566

U.S. EPA (2007). Peer Review Draft of Toxicological Review of Acrylamide. Integrated Risk Information System, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency. Washington, DC.http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=187729. 418811

U.S. EPA (2009). Toxicological review of acrylamide (CAS No. 79-06-1). U.S. Environmental Protection Agency. Washington, DC. NCEA-S-1666. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=187729. 224475

U.S. FDA (2004). Survey data on acrylamide in food: Individual food products . Retrieved 03-FEB-10, from http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Acrylamide/ucm053549.htm#u1003. 399216

U.S. FDA (2009). Acrylamide in food. U.S. Food and Drug Administration. College Park, MD.http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Acrylamide/default.htm. 224481

U.S. FDA (2009). The U.S. FDA Center for Food Safety and Applied Nutrition (CFSAN) site for information on acrylamide and FDA research. U.S. Food and Drug Administration. College Park, MD.http://www.cfsan.fda.gov/-Ird/pestadd.html#acrylamide. 224492

Valdivia RP; Lafuente NM; Katoh M (1989). Acrylamide-induced chromosome-type aberrations in spermiogenic stages evaluated in the first cleavage metaphases in the mouse. Environ Mol Mutagen, 14: 205. 224501

Verlaan M; Te Morsche RH; Roelofs HM; Laheij RJF; Jansen JBMJ; Peters WHM; Drenth JPH (2004). Genetic polymorphisms in alcohol-metabolizing enzymes and chronic pancreatitis. Alcohol Alcohol, 39: 20-24. 224503

Verschueren K (2001). Handbook of environmental data on organic chemicals. New York, NY: John Wiley & Sons, Incorporated. 224505

Vesper HW; Bernert JT; Ospina M; Meyers T; Ingham L; Smith A; Myers GL (2007). Assessment of the relation between biomarkers for smoking and biomarkers for acrylamide exposure in humans. Cancer Epidemiol Biomarkers Prev, 16: 2471-2478. 224511

Vesper HW; Licea-Perez H; Meyers T; Ospina M; Myers GL (2005). Pilot study on the impact of potato chips consumption on biomarkers of acrylamide exposure. Adv Exp Med Biol, 561: 89-96. 224508

Vesper HW; Slimani N; Hallmans G; Tjønneland A; Agudo A; Benetou V; Bingham S; Boeing H Boutron-Ruault MC; Bueno-de-Mesquita HB; Chirlaque D; Clavel-Chapelon F; Crowe F; Drogan D; Ferrari P; Johansson I; Kaaks R; Linseisen J; Lund E; Manjer J; Mattiello A; Palli D; Peeters PH; Rinaldi S; Skeie G; Trichopoulou A; Vineis P; Wirfält E; Overvad K; Strömberg U (2008). Cross-sectional study on acrylamide hemoglobin adducts in subpopulations from the European prospective investigation into cancer and nutrition (EPIC) study. J Agric Food Chem, 50: 6046-6053. 224512

von Tungeln LS; Churchwell MI; Doerge DR; Shaddock JG; McGarrity LJ; Heflich RH; da Costa GG; Marques MM; Beland FA (2009). DNA adduct formation and induction of micronuclei and mutations in B6C3F1/Tk mice treated neonatally with acrylamide or glycidamide. Int J Cancer, 124: 2006-2015. 224513

Voogd CE; van der Stel JJ; Jacobs JJ (1981). The mutagenic action of aliphatic epoxides. DNA Repair (Amst), 89: 269-282. 018782

Walden R; Squibb RE; Schiller CM (1981). Effects of prenatal and lactational exposure to acrylamide on the development of intestinal enzymes in the rat. Toxicol Appl Pharmacol, 58: 363-369. 061391

Walker K; Hattis D; Russ A; Sonawane B; Ginsberg G (2007). Approaches to acrylamide physiologically based toxicokinetic modeling for exploring child-adult dosimetry differences. J Toxicol Environ Health, 70: 2033-2055. 224527

Walker RA; O'Brien ET; Epstein DL; Sheetz MP (1997). N-Ethylmaleimide and ethacrynic acid inhibit kinesin binding to microtubules in a motility assay. Cell Motil Cytoskeleton, 37: 289-299. 224528

Walum E; Flint OP (1993). Selective effects of acrylamide, methylene bisacrylamide, and haloperidol on neuronal development in rat embryo midbrain micromass cultures. Vitro Toxicol, 6: 125-134. 224529

Warr TJ; Parry JM; Callander RD; Ashby J (1990). Methyl vinyl sulphone: a new class of Michael-type genotoxin. Mutat Res, 245: 191-199. 224530

Waters MD; Nolan C (1995). EC/US workshop report: assessment of genetic risks associated with exposure to ethylene oxide, acrylamide, 1,3-butadiene and cyclophosphamide. Mutat Res-Fundam Mol Mech Mutagen, 330: 1-11. 224531

WHO (1985). Environmental health criteria 49: acrylamide. International Programme on Chemical Safety. Geneva, Switzerland.http://www.inchem.org/documents/ehc/ehc/ehc49.htm. 224533

Wilson KM; Balter K; Adami HO; Grönberg H; Vikström AC; Paulsson B; Törnqvist M; Mucci LA (2009). Acrylamide exposure measured by food frequency questionnaire and hemoglobin adduct levels and prostate cancer risk in the cancer of the prostate in Sweden study. Int J Cancer, 124: 2384-2390. 224535

Wilson KM; Mucci LA; Cho E; Hunter DJ; Chen WY; Willett WC (2009). Dietary acrylamide intake and risk of premenopausal breast cancer. Am J Epidemiol, 169: 954-961. 224536

Wise LD; Gordon LR; Soper KA (1995). Developmental neurotoxicity evaluation of acrylamide in Sprague-Dawley rats. Neurotoxicol Teratol, 17: 189-198. 224539

Working PK; Bentley KS; Hurtt ME; Hurtt ME; Mohr KL. (1987). Dominant lethal assay of acrylonitrile and acrylamide in the male rat. Environ Mutagen, 9: 115. 224541

Working PK; Bentley KS; Hurtt ME; Mohr KL (1987). Comparison of the dominant lethal effects of acrylonitrile and acrylamide in the male F344 rat. Mutagenesis, 2: 215-220. 224542

Xiao Y; Tates AD (1994). Increased frequencies of micronuclei in early spermatids of rats following exposure of young primary spermatocytes to acrylamide. Mutat Res, 309: 245-254. 224543

Yamada T; Nakamura J; Murakami M; Okuno Y; Hosokawa S; Matsuo M; Yamada H (1995). Effect of chronic L-DOPA administration on serum luteinizing hormone levels in male rats. Toxicology, 97: 173-182. 224544

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: 388-399. 224545

Zeiger E; Anderson B; Haworth S; Lawlor T; Mortelmans K; Speck W (1987). Salmonella mutagenicity tests III: Results from the testing of 255 chemicals. Environ Mol Mutagen, 9: 61-109. 073869

Zeiger E; Recio L; Fennell TR; Haseman JK; Snyder RW; Friedman M (2009). Investigation of the low-dose response in the in vivo induction of micronuclei and adducts by acrylamide. Toxicol Sci, 107: 247-257. 224546

Zenick H; Clegg ED; Perreault SD; Klinefelter G; Gray LE (1994). Assessment of male reproductive toxicity: a risk assessment approach. In AW Hayes (Ed.),Principles and methods of toxicology (pp. 947). New York, NY: Raven Press. 224553

Zenick H; Hope E; Smith MK (1986). Reproductive toxicity associated with acrylamide treatment in male and female rats. J Toxicol Environ Health, 17: 457-472. 061394

Zhu YJ; Zeng T; Zhu YB; Yu SF; Wang QS; Zhang LP; Guo X; Xie KQ (2008). Effects of acrylamide on the nervous tissue antioxidant system and sciatic nerve electrophysiology in the rat. Neurochem Res, 33: 2310-2317. 224559

APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

The Toxicological Review of AA, external peer review draft (U.S. EPA, 2007, 418811), has undergone formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2006, 194566). In the Spring of 2008, an external review was conducted by members of a U.S. EPA Scientific Advisory Board (SAB) panel who were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A subsequent peer consultation was conducted in late 2009 that focused specifically on revisions made in the Toxicological Review of AA subsequent to the SAB panel's recommendations, related to the use of AA and GA hemoglobin adduct levels and what is known about hemoglobin adduct formation rates method used to estimate internal exposures for a given external dose

The comments from the external peer review and the peer consultation and EPA's responses are presented in chronological order in Appendix A, with the SAB review first.

A.1. EPA'S SCIENCE ADVISORY BOARD (SAB) REVIEW OF THE DRAFT TOXICOLOGICAL REVIEW OF AA

The SAB Panel deliberated on the charge questions during a March 10–11, 2008 face-to-face meeting, and discussed an SAB draft report in a subsequent conference call on July 16, 2008. The final draft of the panel's report was released on December 19, 2008.

A summary of the significant comments made by the SAB reviewers and EPA's responses to these comments follow. In many cases the comments of the individual reviewers have been synthesized and paraphrased in the development of Appendix A. EPA also received scientific comments from the public. These comments and EPA's responses are included in a separate section of this appendix.

The complete list of charge questions to the SAB are presented below followed by a summary of the SAB reviewers' comments, and EPA's response.

A.1.1. Charge Questions for EPA's Science Advisory Board (SAB) - IRIS Toxicological Review of AA

The U.S. Environmental Protection Agency (EPA) is releasing an external review draft of the revised IRIS Toxicological Review of AA that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). This revised version will replace the previous version of the IRIS Toxicological Review of AA that was posted in 1988. Science Advisory

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS).

Board (SAB) review of this assessment is considered vital to the external review process. The IRIS Toxicological Review(s) is a compilation and summary of the available information on the potential for cancer and noncancer hazardous effects in humans from exposure to AA. If information is sufficient to develop a quantitative characterization of the dose-response relationship for sensitive endpoints, toxicity values are derived that can be used for risk assessment including values for an oral reference dose (RfD), inhalation reference concentration (RfC), cancer oral slope factor, and IUR.

In reviewing each of the chapters in the IRIS Toxicological Review of AA, the SAB is asked to comment on (1) whether the document is logical, clear and concise, (2) if the discussion is objectively and transparently represented, and (3) if it presents an accurate synthesis of the scientific evidence for noncancer and cancer hazard. The SAB is also asked to identify any additional relevant studies that should be included in the evaluation of the noncancer or cancer health effects of AA, or in derivation of toxicity values. In addition, the SAB is asked to provide advice on the following specific charge questions related to the derivation of a proposed oral reference dose (RfD), and inhalation reference concentration (RfC) for non-cancer endpoints; cancer descriptor, oral slope factor, and IUR for AA.

A.1.2. Selection of Studies and Endpoints for the Oral Reference Dose

In the draft assessment, the proposed most sensitive noncancer effect from exposure to AA is neurotoxicity. This endpoint is based on an extensive database of animal and human studies. The next most sensitive effect is reproductive toxicity, which was in the three- to fivefold higher exposure range for a no effect response in animal studies. No human data were identified for AA related reproductive effects. Heritable germ cell effects, a potentially serious noncancer effect, have been observed in male mice, however, the lowest dose levels tested are considerably higher (two orders of magnitude) than the doses where neurotoxicity were observed, and there is uncertainty about the shape of the low-dose-response relationship.

1. Please comment on the selection of neurotoxicity as the most appropriate choice for the most sensitive endpoint (in contrast to reproductive toxicity, heritable germ cell effects, or other endpoint) based upon the available animal and human data.
2. Please comment on the discussion of mode of action for AA-induced neurotoxicity. Is the discussion clear, transparently and objectively described, and accurately reflective of the current scientific understanding?
3. Please comment on the qualitative discussion of AA's heritable germ cell effects and whether the discussion is clear, transparently and objectively described, and reflective of the current science.

A.1.3. Derivation of the Reference Dose

The proposed RfD (0.003 mg/kg-day) for AA is based on a benchmark dose analysis of the dose-response relationship for neurotoxicity in two chronic drinking water exposure bioassays using Fischer 344 rats. Uncertainty factors and a PBTK model are used to extrapolate the animal dose-response to a human equivalent dose-response in the derivation of the RfD.

4. Please comment on whether the selection of the Friedman et al. (1995, 224307) and Johnson et al. (1986, 061340) studies as co-principal studies has been scientifically justified. Although EPA considers Friedman et al. and Johnson et al. to be co-principal studies, the final quantitative RfD value is derived only from the Johnson study. Please comment on this aspect of EPA's approach. Please also comment on whether this choice is transparently and objectively described in the document. Please identify and provide the rationale for any other studies that should be selected as the principal study(s).

5. Please comment on the benchmark dose methods and the choice of response level used in the derivation of the RfD, and whether this approach is accurately and clearly presented. Do these choices represent the most scientifically justifiable approach for modeling the slope of the dose-response for neurotoxicity? Are there other response levels or methodologies that EPA should consider? Please provide a rationale for alternative approaches that should be considered or preferred to the approach presented in the document.

6. Please comment on the selection of the uncertainty factors (other than the interspecies uncertainty factor) applied to the point of departure (POD) for the derivation of the RfD. For instance, are they scientifically justified and transparently and objectively described in the document? (Note: This question does not apply to the interspecies uncertainty factor which is addressed in the questions on the use of the PBTK model; see PBTK model questions below)

7. Please provide any other comments on the derivation of the RfD and on the discussion of uncertainties in the RfD.

A.1.4. Use of a PBTK Model in the Derivation of the RfD and the Inhalation Reference Concentration

A physiologically-based toxicokinetic (PBTK) model originally developed by Kirman et al. (2003, 087778), and recalibrated by EPA with more recent kinetic and Hb binding data in rats, mice, and humans (Boettcher et al., 2005, 224446; Doerge et al., 2005, 224344; Doerge et al., 2005, 224348; Fennell et al., 2005, 224299) was used in the derivation of the RfD to extrapolate from the animal dose-response relationship (observed in the co-principal oral exposure studies for neurotoxicity) to derive a HED. The HED is the external AA exposure level that would produce the same internal level of parent AA (in this case the area under the curve [AUC] of AA in the blood) that was estimated to occur in the rat following an external exposure

to AA at the level of the proposed point of departure, and related to a response level of 5% (i.e., the $BMDL_5$). The model results were used in lieu of the default interspecies uncertainty factor for toxicokinetics differences of $10^{1/2}$, which left a factor of $10^{1/2}$ (which is rounded to 3) for interspecies differences in toxicodynamics.

With respect to the RfC, there are presently insufficient human or animal data to directly derive an RfC for AA. The PBTK model was thus used to conduct a route-to-route extrapolation (oral-to-inhalation) to derive an RfC based on the dose-response relationship observed in the co-principal oral exposure studies for neurotoxicity. In this case, the HEC was based on a continuous inhalation exposure to AA in the air that would yield the same AUC for the parent AA in the blood as that estimated for the rat following an external oral exposure to AA at the level of the proposed point of departure (i.e., the HED_{BMDL5}).

8. Please comment on whether the documentation for the recalibrated Kirman et al. (2003, 087778) PBTK model development, evaluation, and use in the assessment is sufficient to determine if the model was adequately developed and adequate for its intended use in the assessment. Please comment on the use of the PBTK model in the assessment, e.g., are the model structure and parameter estimates scientifically supportable? Is the dose metric of area-under-the-curve (AUC) for AA in the blood the best choice based upon what is known about the mode of action for neurotoxicity and the available kinetic data? Please provide a rationale for alternative approaches that should be considered or preferred to the approach presented in the document.

9. Is the Young et al. (2007, 224545) PBTK model adequately discussed in the assessment with respect to model structure, parameter values, and data sets used to develop the model? Do you agree with the conclusion (and supporting rationale) that the recalibrated Kirman et al. (2003, 087778) model (model structure and parameter values presented in the Toxicological Review) currently represents the best model to use in the derivation of the toxicity values?

10. According to US EPA's RfC Methodology (1994, 006488), the use of PBTK models is assumed to account for uncertainty associated with the toxicokinetic component of the interspecies uncertainty factor across routes of administration. Does the use of the PBTK model for AA objectively predict internal dose differences between the F344 rat and humans, is the use of the model scientifically justified, and does the use of the PBTK reduce the overall uncertainty in this estimate compared to the use of the default factor? Are there sufficient scientific data and support for use of this PBTK model to estimate interspecies toxicokinetic differences and to replace the default interspecies factor for toxicokinetic differences (i.e., $10^{1/2}$)? Is the remaining uncertainty factor for toxicodynamic differences scientifically justified, appropriate and correctly used?

11. Please comment on whether the PBTK model is adequate for use to conduct a route-to-route extrapolation for AA to derive an RfC in the absence of adequate inhalation animal or human dose-response data to derive the RfC directly. Was the extrapolation correctly performed and sufficiently well documented?
12. Please provide any other comments on the derivation of the RfC and on the discussion of uncertainties in the RfC.

A.1.5. Margin of Exposure Analysis

IRIS assessments do not include exposure assessments, which precludes the ability to conduct a Margin of Exposure (MOE) analysis. It has been suggested, however, that the AA assessment include a Table that lists points of departure for various endpoints to facilitate a MOE evaluation by EPA's Regional or Program offices, or by other end users of the assessment.

13. Would you suggest that EPA include a Table that lists points of departure (e.g., NOAELs, BMDs, etc.) for various endpoints that could be used, in conjunction with exposure assessments, to conduct a MOE analysis?

A.1.6. Quantitating Heritable Germ Cell Effects

The Toxicological Review includes a discussion of methods to quantitate the risk for heritable germ cell effects (Section 5.4). The questions below address the uncertainty and utility of the quantitative results.

14. Please comment on the discussion of methods to quantitate the dose-response for heritable germ cell effects as to whether it is appropriate, clear and objective, and reflective of the current science. Has the uncertainty in the quantitative characterization of the heritable germ cell effects been accurately and objectively described?
15. Please comment on the scientific support for the hypothesis that heritable germ cell effects are likely to occur at doses lower than those seen for neurotoxicity? What on-going or future research might help resolve this issue?
16. The risks of heritable germ cell effects (i.e., number of induced genetic diseases per million offspring) for some estimated exposure in workers and the population are presented in Table 5-11, and are based on the quantitative methods and parameter estimates discussed in Section 5.4 of the Toxicological Review (U.S. EPA, 2007, 418811). Please comment on whether or not the quantitation of heritable germ effects should be conducted, the level of uncertainty in the results, if Table 5-11 is useful for risk assessment purposes, and if the RfD should be included in the Table as one of the exposure levels.
17. Do you know of any additional data or analyses that would improve the quantitative characterization of the dose-response for AA-induced heritable germ cell effects? Would

these data also support the quantitative characterization of "total" male-mediated reproduction risks to offspring (i.e., lethality + heritable defect)? If data are not available, do you have any recommendations for specific needed studies?

A.1.7. Carcinogenicity of AA

In accordance with EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005, 086237), AA is described as *likely to be carcinogenic to humans* based on: (1) significant 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) initiation of skin tumors following oral, intraperitoneal, or dermal exposure to AA and the tumor promoter, TPA, in two strains of mice; and (3) increased incidence of lung adenomas in another mouse strain following intraperitoneal injection of AA. Evidence from available human studies is judged to be limited to inadequate.

The mechanisms by which AA may cause cancer are poorly understood, but EPA has determined that the weight of the available evidence supports a mutagenic mode of carcinogenic action, primarily for the AA epoxide metabolite, glycidamide (GA). Other mode(s) of action (MOA) have been proposed for the carcinogenicity of AA, but there is less support.

18. Have the rationale and justification for the cancer designation for AA been clearly described? Is the conclusion that AA is a likely human carcinogen scientifically supportable?

19. Do you agree that weight of the available evidence supports a mutagenic mode of carcinogenic action, primarily for the AA epoxide metabolite, glycidamide (GA)? Has the rationale for this MOA been clearly and objectively presented, and is it reflective of the current science?

20. Are there other MOAs that should be considered? Is there significant biological support for alternative MOAs for tumor formation, or for alternative MOAs to be considered to occur in conjunction with a mutagenic MOA? Please specifically comment on the support for hormonal pathway disruption. Are data available on alternate MOAs sufficient to quantitate a dose-response relationship?

21. Two chronic drinking water exposure bioassays in Fischer 344 rats (Friedman et al., 1995, 224307; Johnson et al., 1986, 061340) were used to derive the oral slope factor, and to identify the tumors of interest for the MOA discussion. Are the choices for the studies, tumors, and methods to quantify risk transparent, objective, and reflective of the current science? Do you have any suggestions that would improve the presentation or further reduce the uncertainty in the derived values?

22. The cancer slope factor (CSF) derivation includes an adjustment for early mortality (i.e., time-to-tumor analysis). Is this adjustment scientifically supported in estimating the risk from the 2-year bioassay data for increased incidence of tumors in the rats?

23. The dose metric used in the PBTK model analysis to derive the human equivalent concentration was area under the curve (AUC) in the blood for the putative genotoxic metabolite, glycidamide. Please comment on whether AUC for glycidamide is the best choice of the dose metric in estimating the human equivalent concentration to derive the oral slope factor. If other dose metrics are preferable, please provide the scientific rationale for their selection.

24. As with the RfC, there were insufficient cancer inhalation data to derive an inhalation unit risk (IUR). The PBTK model was used in a route-to-route extrapolation of the dose-response relationship from the oral data, and to estimate the human equivalent concentration for inhalation exposure to AA. Please comment on whether this extrapolation to derive the inhalation unit risk was correctly performed and sufficiently well documented.

25. The recommendation to use the age-dependent adjustment factors (ADAFs) is based on the determination of a mutagenic MOA for carcinogenicity. Is this recommendation scientifically justifiable and transparently and objectively described

26. Please provide any other comments on the CSF or IUR, and on the discussion of uncertainties in the cancer assessment.

A.1.8. Summary of the SAB Reviewers' Comments and EPA Responses:

1. **Comment:** SAB reviewers noted that the determination of accurate BMDs for AA-induced neurotoxicity in the principal studies for the RfD and RfC may be compromised by a lack of functional testing of neurotoxicity and the use of a relatively insensitive measure, peripheral axonopathy detected by light microscopy, as the primary index of neurotoxicity. Of concern was the possibility that, in looking at axonal degeneration, preceding terminal degeneration may have been missed, particularly at lower doses.

   Response: Additional text was added to Section 5.1.1 (Choice of Principal Study and Critical Effect—with Rationale and Justification) to discuss this area of uncertainty in selecting the critical effect and principal study for the derivation of the RfD and RfC.

2. **Comment:** SAB reviewers noted that future studies may demonstrate AA effects on male reproductive function at lower exposure levels than those associated with neurotoxicity in animal studies. Of particular concern to the reviewers were: (1) observations that impaired male reproductive performance (e.g., male-mediated implantation losses) occurred in laboratory animals at oral exposure levels (~3–13

mg/kg-day) that are only "somewhat above" the lowest chronic doses associated with neurotoxicity (1–2 mg/kg-day); (2) in-depth animal studies of dose-response relationships for heritable germ cell effects are not available; (3) possible associations between human exposure to AA and reproductive effects (especially heritable germ cell effects and effects on sperm endpoints) have not been adequately studied; and (4) growing evidence that cigarette smoking, a known source of AA exposure, is associated with altered male reproductive tissue endpoints, including sperm concentration, morphology, motility, and DNA fragmentation.

Response: Several changes were made in Chapter 5 to explicitly note the SAB's concerns about heritable germ cell effects and to strongly encourage further research, including text to clearly state that: (1) the lower end of the range of animal oral doses associated with lethal germ cell effects, particularly male-mediated implantation losses, is close to the lowest exposure levels associated with neurotoxicity in orally exposed animals; (2) dose-response relationship for heritable germ cell effects in AA-exposed animals are not well described, particularly at dose levels below 50 mg/kg-day; and (3) possible associations between human exposure to AA and altered sperm characteristics have not been adequately studied. In addition, text was added to Chapters 5 and 6 to note that any future studies of possible associations between AA exposure and sperm characteristics should adjust for smoking history and alcohol consumption, especially due to the growing evidence of associations between cigarette smoking and altered sperm endpoints.

The database deficiency UF used in the derivation of the RfD and RfC ($UF_{DB} = 1$) was not changed in response to these comments, because, as discussed in several places in Chapter 5, the animal database for oral exposure to AA is robust. Although the human data are limited, they clearly demonstrate neurotoxicity as the predominant observable noncancer adverse effect. Although animal studies for inhalation exposures are limited, kinetic studies in animals and humans indicate no portal of entry effects (i.e., lung or gastrointestinal effects that would be unique to that route of exposure). 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.

3. **Comment:** SAB reviewers noted that the discussion of MOA(s) for neurotoxicity in two sections of the 2007 document (Section 4.6.1, pp 123–124; and Section 4.7.3, pp 134–136) was confusing and recommended their incorporation into a single section. In

addition, several deficiencies in the discussion were noted (inadequate discussion of the current molecular understanding of the MOAs, AA adduct chemistry pertinent to AA MOAs for neurotoxicity, and the respective roles of AA, the parent compound, and its epoxide metabolite, GA). Panel members provided more specific text to address these deficiencies and offered the text to EPA for consideration in revising the document.

Response: In response to this comment, the two sections were consolidated into one section (Section 4.7.3.1 in the current version) and the recommended text was incorporated into the revised discussion of the MOAs for neurotoxicity.

4. **Comment:** SAB reviewers commented that the discussion of heritable germ cell effects studies in Section 4.4 should be modified to add synthesis, analysis, and scrutiny. Recommended issues to be discussed further include considerable deficiencies in the database (e.g., characterization of dose-response relationships for certain endpoints), the significance of the effect, and MOAs for heritable germ cell effects (including DNA adduct formation leading to mutagenicity, as well as clastogenic and mitotic spindle effect mechanisms).

   Response: Section 4.4 (Heritable Germ Cell Effects) was rewritten to add a new section *"Synthesis, and evaluation of heritable germ cell effects"*, as well as further discussion of potential MOAs for heritable germ cell effects.

5. **Comment:** SAB reviewers considered studies other than the 2-year rat study by Johnson et al. (1986, 061340) that could serve as the basis of the RfD and RfC, noting that the critical effect of axonal degeneration detected by light microscopy is relatively less sensitive than neuronal changes visible under the electron microscope or functional/behavioral alterations. The Panel suggested that EPA generate an RfC from the Calleman et al. (1994, 202900) study of AA-exposed workers for the purpose of comparison with the RfC based on the rat data from Johnson et al. (1986, 061340), despite the limitations of this human study including the small sample size and restriction to young adult males, the bias toward the null from the healthy worker effect, the effect of confounding exposures to other neurotoxicants, and the short duration of exposure. The Panel saw this exercise as a type of sensitivity analysis to help to determine whether the RfD and RfC based on the relatively insensitive measure of neurotoxicity in the rat study appears to be adequately health protective. The SAB reviewers noted that the Burek et al. (1980, 061311) study was limited in being a subchronic study, although one reviewer proposed that EPA still consider generating an RfD based on the data in Burek et al. (Burek et al., 1980, 061311).

Response: As suggested, EPA conducted an alternate derivation of the RfC based upon the Calleman et al. (1994, 202900) study of worker exposure to AA via the inhalation and dermal routes. This analysis can be found in Appendix F, and the details of Calleman et al. (1994, 202900) study are presented in Section 4.1. EPA notes that the human data from the Calleman et al. (1994, 202900) study are very limited due to the small number of subjects (n = 41), the narrow representation of the general public (i.e., workers), and a number of potentially confounding factors including concurrent exposure to another neurotoxin (acrylonitrile), aggregate exposure via both dermal and inhalation routes, a composite index of neurotoxicity, and control groups of varying size and composition. There are also no other human inhalation toxicity studies to support or challenge the reproducibility or validity of the Calleman et al. (1994, 202900) study results.

With respect to the Burek et al. (1980, 061311) study, EPA determined that an RfD based on the Burek et al. (1980, 061311) study would not be a useful comparison to the RfDs based on the BMD analysis of the data from the two year bioassays EPA due to the following deficiencies in Burek et al. (1980, 061311) study data including:

- The number of rats per group in Burek et al. (1980, 061311) subchronic study was small, and the subset used for the electron microscopy was limited to only three male rats per group evaluated.
- The severity categories for the difference in response were not related to any increase in the severity of the clinical effects.
- There was considerable variability among the controls for the mid-level category, and the statistical significance of the differences in response between treatment groups and controls was not reported.
- The available data were insufficient to conduct a BMD analysis.
- In the absence of a POD based on a BMD analysis , the choice of the NOAEL (i.e., 0.2 mg/kg-day) is limited to the doses specified in the Burek et al. (1980, 061311) study design, and would not provide a POD that takes into account the shape of the dose-response curve (as is the case for the RfDs based on the 2 year bioassays).

6. **Comment:** The SAB panel could not reach consensus about EPA's decision that a database deficiency UF greater than 1 was not needed in the derivation of the RfD and RfC. Some panel members agreed with EPA's position, whereas others argued that deficiencies in the database could lead to lower values for the RfD and RfC when they are filled, thereby warranting a database deficiency UF. Database deficiencies pointed out included: (1) the absence of robust neurotoxicity evaluations including histopathology and electron microscopy coupled with systemic evaluation of functional

or behavioral endpoints at multiple time points in adult animals and in animals exposed during early development to determine whether critical lifestage differences exist in susceptibility to AA neurotoxicity; (2) the chronic neurotoxicity of AA has only been assessed in rats; and (3) heritable germ cell effects have not been fully characterized, especially dose-response relationships.

Response: See response to Question 2. Although the database deficiency UF used in the derivation of the RfD and RfC ($UF_{DB} = 1$) was not changed in response to these comments, it is agreed that 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. Text changes were made in several places in Sections 5.1, 5.2, and 5.3 to emphasize these points.

7. **Comment:** The SAB panel recommended that the document should include some discussion of the potential for cumulative effects from exposure to AA and other type-2 alkenes, which can produce similar noncancer effects via common mechanisms of action. Evaluating the cumulative effects of type-2 alkenes was noted to be particularly germane since human exposure can be pervasive due to environmental pollution (e.g., acrolein, acrylonitrile), contamination of food (e.g., AA, methyl acrylate), and endogenous generation (e.g., acrolein, 4-hydroxy-2-nonenal, as reviewed by LoPachin et al. (2008, 224560).

   Response: Text was added to Section 6.1 regarding this issue.

8. **Comment:** The SAB panel noted that the recalibrated Kirman et al. (2003, 087778) PBTK model was superior to the Young et al. (2007, 224545) model, but requested that EPA provide further descriptions of the model and its parameterization/development and consider further evaluation and refinement of the model with recently available toxicokinetics data. To justify the latter actions, the Panel noted several discrepancies between the PBTK predicted and measured dose metrics and that the use of other available kinetic data (e.g., Fennell et al., 2003, 224295; Hartmann et al., 2008, 224480; Vesper et al., 2007, 224511; Vesper et al., 2008, 224512) to refine the model may help to resolve the apparent discrepancies.

   Response: The recalibrated Kirman et al. (Kirman et al., 2003, 087778) model used in the external review draft of the Toxicological Review of AA (U.S. EPA, 2007, 418811), has since been published by Walker et al. (2007, 224527). The Walker et al. (2007, 224527) report provides additional supporting model documentation. EPA agrees with the SAB that an update of the Walker et al. (2007, 224527) PBTK model parameter values, as well as the model structure, are needed based upon more recent kinetic data, and the need to represent first pass lung clearance for an inhalation exposure. For the

purposes of the current assessment, however, recent kinetic data from Doerge et al. (2005, 224348; 2005, 224355; 2005, 224344) and Tareke et al. (2006, 224387) in conjunction with the human adduct data from Fennel et al. (2005, 224299) are sufficient to conduct a direct extrapolation of the rat dose-response POD to a human equivalent administered dose based on equivalent AUCs in the blood for AA or GA. Because AA or GA-AUC in the blood was also the dose metric that would be simulated with a PBTK model, the equivalent AUC method is a viable alternative in lieu of using the UF for interspecies toxicokinetic differences, and thus EPA chose not to delay the assessment pending update and peer review of a revised AA PBTK model. The equivalent AUC method also has fewer, relatively well supported parameters, and thus has inherently less uncertainty than a PBTK model. An AA PBTK model, of course, has far greater applicability to generate dose metrics other than the AUC in blood, to compare different dosing regimens, to simulate a far greater range of data, and to conduct variability analyses. Thus, EPA recognizes and supports the important use of PBTK models in risk assessment, and encourages the research community to continue to develop and submit peer reviewed AA PBTK models for consideration. In future reviews or revisions to this document, EPA will consider available AA PBTK model results, and if needed, revise accordingly the reference values derived in this current version of the Toxicological Review of AA.

9. **Comment:** The SAB panel noted that Hb adduct data and other data in several recent publications (Fennell et al., 2005, 224299; Hartmann et al., 2008, 224480; Vesper et al., 2008, 224512) provide a means of estimating Human Equivalent Dose (HED) by alternative empirical approaches that might be compared with the predictions from the PBTK models.

Response: The HED is an estimate of the human internal dose of either AA or GA that is equivalent to the test animal internal dose following external exposure to a given level of AA. There are various measures of internal dose that can be used, and for the purposes of this assessment, the chosen internal dose metric is area under the time-concentration (AUC) for AA in the blood, as the basis for deriving the noncancer reference values, and AUC for GA in the blood for the cancer reference values. In the previous draft of the Toxicological Review for AA a PBTK model was used to estimate the AA-AUC and the GA-AUC. More recent kinetic data have become available that require an update of the PBTK model, but that are also sufficiently detailed and appropriate for a direct estimate of the AA-AUC and GA-AUC precluding the need to use a PBTK model for this purpose. In accordance with the SAB recommendation to evaluate this latter method, EPA extensively reviewed and evaluated the more recent kinetic data on Hb adducts and serum AUCs for AA or GA following a specified administered dose. EPA concluded that the recent data are insightful and that the direct estimate of the AUC based on the data is indeed, a viable and more supportable (i.e., fewer parameters) method to derive the HEC compared with the use of a PBTK model. The use of AA and GA Hb adducts as biomarkers of exposure dose is also an established method that has been employed in a

number of peer reviewed studies (Bergmark, 1997, 224422; Bergmark et al., 1993, 224424; Calleman et al., 1994, 202900; Fennell et al., 2005, 224299; Hartmann et al., 2008, 224480; Hays and Aylward, 2008, 224507; Törnqvist et al., 2008, 224428). Thus, the direct estimate of the AUC based on Hb adduct levels was used in this current version of the assessment. Detailed text has been added in Sections 3.5 and 5.1.2 that presents the equations, assumptions, and results in this method. Appendix E provides additional details of the data and calculations used to develop the in vivo second order adduct formation rate constants, and how the AUCs were normalized to the administered dose.

10. **Comment:** The SAB panel noted there are recent data indicating human variability in the metabolism and toxicokinetics of AA (e.g., Fennell et al., 2003, 224295; Hartmann et al., 2008, 224480; Heudorf et al., 2009, 224517; Vesper et al., 2007, 224511; Vesper et al., 2008, 224512) and asked EPA to consider how to incorporate this information into the PBTK model.

    Response: EPA agrees that human variability in both internal dose and response to AA and GA is an important consideration in risk assessment, and an updated and peer reviewed PBTK model could account for altered disposition of AA or GA (e.g., due to variability in metabolism). More data, however, are needed to identify both the critical factors and level of variability at low dose exposures in the general human population. Assessing the impact of altered internal levels of AA and GA on the response is also challenging because both are toxins, thus variability in metabolism leading to decreased GA and increased AA might change the "spectrum" of adverse effects.

11. **Comment:** The SAB panel agreed with the use of the PBTK models to conduct route-to-route extrapolations for noncancer effects and cancer. The panel noted that the value generated in the default approach to estimating a human equivalent dose was very similar to the value derived using the PBTK model.

    Response: In the external review draft of the Toxicological Review of AA (U.S. EPA, 2007, 418811), the default approach to generate an RfD uses a $UF_{A\text{-}TK}$ of 3, and resulted in an RfD similar to the value derived from the results with the recalibrated Kirman et al. (2003, 087778) PBTK model. This could possibly have been due to the dose metric used in the model simulation, which was AA-AUC in blood, and which apparently scaled roughly comparably to the uncertainty value of 3. The default approach, however, would result in a considerably different value for the oral slope factor than the current value, because the default approach is based on the external dose of the parent compound and the use of a $UF_{A\text{-}TK}$ of 3, and the putative mutagen leading to cancer is the GA metabolite. The default approach does not estimate the internal dose of GA. The AUC for GA is estimated either with use of a PBTK model, or the currently used equivalent AUC method that estimates the AUC of GA based on GA-Hb adducts levels. The oral slope factor based on the internal dose of GA is thus a more accurate and scientifically supportable value compared to the value based on the default approach

12. **Comment:** The SAB panel recommended including a table displaying relevant outcomes from reliable and well-performed studies for the following categories of noncancer effects: neurotoxicity in the adult and developing organism, reproductive toxicity including heritable germ cell effects, developmental toxicity, and general systemic toxicity following various durations of exposure, as appropriate.

    Response: A data array figure (Figure 5-1) and an accompanying table of NOAELs and LOAELs suitable for an MOE analysis (Table 5-1) was added to Section 5.1.1 with reliable NOAELs and LOAELs for the following categories of noncancer effects from oral exposure studies: subchronic and chronic effects; reproductive effects (including testicular and sperm effects, male-mediated implantation losses, and female reproductive performance effects); and developmental effects (including fetal effects in standard developmental bioassays and neurological assessments in offspring exposed during gestation and beyond). The text in Section 5.1 was modified to guide the reader through the data in the figure in defense of selecting degenerative nerve changes as the critical effect for the RfD and RfC. There are no studies of heritable germ cell effects in orally exposed animals, but the text in Section 5.1 discusses the results of the i.p. and dermal exposure studies on this endpoint.

13. **Comment:** The SAB panel agreed with the inclusion of Section 5.5, *Quantitating Risk for Heritable Germ Cell Effects*, in the document, but asked that: (1) the risk extrapolation factors (REFs) be explained in more detail; (2) the basis of the assumed number of human loci capable of mutating to dominantly expressed disease alleles (1,000) be clarified in the modified direct approach; and (3) how, in the doubling dose approach, the four data sets, each of which used high AA dosing rates, could accurately predict the number of new disease in the offspring at low doses.

    Response: Additional clarifying text was added to Section 5.5 to provide more information on the bases for the REFs; however, the basis of the assumed number of 1,000 mutable genes in the modified direct approach was not available. It is agreed that, in the doubling dose approach, extrapolation from the high-dose studies to low-dose human exposure scenarios is of highly uncertain accuracy. The following statement was added to emphasize this uncertainty: "Nevertheless, the accuracy of extrapolation of these high exposure rates to the expected human exposure scenarios presented in Table 5-20 is another major uncertainty in the calculations." EPA also provides recommendations in Section 5.5 and elsewhere in the document for further research and data to fill the critical data gaps: in the REFs, the quantitative relationship between genetic alterations in germ cells and heritable disease, and the shape of the low-dose relationship.

14. **Comment:** The SAB panel agreed that the rationale and justification for the "likely to be carcinogenic to humans" hazard descriptor was clearly described and that the

conclusion was scientifically supportable. The panel suggested that the rationale and justification could be further expanded by:

a) Noting that the NTP and IARC have placed AA in cancer classification groups similar to EPA's "likely to be carcinogenic to humans" category;
b) Emphasizing that concordance between tumor sites in animals and humans is not as important as the observed concordance that pertinent modes of action (e.g., somatic cell mutagenicity) operate in cells of humans and animals;
c) Adding primary CNS tumors to the list of experimental tumors induced by AA;
d) Emphasizing that the spectrum of tumors seen in AA-exposed rats is completely consistent with a DNA-reactive MOA, based on published data about other substances that induce or initiate the same kinds of neoplasms; and
e) Emphasizing that the demonstration of AA's tumor initiation activity by multiple routes of administration provides strong support that AA causes cancer by a DNA-reactive MOA.

Response: Additional text was added to Section 4.8 in general accordance with the Panel's recommendations. It should be noted that the Agency generally does not include information regarding other agencies determinations, such as IARC, in Toxicological Reviews.

15. **Comment:** The SAB panel noted that rationale and justification for the weight of evidence for a mutagenic MOA for AA carcinogenicity was sound and clearly and objectively presented. The SAB panel further noted that hormonal disruption MOAs proposed for AA are highly speculative and supported by, at most, limited evidence. The Panel made several recommendations for improving the presentation as follows:

a) Expand the discussion of biological plausibility and coherence beyond DNA adducts and expand the human relevance section;
b) Reconsider the statement regarding the lack of relationship of cytogenetic damage to a mutagenic mode MOA because the literature is full of such correlations;
c) Consider adding the results of the case-control study of post-menopausal breast cancer by Olesen et al. (2008, 224303) (reporting an association between AA-Hb level and risk for breast cancer after adjustment for smoking status) to the discussion;
d) Emphasize that observations in humans of GA-Hb adducts and GA urinary metabolites demonstrate that internal exposure to GA, the mutagenic AA metabolite, occurs in the general population at low levels of AA exposure;
e) Add discussion that AA/GA is not unique among DNA-reactive epoxides (e.g., glycidol, ethylene oxide) in displaying carcinogenic action in the thyroid, peritesticular mesothelium and mammary tissue and

f) Add discussion that CNS tumors were observed in both chronic bioassays and that this observation represents strong evidence for a DNA-damaging mechanism;
g) Add discussion of observations that short-term exposure to high doses of AA in male F344 rats found no evidence for hormonal dysregulation in the hypothalamus-pituitary-thyroid axis, yet some studies report associations with hormonal changes, low level AA exposure, and cancer; and
h) The lack of data to describe dose-response relationships for DNA adducts or pertinent mutagenic events in animals exposed to low levels of AA.

Response: Additional text was added to Section 4.8 in accordance with the Panel's recommendations.

16. **Comment:** The SAB panel recommended that data from the two chronic bioassays in F344 rats (Friedman et al., 1995, 224307; Johnson et al., 1986, 061340) be modeled for the purpose of deriving oral slope factors, noting that they did not agree that the Friedman study was a better basis for the oral slope factor, that both are reasonably strong studies, and that the strengths and limitation of both studies should be discussed in greater depth.

The Panel also suggested that tests for dose-related trends be conducted and presented for all tabulated sites, and that a footnote be added to the text to indicate with respect to the clitoral gland findings in the Johnson et al. study, histology was done only on clitoral tissues observed with gross masses.

Response: In accordance with the Panel's concerns, the tumor incidence data from the Johnson et al. (1986, 061340) bioassay were analyzed and compared with the results of the analysis of the Friedman et al. (1995, 224307) bioassay. The numerical value of the oral slope factor was indeed changed (increased risk) based on using the summed risk for increased incidence of tumors in the Johnson et al. (1986, 061340) male rats. Both studies were needed to support the use of the Johnson et al. (1986, 061340) data, and are now considered to be co-principal studies. Text in Section 5.4 was modified to compare the strengths and limitations of the two bioassays, and to describe the change in the derivation of the oral slope factor and IUR. Appropriate additions were also made to Appendix D to provide details of the analysis for the tumor incidence data from the Johnson et al. (1986, 061340) study.

With respect to the suggestion that test for dose-related trends be conducted, EPA agrees that some tumor responses at doses below 2.0 mg/kg-day in Johnson et al. (1986, 061340) were consistent with dose-related trends, and that trend testing is generally superior to pairwise comparisons for judging the significance of tumor incidence data. In this case, however, the use of trend testing would not have identified any additional important sites for the derivation of the cancer slope factor (i.e., the adenomas would not be pursued).

17. **Comment: In response to charge Question #21,** the SAB panel noted that the cancer dose-response analysis did not include a factor to scale for pharmacodynamic differences in potency between animals and humans, that such a factor should be considered as per the EPA Cancer Guidelines, and that the potential for human pharmacokinetic variability to influence the cancer potency estimate should be discussed qualitatively and quantitatively.

    Response: For a mutagenic carcinogen, as is the case here, the current EPA Cancer Guidelines do not include an adjustment for pharmacodynamic differences in extrapolating from animals to humans. Rather, the method used is a low dose linear extrapolation from the BMDL as a POD. The text does discuss variability (kinetic and dynamic) in the human population as a source of uncertainty.

18. **Comment:** The SAB panel agreed that the AUC for GA is the best choice for the internal dose metric used in deriving the oral slope factor, but asked EPA to consider the inclusion of additional human data on variability to form GA from AA.

    Response: Additional references and text have been added in Sections 3.3 and 3.5 that address variability in human metabolism of AA and GA, and that present epidemiology data on differences in GAVal adduct levels, and urinary metabolites.

19. **Comment:** The SAB panel agreed that the recommendation to use the ADAFs is well justified and transparently and objectively described. The panel noted that using the PBTK model to evaluate the effect of lifestage on CYP2E1 and GSH levels on internal exposure to GA and that such analysis could be used to develop chemical-specific adjustment factors for early life exposure.

    Response: EPA has developed specific guidelines for ADAFs. There are also insufficient data to determine whether children would be more or less susceptible to AA induced toxicity because both the parent and GA metabolite are toxic. Differences in AA or GA metabolism, or other kinetic drivers for different age groups may alter the internal disposition of AA or GA, but the effects on the resulting spectrum of adverse effects are not known at present.

20. **Comment:** The SAB panel noted that the discussion of uncertainties in the cancer assessment and toxicity values was good, but could be improved by expanding the discussion of human variability (specifically how human polymorphisms, or age-related changes, in CYP2E1 and GSTs may influence cancer risk) and of the limitation of not having another rodent species.

    Response: Additional data and discussion have been added to Section 3.3 and 3.5, however, as in the response to comment 19, it is difficult to predict the ultimate impact on

the spectrum of adverse effect from altered disposition of AA and GA due to polymorphisms or enzyme status.

A.1.9. Public Comments and Disposition

General comments are summarized below with a EPA response given for each. Specific comments related to each subject area are provided in a bulleted list with the reviewer attribution noted.

21. **General comment:** A hormonal MOA for AA carcinogenesis is possible.

    Response: EPA agrees that disruption of hormone levels or signaling is a possible, although well less supported, MOA for AA carcinogenesis. The experimental data supporting this MOA are discussed in Section 4.8.3.2 of the Toxicological Review.

22. **General comment:** Multiple MOAs are likely to be responsible for AA-induced cancer.

    Response: EPA agrees that a mixed MOA is possible, i.e., an increased mutagenic burden in hormonally-sensitive tissues with or without disruption of the hormonal pathways (see Section 4.8.3.3 of the Toxicological Review).

23. **General comment:** The hormonal MOA is the most plausible MOA for AA carcinogenesis.

    Response: EPA disagrees with this conclusion and considers the data for the hormonal MOA to be limited or lacking (see Section 4.8.3 of the Toxicological Review). The SAB Review Panel agreed with EPA and considered the hormone disruption MOA to be highly speculative. In addition, the SAB Panel concluded that the existing short-term mouse studies in SENCAR, ICR (skin), and A/J (lung) show no such selectivity of carcinogenicity for hormonally regulated tissues. Also, the CNS tumors observed in both chronic AA cancer bioassays were considered strong evidence for a DNA-damaging mechanism. The SAB Panel cited a short-term, high dose study of AA in male F344 rats that found essentially no evidence for hormonal dysregulation in the hypothalamus-pituitary-thyroid axis based on measurements of gene expression, neurotransmitters, hormones, and histopathology (Bowyer et al., 2008, 224470).

24. **General comment:** Evidence for a mutagenic MOA for cancer is weak. DNA adducts are found in both target and nontarget tissues. Genotoxicity findings interpreted as mutagenicity may actually represent chromosome deletion. Genotoxic effects such as micronuclei formation may exhibit a nonlinear dose-response.

    Response: EPA disagrees with the conclusion that the weight of evidence for a mutagenic MOA is weak. Section 4.8.3 of the Toxicological Review illustrates that the majority of the data support a mutagenic MOA for AA carcinogenicity. The SAB panel agreed, indicating that a sound rationale and justification for a mutagenic MOA were provided in the Toxicological Review. The Panel discussed the fact that AA/GA is not unique among DNA-reactive epoxides for carcinogenic action in thyroid, peritesticular mesothelium, and mammary tissue (e.g., glycidol, ethylene oxide). In addition, the SAB panel cited new data further supporting a mutagenic MOA (i.e., recent studies showing

GA-Hb adducts or GA urinary metabolites in humans suggesting internal exposure at low environmental concentrations). The observation that DNA adducts are found in both target and nontarget tissue does not alter the conclusion that adduct formation is likely related to cancer. Target organ responses may be related to differences in DNA repair and organ susceptibility to cancer. Findings in the mouse lymphoma and Big Blue mouse assays are considered relevant for the identification of DNA reactive carcinogens. *EPA Guidelines for Carcinogen Risk Assessment* indicate that linear low dose extrapolation should be used for agents that are DNA-reactive and demonstrate mutagenic activity (U.S. EPA, 2005, 086237).

25. **General comment:** Combination of tumor types is not appropriate for hazard identification or dose-response assessment of AA.

    Response: The *EPA Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005, 086237) allow for the combination of tumor data using several possible options. These include adding risk estimates derived from different tumor sites and representing the overall response in each experiment by counting animals with any tumor showing a statistically significant increase.

26. **General comment:** Mortality adjustment of tumor data was not necessary and the use of a time-to-tumor model was not warranted.

    Response: Although not strictly necessary, EPA performs a mortality adjustment of tumor data, when the raw data are available, to assess the impact of survival across dose groups on cancer incidence.

27. **General comment:** The development and validation of the PBTK model for AA is not fully described.

    Response: The recalibrated Kirman et al. (2003, 087778) model used in the external review draft of the Toxicological Review of Acrylamide (U.S. EPA, 2007, 418811), has since been published by Walker et al. (2007, 224527). EPA agrees that an update of the Walker et al. (2007, 224527) PBTK model parameter values, as well as the model structure, are needed based upon more recent kinetic data, and the need to represent first pass lung clearance for an inhalation exposure. For the purposes of the current assessment, however, recent kinetic data from Doerge et al. (2005, 224348; 2005, 224355; 2005, 224344) and Tareke et al. (2006, 224387) in conjunction with the human adduct data from Fennel et al. (2005, 224299) are sufficient to conduct a direct extrapolation of the rat dose-response POD to a human equivalent administered dose based on equivalent AUCs in the blood for AA or GA. Because AA or GA-AUC in the blood was also the dose metric that would be simulated with a PBTK model, the equivalent AUC method is a viable alternative in lieu of using the UF for interspecies toxicokinetic differences, and thus EPA was not subject to delay of the assessment pending update and peer review of a revised AA PBTK model. The equivalent AUC method also has fewer, relatively well supported parameters, and thus has inherently less uncertainty than a PBTK model. An AA PBTK model, of course, has far greater applicability to generate dose metrics other than the AUC in blood, to compare different dosing regimens, to simulate a far greater

range of data, and to conduct variability analyses. Thus, EPA recognizes and supports the important use of PBTK models in risk assessment, and encourages the research community to continue to develop and submit peer reviewed AA PBTK models for consideration. In future reviews or revisions to this document, EPA will consider available AA PBTK model results, and if needed, revise accordingly the reference values derived in this current version of the Toxicological Review of AA.

A.2. PEER CONSULTATION OF THE METHOD USED TO DERIVE AN INTERNAL DOSE (AUC) FOR A GIVEN EXPOSURE IN THE TOXICOLOGICAL REVIEW FOR ACRYLAMIDE

A peer consultation was conducted from December 2009 to January 2010 of the method used in the Toxicological Review for Acrylamide to derive the area-under-the concentration versus time curve (AUC) for acrylamide in the blood of rats based on hemoglobin adduct levels following a specified dose, as well as to estimate the external dose needed to produce an equivalent AUC in humans, also based on human hemoglobin adduct data.

This methodology was suggested during the previous Science Advisory Board review of the draft acrylamide assessment, and because acrylamide is one of the few chemicals where there are sufficient kinetic data to conduct this analysis.

The peer consultants for this task were chosen by an EPA peer review contractor according to scientific credentials and particular areas of expertise. The final selection included:

- Lesa L. Aylward, Ph.D. – Principal, Summit Toxicology, LLP.
- Margareta Törnqvist, Ph.D. - Associate Professor, Department of Environmental Chemistry, Stockholm University.
- Hermann M. Bolt, Dr. Med., Dr. Rer. Nat. - Professor Emeritus, Leibniz Research Centre for Working Environment and Human Factors.

Consultants were asked to address the following specific charge questions.

A.2.1. Charge Questions:

1. Please comment on the discussion and presentation of the method used to estimate the AUC in the ToxRev. Were the discussion and presentation clear and sufficiently detailed to support an understanding of what was done?
2. Is the method that EPA used to estimate AUC (based upon hemoglobin adducts levels as a biomarker of exposure) consistent with previously published methods in the peer reviewed literature that also used hemoglobin adduct levels, adduct formation rates, and what is known about the kinetics of acrylamide?
3. There are a number of calculations and numerical results in the derivation of the AUC and the HED/HEC. Have the equations and the resulting numbers been clearly and transparently presented so that the numbers can be checked for numerical accuracy? Please provide and comment on any errors in the calculations or the equations.

4. EPA derived hemoglobin adduct formation rate constants based on recently published data from in vivo studies to replace the formation rates based on more dated *in vitro* data. The rationale is that rate constants based upon levels of acrylamide (or glycidamide) and adducts in the blood from test animals receiving an oral exposure of acrylamide support a more accurate and relevant estimate of the internal exposure to the test animals in the two chronic (2-year) drinking water studies (Friedman et al., 1995, 224307; Johnson et al., 1986, 061340) compared to the use of formation rates based upon *in vivo* studies where acrylamide is directly added to blood.

   Please comment on whether or not you agree with EPA's rationale that rate constants based on the in vivo data are preferable to the use of rate constants based upon *in vitro* data. Please also comment on the appropriateness of the method that EPA used to derive the in vivo rate constants.

5. Please provide any additional comments you may have on the method used to estimate the AUC and the HED/HEC.

A.2.2. Peer Consultant Comments on the Charge Questions:

Summary Comments

All three of the reviewers endorsed the use of hemoglobin adducts and in vivo (versus in vitro)[1] formation rate constants to estimate the AUC of acrylamide in the blood of the principal study test animal (the rat), and to derive a human equivalent dose based on an equivalent AUC in humans.

Excerpts include:

- "The method that EPA used to estimate AUC based on Hb adducts is consistent with previously published methods and appears to me as the best possible way at present to bridge the animal and human data……I very strongly agree with the view of EPA that in vivo data are preferable to in vitro data in deriving the rate constants."
- "The general approach used by EPA is scientifically sound and represents a sophisticated and appropriate use of the detailed dosimetry and toxicokinetic data available from the Doerge and Tareke publications……I think that the overall approach and decision to use the in vivo-derived second order rate constants is a scientifically sound approach, and I congratulate EPA on synthesizing the detailed available data in a useful way."
- "The model used to calculate AUC from Hb adduct level …is consistent with previous literature….. The approach applied in the IRIS review to derive the second order rate constants from AUC in single dose experiments in vivo (in rats and mice) seems to be appropriate given the differences of the determined in vitro rate constants…. This referee.. is pleased to see that the value of the approach has been recognized and that the approach is further developed."

[1] This endorsement is with the implicit assumption that the in vivo data are of at least comparable quality to the in vitro data, i.e., in vivo rates are preferable, all other things being equal.

Response: EPA appreciates the external peer reviewer's support of, and concordance on, the use of AA and GA hemoglobin adducts as a viable biomarker of exposure.

Comment: All three reviewers provided a number of edits and suggestions for improving the clarity and presentation of the results.

Response: EPA appreciates the reviewer's identification of editorial errors, the identification of confusing or ambiguous text, and especially the rigorous and detailed scrutiny of the consistency of terms and units, as well as mathematical accuracy, of the equations used in the modeling and derivation of the internal doses. EPA has incorporated many of the reviewer's suggested text and editorial revisions into the current version of the Toxicological Review.

Comment: One reviewer suggested that EPA discuss the possibility that GA is the putative toxin for neurotoxic effects, and offered that an alternate analysis may be useful for the purpose of comparison.

Response: EPA has included text in Section 4.7.3.1 that discusses, in some detail, various hypothetical modes of action for AA versus GA neurotoxicity. There are studies supporting just parent AA as the putative toxin based on the adduct chemistries and the toxicity pathway for blockade of NO signaling (LoPachin and Barber, 2006, 224552; LoPachin et al., 2008, 224560; LoPachin et al., 2009, 224561). There are, however, also studies supporting either AA or GA as potential toxicants for the toxicity pathway involving kinesin inhibition (Sickles et al., 2002, 224497). Resolving the relative roles of AA versus GA, and the precise molecular mechanisms and sites of neurotoxicity, is clearly a research priority. With respect to the derivation of the reference value, however, the parent AA chemical is known to produce neurotoxicity, and will be considered the primary toxicant until there is sufficient evidence to support GA as a more potent toxin than AA either due to dynamic or kinetic differences in how it interacts with the body.

Comment: Two of the reviewers questioned the use of a gender specific in vivo adduct formation rate constant as not supported by the data or a biological rationale. On reviewer noted that the main difference in rates between male and female rats derived is primarily the result of a lower AUC in females from the oral gavage exposure, data that also had the largest standard deviation (i.e., was the most variable, least certain/precise).

Response: EPA reviewed the additional data analyses provided by the peer reviewers, and agrees that the more robust value for the in vivo adduct formation rate is the one derived from the pooled set of data for both male and female rats in the Tareke et al. (2006, 224387) and Doerge et al. (2005, 224355) studies. Revisions have been made accordingly in the assessment

text and reference value derivations to reflect this change. The absolute change in the reference values was small, but the values are now more scientifically sound.

Comment: Two reviewers questioned the use of gender specific elimination rates, and one reviewer provided an alternate model for estimating the AUC that differed primarily in the use of a term for erythrocyte degradation in conjunction with a first order elimination rate for other (unspecified) loss of adducts.

Response: EPA conducted an initial review of the alternate model, and considered the option of using the same elimination rate constant for genders and simulations. The results from the alternate model and EPA's model were very similar because the predominant contributor to the first order elimination rate used in the EPA model is erythrocyte turnover (i.e., a half-life of 12 days results in a nearly complete loss of adduct in 5 cycles (60 days), the proposed life span of an erythrocyte. The difference in model results from the use of an average 12 day half life as opposed to the gender specific rates of from 11-13 days, also had little effect on model results, and the final value of the reference value. For this rate, then, EPA choose to use the model presented in Appendix E with the gender specific elimination rates because these rates were data based for adduct losses in male and female rats (the species of interest) following a drinking water exposure, they represent all contributors to adduct loss including erythrocyte turnover as well as chemical instability, toxicity, or any gender specific process; without the need for assumptions or additional parameters to improve the model fit to the data, and the Tareke et al. (2006, 224387) data come from the same laboratory where the adduct formation rate data were generated (i.e., and presumably have more internal consistency [less impact on discrepancies in values due to systemic errors or bias] compared to rates derived from different laboratories).

APPENDIX B. MUTAGENICITY TEST RESULTS

Table B-1. Results of AA 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, 073869)
	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, 224547)
	S. typhimurium TA1535, TA1537, TA98, TA100 *Escherichia coli* WP2 *uvrA*$^-$	0.5–50 mg/plate ± S9 activation	50	Negative in both systems	Tsuda et al. (1993, 224441)
	S. typhimurium TA1535	Up to 5 mg/plate ± S9 activation	5	Negative	Müller et al. (1993, 224602); Jung et al. (1992, 224612)
	S. typhimurium TA1535, TA1537, TA1538, TA98, TA100	Up to 1 mg/plate ± S9 activation	1	Negative	Lijinsky and Andrews (1980, 017361)
	S. typhimurium TA1535, TA1537, TA1538, TA98, TA100	0.5–5,000 µg/plate ± S9 activation	5,000	Negative	Hashimoto and Tanii (1985, 224504)
Fluctuation test	*K. pneumoniae* ur$^-$ pro$^-$	2–10 mg/mL	10	Negative	Knaap et al. (1988, 224547)
Nonmammalian gene mutation assays in vivo					
Sex-linked recessive lethal	*D. melanogaster*	40–50 mM abdominal injection	50	Negative	Knaap et al. (1988, 224547)
	D. melanogaster	0.24–5 mM larvae feeding	1	Positive	Tripathy et al. (1991, 224435)

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS).

Assay	Test system[a]	Dose/concentration	HID or LED[b]	Result	Reference
Somatic mutation, recombination	*D. melanogaster*	1–1.5 larvae feeding (unit unspecified)	1	Weakly positive	Knaap et al. (1988, 224547)
	D. melanogaster	1–1.5 mM larvae feeding	1	Positive	Batiste-Alentorn et al. (1991, 224419)
	D. melanogaster	0.25–5 mM larvae feeding	1	Positive	Tripathy et al. (1991, 224435)
Mammalian gene mutation assays in vitro					
	Mouse lymphoma L5178Y $TK^{+/-}$, tk locus	10 mM	10	Positive (more pronounced without activation)	Barfknecht et al. (1988, 224417)
	Mouse lymphoma L5178Y $TK^{+/-}$, tk locus	0–0.85 mg/mL without activation	0.5	Positive	Moore et al. (1987, 224589)
	Mouse lymphoma L5178Y/$TK^{+/-}$, tk locus	0–18 mM no activation	12	Positive	Mei et al. (2008, 224585)
	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, 224547)
	Mouse lymphoma L5178Y $TK^{+/-}$, HPRT locus	0.1–0.5 mg/mL with cocultivated mammalian cells	0.3	Positive	Knaap et al. (1988, 224547)
	Chinese hamster V79H3 cells, HPRT locus	1–7 mM no activation	7	Negative	Tsuda et al. (1993, 224441)
	Human promyelocytic leukemia HL-60 and NB4 cells, HPRT locus	0–700 mg/L no activation	700	Positive	Ao et al. (2008, 224395)
Mammalian gene mutation assays in vivo					
	Mouse $B6C3F_1/Tk^{+/-}$, (M, F) spleen lymphocytes tk and HPRT loci	0–0.70 mmol/kg i.p. injection postnatal d 1, 8, 15	0.70	Negative	Von Tungeln et al. (2009, 224513)
	Mouse $B6C3F_1/Tk^{+/-}$, (M, F) spleen lymphocytes tk and HPRT loci	0–0.70 mmol/kg i.p. injection postnatal d 1–8	0.14	Positive	Von Tungeln et al. (2009, 224513)
Transgenic mouse liver cII, lymphocyte HPRT	BB Mouse (M, F)	100, 500 mg/L AA or GA drinking water for 3–4 wks	100 (est. 19–25 mg/kg-day)	Positive	Manjanatha et al. (2006, 224572)

Assay	Test system[a]	Dose/concentration	HID or LED[b]	Result	Reference
Transgenic mouse *lacZ*	Muta® Mouse	5×50 mg/kg-d i.p. injection	5×50	Weakly positive, no statistical analysis	Hoorn et al. (1993, 224524)
	Muta® Mouse	50–100 mg/kg i.p. injection	100	Negative	Krebs and Favor (1997, 224526)
Mouse spot test	Mouse embryos $(T \times HT)F_1$	1 × 50 or 75 mg/kg i.p. injection	50	Positive	Neuhäuser-Klaus and Schmahl (1989, 224607)
		3 × 50 or 75 mg/kg i.p. injection	3 × 50	Positive	
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, 224406)
	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, 224391)
Chromosomal alterations in mammalian cells in vitro					
CAs	Chinese hamster cells	0.5–5 mM no activation used	2	Positive	Tsuda et al. (1993, 224441)
	Chinese hamster cell line (V79)	0.1–3 mg/mL ± S9 activation	1	Positive, with or without metabolic activation	Knaap et al. (1988, 224547)
	Chinese hamster cell line (V79)	0–2,000 µM no activation	2,000	Weakly positive	Martins et al. (2007, 224580)
	Mouse lymphoma L5178Y $TK^{+/-}$ -3.7.2 cells	0.65–0.85 mg/mL without activation	0.75	Positive	Moore et al. (1987, 224589)
Cell division aberration	Chinese hamster lung cell line DON:Wg3h	0.2–1 mg/mL	0.2	Positive	Warr et al. (1990, 224530)
	Chinese hamster lung fibroblast LUC2 p5	0.01–1 mg/mL	0.01	Positive	Warr et al. (1990, 224530)
Chromosome enumeration	Chinese hamster lung fibroblast LUC2 p5	0.0125–0.5 mg/mL	0.5	Positive	Warr et al. (1990, 224530)
Polyploidy	Chinese hamster cell line (V79)	0.5–5 mM	1	Positive	Tsuda et al. (1993, 224441)

Assay	Test system[a]	Dose/concentration	HID or LED[b]	Result	Reference
Spindle disturbances	Chinese hamster cell line (V79)	0.01–1 mg/mL	0.01	Positive	Adler et al. (1993, 224310)
MN	Seminiferous tubular segments (spermatids from Sprague-Dawley rats)	5–50 µg/mL	50	Negative	Lähdetie et al. (1994, 224500)
	Human hepatoma G2 cells	0–2.5 mM	0.625	Positive	Jiang et al. (2007, 224388)
Chromosomal alterations in mammalian cells in vivo					
CAs	Mouse (101/E1 × C3H/E1)F_1 (bone marrow cells)	50–150 mg/kg i.p. injection	50	Positive	Adler et al. (1988, 224301)
	Mouse (ICE-SPF) (bone marrow cells)	100 mg/kg i.p. injection	100	Positive	Čihák and Vontorková (1988, 224270)
	Mouse (ddY) (bone marrow cells)	100–200 mg/kg i.p. injection	200	Negative	Shiraishi (1978, 224490)
	Mouse (ddY) (bone marrow cells)	500 ppm in diet for 7–21 days (78 mg/kg-day)	78	Negative	Shiraishi (1978, 224490)
	Rat (bone marrow cells)	100 mg/kg i.p. injection	100	Negative	Krishna and Theiss (1995, 224525)
	Mouse (C57BL/6J) (spleen lymphocytes)	50–125 mg/kg i.p. injection	125	Negative	Backer et al. (1989, 224404)
	Mouse (C57BL/6) (splenocytes)	100 mg/kg i.p. injection	100	Negative	Kligerman et al.
	Mouse (101/E1 × C3H/E1)F_1 (spermatogonia)	50–150 mg/kg i.p. injection	150	Negative	Adler et al. (1988, 224301)
	Mouse (C57BL/6J) (spermatogonia)	50–125 mg/kg i.p. injection	125	Negative	Backer et al. (1989, 224404)
	Mouse (102/E1 × C3H/E1)F_1 (spermatogonia)	5×50 mg/kg-day i.p. injection	5×50	Negative	Adler (1990, 224296)
	Mouse (102/E1 × C3H/E1)F_1 (spermatocytes)	100 mg/kg i.p. injection	100	Positive	Adler (1990, 224296)
	Mouse (CF_1) (first cleavage embryos)	150 mg/kg i.p. injection	150	Positive in embryos from which the males had mated 6–8 d following treatment (early spermatozoa stage)	Valdivia et al. (1989, 224501)
	Mouse ($B6C3F_1$) (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, 224316)

Assay	Test system[a]	Dose/concentration	HID or LED[b]	Result	Reference
	Mouse ($B6C3F_1$) (first cleavage zygotes, examined after mating)	50 mg/kg i.p. injection (males) for 5 days before mating	50	Positive	Marchetti et al. (1997, 224573)
Polyploidy or aneuploid	Mouse bone marrow cells	100–200 mg/kg i.p. injection	100	Positive	Shiraishi (1978, 224490)
	Mouse bone marrow cells	500 ppm in the diet for 7–21 days (78 mg/kg-day)	78	Positive	Shiraishi (1978, 224490)
Spindle disturbances	Mouse (102/E1 × C3H/E1) bone marrow cells	120 mg/kg i.p. injection	120	Negative	Adler et al. (1993, 224310)
MN	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, 224301)
	Mouse (ICR-SPF) bone marrow cells (M)	100 mg/kg i.p. injection	100	Positive	Čihák and Vontorková (1988, 224270)
	Mouse (ICR-SPF) bone marrow cells (M)	25–100 mg/kg-day for 2 days i.p. injection	25	Positive	Čihák and Vontorková (1988, 224270)
	Mouse (Swiss NIH) bone marrow cells (M, F)	136 mg/kg i.p. injection	136	Positive	Knaap et al. (1988, 224547)
	Mouse (ICR-SPF) bone marrow cells (M, F)	42.5–100 mg/kg-day (1, 2, or 3 d) i.p. injection	M: 42.5 F: 55	Positive	Čihák and Vontorková (1990, 224271)
	Rat (Sprague-Dawley) bone marrow cells (M)	100 mg/kg i.p. injection	100	Negative	Paulsson et al. (2002, 224334)
	Rat bone marrow cells	100 mg/kg i.p. injection	100	Negative	Krishna and Theiss (1995, 224525)
	Mouse (BALB/c) reticulocytes	50–100 mg/kg i.p. injection	50	Positive	Russo et al. (1994, 224409)
	Mouse (CBA) reticulocytes	25–50 mg/kg i.p. injection	25	Positive, but results were not analyzed statistically	Paulsson et al. (2002, 224334)
	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, 224340)
	Mouse ($B6C3F_1$) reticulocytes and normochromatic erythrocytes (M, F)	0, 0.14, 0.70 mmol/kg i.p. injection PNDs 1, 8, 15	0.70	Negative	Von Tungeln et al. (2009, 224513)
	Mouse ($B6C3F_1$) reticulocytes and normochromatic erythrocytes (M, F)	0, 0.14, 0.70 mmol/kg i.p. injection PNDs 1–8	0.70	Negative	Von Tungeln et al. (2009, 224513)

Assay	Test system[a]	Dose/concentration	HID or LED[b]	Result	Reference
	Mouse ($B6C3F_1$) reticulocytes	0–24 mg/kg-day for 28 days gavage	6	Positive	Zeiger et al. (2009, 224546)
	Mouse ($B6C3F_1$) normochromatic erythrocytes	0–24 mg/kg-day for 28 days gavage	4	Positive	Zeiger et al. (2009, 224546)
	Mouse (wild-type or CYP2E1- null) erythrocytes (F)	0, 25, 50 mg/kg-days i.p. injection for 5 days	25	Positive (wild-type mice only)	Ghanayem et al. (2005, 224354)
	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, 224340)
	Mouse (C57BL/6J) (M) spleen lymphocytes	50–125 mg/kg i.p. injection	50	Positive	Backer et al. (1989, 224404)
	Mouse (C57BL/6) (M) splenocytes	100 mg/kg i.p. injection	100	Positive	Kligerman et al. (1991, 006236)
	Mouse (C57BL/6J) spermatids	10–100 mg/kg i.p. injection	50	Positive	Collins et al. (1992, 224280)
	Mouse (BALB/c) spermatids	50–100 mg/kg or 4 × 50 mg/kg-day i.p. injection	50	Positive	Russo et al. (1994, 224409)
	Rat (Lewis) spermatids	50–100 mg/kg or 4 × 50 mg/kg-day i.p. injection	100	Positive	Xiao and Tates (1994, 224543)
	Rat (Sprague-Dawley) spermatids	50–100 mg/kg or 4 × 50 mg/kg-day i.p. injection	4×50	Positive	Lähdetie et al. (1994, 224500)
Synaptonemal complex aberrations	Mouse (C57BL/J6) (M) germ cells	50–150 mg/kg i.p. injection	150	Negative	Backer et al. (1989, 224404)
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, 224404)
Heritable translocations	Mouse (C3H × 101)F_1 (M)	5 × 40–50 mg/kg-day i.p. injection	40	Positive	Shelby et al. (1987, 088819)
	Mouse (C3H/E1) (M)	50–100 mg/kg i.p. injection	50	Positive	Adler et al. (1994, 224314)
	Mouse (C3H/E1) (M)	5 × 50 mg/kg-day dermal	50	Positive	Adler et al. (2004, 224343)
Reciprocal translocations	Mouse (C3H/E1) (M)	5 × 50 mg/kg-day i.p. injection	50	Positive	Adler (1990, 224296)
DNA damage and repair and DNA adduct formation					
Spore rec assay	*Bacillus subtilis* H17 (rec^+) and M45 (rec^-)	1–50 mg/disk	10	Positive	Tsuda et al. (1993, 224441)
In vitro DNA breakage (comet assay)	Human hepatoma G2 cells	0–20 mM	2.5	Positive	Jiang et al. (2007, 224388)

Assay	Test system[a]	Dose/concentration	HID or LED[b]	Result	Reference
In vivo DNA breakage (comet assay)	Mouse (C3H × C57BL/10)F_1 (M)	25–125 mg/kg i.p. injection	25	Positive	Sega and Generoso (1990, 224465)
	Mouse Pzh:SFIS (M) bone marrow, spleen, liver, kidney, lungs, testes	0–125 mg/kg i.p.injection	50	Positive	Dobrzynska (2007, 224338)
	Mouse (wild-type or CYP2E1-null) leukocytes, liver, lung (F)	0, 25, 50 mg/kg-day i.p injection, for 5 days	25	Positive (wild-type mice only)	Ghanayem et al. (2005, 224354)
Oxidative DNA damage	Human hepatoma G2 cells	0–20 mM	5	Positive	Jiang et al. (2007, 224388)
In vitro UDS	Rat primary hepatocytes	5–20 mM	17.5	Weakly positive	Barfknecht et al. (1988, 224417)
	Rat (F344) (M) primary hepatocytes	0.01–1 mM	1	Negative	Butterworth et al. (1992, 202898)
	Human mammary epithelial cells	1–10 mM	1	Positive	Butterworth et al. (1992, 202898)
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, 202898)
	Rat (F344) (M) spermatocytes	1 × 100 mg/kg 5 × 30 mg/kg-day gavage	5 × 30	Positive	Butterworth et al. (1992, 202898)
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, 224482)
In vitro DNA adducts	Chinese hamster cells (V79)	0–2,000 µM	2,000	Positive	Martins et al. (2007, 224580)
	Mouse lymphoma cells L5178Y/$TK^{+/-}$	0–20 mM	20	Negative	Mei et al. (2008, 224585)
	BB mouse embryonic fibroblasts (with lambda phage cII transgene)	0, 0.0032, 0.320, 16 mM	0.0032	Positive	Besaratinia and Pfeifer (2004, 224427)
	Human bronchial epithelial cells (with lambda phage cII transgene)	0, 0.320, 3.2 mM	0.320	Positive	Besaratinia and Pfeifer (2004, 224427)
In vivo DNA adducts	Mouse (C3H × BL10)F_1 testis	46 mg/kg i.p. injection	46	Positive	Sega et al. (1990, 224482)
	Mouse (C3H × BL10)F_1 (M) liver	46 mg/kg i.p. injection	46	Positive	Sega et al. (1990, 224482)

Assay	Test system[a]	Dose/concentration	HID or LED[b]	Result	Reference
	Rat (Sprague-Dawley) liver, lung, kidney, brain, testis	46 mg/kg i.p. injection	46	Positive	Segerbäck et al. (1995, 224485)
	Mouse (BALB/c) liver, kidney, brain	53 mg/kg i.p. injection	53	Positive	Segerbäck et al. (1995, 224485)
	Neonatal mouse ($B6C3F_1$) whole-body	50 mg/kg i.p. injection	50	Positive	Gamboa da Costa et al. (2003, 194572)
	Mouse (C3H/HeNMTV) (M) and (C57B1/CN) (F) liver, lung, kidney	50 mg/kg i.p. injection	50	Positive	Gamboa da Costa et al. (2003, 194572)
	Mouse (C3H/HeNMTV) (M) liver, lung	0–50 mg/kg i.p. injection	1	Positive	Gamboa da Costa et al. (2003, 194572)
	Mouse ($B6C3F_1$) lung, liver, spleen, bone marrow	0, 0.14, 0.70 mmol/kg i.p. injection PNDs 1, 8, 15	0.14	Positive	Von Tungeln et al. (2009, 224513)
	Mouse ($B6C3F_1$) lung, liver, spleen, bone marrow	0, 0.14, 0.70 mmol/kg i.p. injection postnatal day 1–8	0.14	Positive	Von Tungeln et al. (2009, 224513)
	Mouse ($B6C3F_1$) (M) liver	0–24 mg/kg-day for 28 days gavage	0.125	Positive	Zeiger et al. (2009, 224546)
	Mouse ($B6C3F_1$) (M, F) liver, lung, kidney, leukocytes, testis	50 mg/kg i.p.injection	50	Positive	Doerge et al. (2005, 224344)
	Mouse ($B6C3F_1$) (M, F) liver	1 mg/kg-day drinking water	1	Positive	Doerge et al. (2005, 224344)
	Rat (F344) (M, F) liver, brain, thyroid, leukocytes, mammary gland, testis	50 mg/kg i.p.injection	50	Positive	Doerge et al. (2005, 224344)
	Rat (F344) (M, F) liver	1 mg/kg-day drinking water	1	Positive	Doerge et al. (2005, 224344)
SCE					
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, 224547)
	Chinese hamster V79 cells	0.5–2.5 mM no activation used	1	Positive	Tsuda et al. (1993, 224441)
	Chinese hamster V79 cells	0–2,000 µM no activation	2,000	Positive	Martins et al. (2007, 224580)

Assay	Test system[a]	Dose/concentration	HID or LED[b]	Result	Reference
In vivo	Mouse (C57BL/6J) (M) spleen lymphocytes	50–125 mg/kg i.p. injection	50	Positive	Backer et al. (1989, 224404)
	Mouse (C57BL/6) (M) splenocytes	100 mg/kg i.p. injection	100	Positive	Kligerman et al. (1991, 006236)
	Mouse (BALB/c) differentiating spermatogonia	50–100 mg/kg	50	Positive	Russo et al. (1994, 224409)
Cell transformation					
	Mouse C3H/10T1/2 clone 8 cells	25–200 µg/mL	50	Positive	Banerjee and Segal (1986, 224412)
	Mouse NIH/3T$_3$ cells	2–200 µg/mL	0.0125	Positive	Banerjee and Segal (1986, 224412)
	Mouse C3H/10T1/2 cells	0.01–0.3 mg/mL	0.3	Negative	Abernethy and Boreiko (1987, 224278)
	Mouse BALB/c 3T$_3$ cells	0.5–2 mM	1	Positive	Tsuda et al. (1993, 224441)
	SHE cells	0.1–0.7 mM	0.5	Positive	Park et al. (2002, 224330)
	SHE cells	0.001–10 mM	10	Negative	Kaster et al. (1998, 224588)
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, 224477)
Sperm head protamine alkylation	Mouse (C3H × 101)F$_1$	125 mg/kg i.p. injection	125	Positive	Sega et al. (1989, 224477)
Sperm head abnormalities	Mouse (ddY)	0.3–1.2 mM in drinking water for 4 wks	1.2	Positive	Sakamoto and Hashimoto (1986, 224442)
Sperm aneuploidy	Mouse (102/ElxC3H/ElF$_1$) (M)	0, 60, 120 mg/kg i.p. injection	120	Negative	Schmid et al. (1999, 224458)

[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 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, 224307; Johnson et al., 1986, 061340). The data that were modeled are shown below in Table C-1. The BMR predicted to affect 5% of the population (BMR_5) was selected for the POD. A BMR of 5% extra risk was selected for the following reasons: (1) this effect level is considered to be a minimal biologically significant change given the critical effect of degenerative nerve changes; (2) the $BMDL_5$ remained near the range of observation; and (3) the 5% extra risk level is supportable given the relatively large number of animals used in the principal studies.

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

Reference	Dose (mg/kg-day)							
	0	0	0.01	0.1	0.5	1.0	2.0	3.0
Johnson et al. (Johnson et al., 1986, 061340) (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.[d] (Friedman et al., 1995, 224307) (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."

All models provided adequate fits to the data for changes in tibial nerves in male and female rats in the Johnson et al. (1986, 061340) study, as assessed by a χ^2 goodness-of-fit test (see Tables C-2 and C-3 and following plots [Figures C-1 and C-2] of observed and predicted

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS).

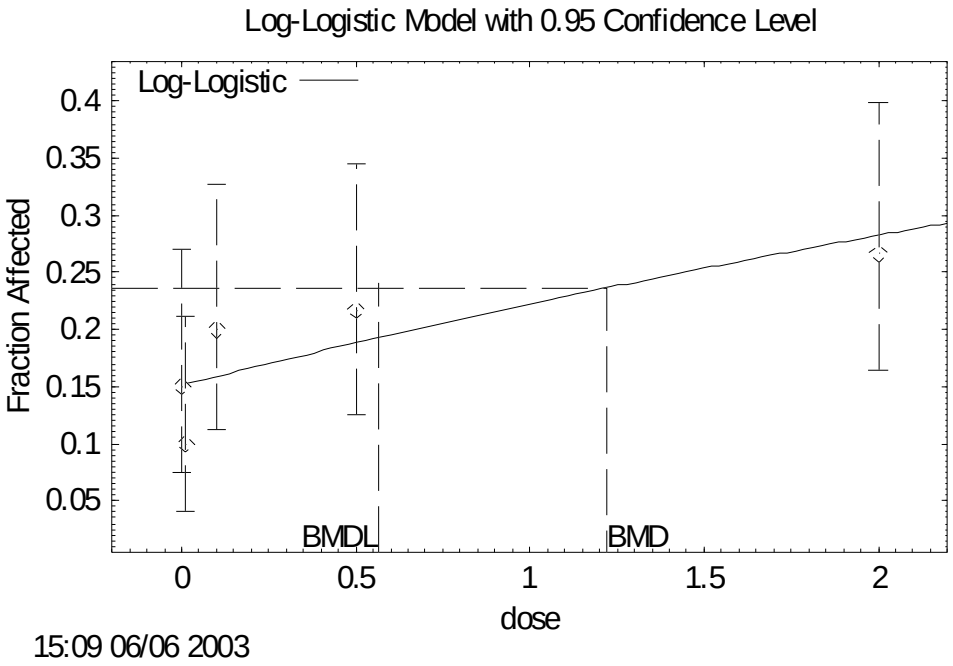
values from the various models). The log-logistic model provided the best fit for the male rat data as assessed by Akaike's Information Criterion (AIC) and was thus selected to estimate a BMD from the Johnson et al. (1986, 061340) data. The probit model provided the best fit of the female rat data. Table 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, 061340) 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 AA 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.

Source: Johnson et al. (1986, 061340).



Source: Johnson et al. (1986, 061340).

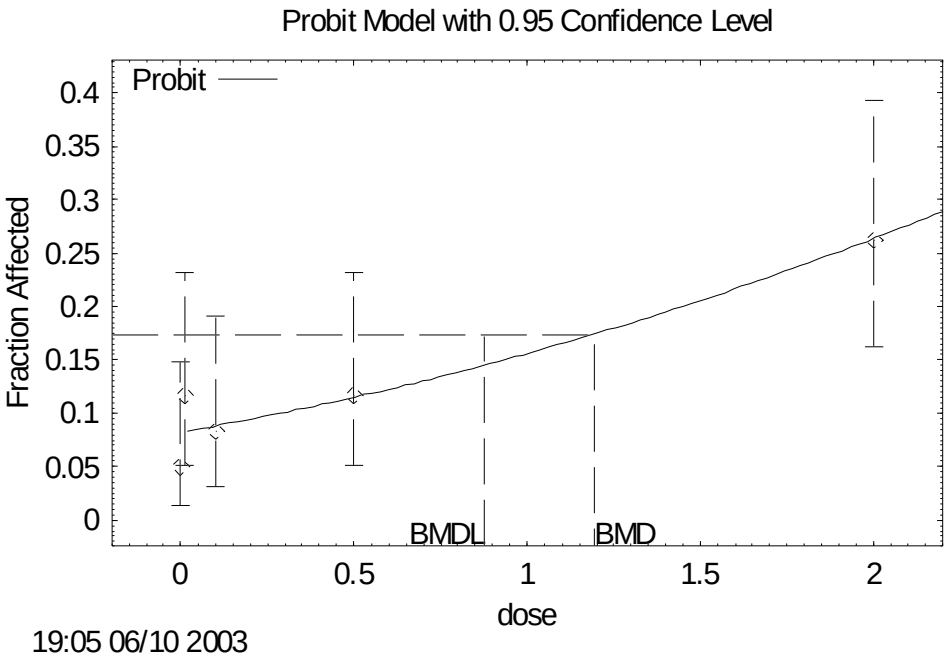
Figure C-1. Observed and predicted incidences for nerve changes in male rats exposed to AA in drinking water for 2 years. (Log-Logistic Model)

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 AA 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.

Source: Johnson et al. (1986, 061340).



Source: Johnson et al. (1986, 061340).

Figure C-2. Observed and predicted incidences for nerve changes in female rats exposed to AA in drinking water for 2 years. (Probit Model)

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 AA 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, 061340).

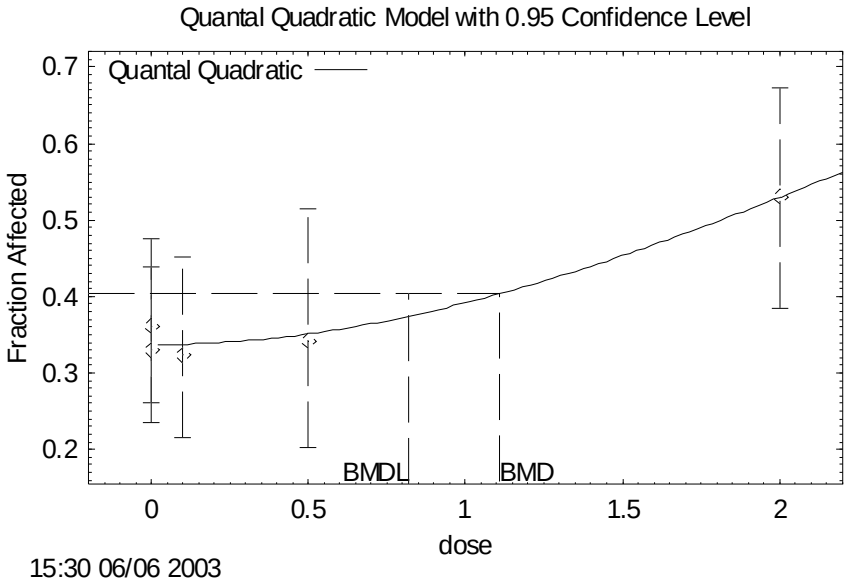
Several models in the software provided adequate fits to the data for minimal to mild changes in sciatic nerves in male and female rats in the Friedman et al. (1995, 224307) study, as assessed by a χ^2 goodness-of-fit test (see Tables C-5 and C-6 and following plots [Figures C-3 and C-4] of observed 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, 224307) 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 AA 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.

Source: Friedman et al. (1995, 224307).



Source: Friedman et al. (1995, 224307).

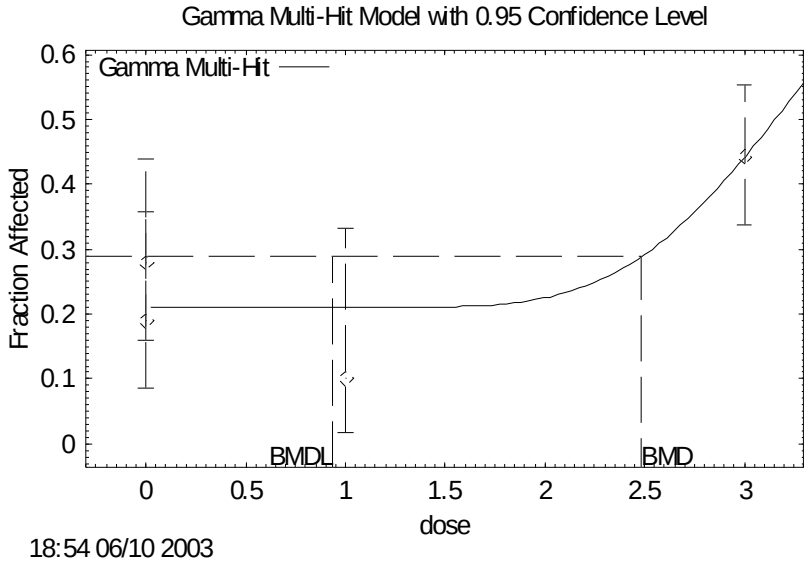
Figure C-3. Observed and predicted incidences for nerve changes in male rats exposed to AA in drinking water for 2 years. (Quantal Quadratic Model)

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 AA 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-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.

Source: Friedman et al. (1995, 224307).



Source: Friedman et al. (1995, 224307).

Figure C-4. Observed and predicted incidences for nerve changes in female rats exposed to AA in drinking water for 2 years. (Gamma Multi-Hit Model)

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 AA 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, 224307).

APPENDIX D. DOSE-RESPONSE MODELING FOR CANCER

D.1. METHODS

D.1.1. Data

Tumor data from the 2-year bioassays with F344 rats (Friedman et al., 1995, 224307; Johnson et al., 1986, 061340) were modeled to obtain potential PODs for deriving an oral slope factor and IUR (Tables D-1 and D-2).

Table D-1. Incidence of tumors with statistically significant increases in the Friedman et al. (1995, 224307) bioassay with F344 rats exposed to AA in drinking water

Reference/tumor type	Dose (mg/kg-day)						
	0	0	0.1	0.5	1.0	2.0	3.0
Males[a]							
Follicular cell adenoma/carcinoma	3/100	2/102[e]	12/203	5/101	–	17/75[i]	–
TVM[b]	4/102	4/102	9/204	8/102	–	13/75[i]	–
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, 224307) and not in the reevaluation study by Iatropoulos et al. (1998, 224628).
[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, 224307) lists one follicular cell adenoma in the second control group; however, the raw data obtained in the Tegeris Laboratories (1989, 224400) 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, 224307).

Table D-2. Incidences of tumors with statistically significant increases in the Johnson et al. (1986, 061340) bioassay with F344 rats exposed to AA in drinking water

Tumor type	Dose (mg/kg-day)				
	0	0.01	0.1	0.5	2.0
Males					
Thyroid (follicular cell) adenoma (no carcionomas found)	**1/60**	**0/58**	**2/59**	**1/59**	**7/59[a]**
TVM	**3/60**	**0/60**	**7/60**	**11/60[a]**	**10/60[a]**
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]
Mammary malignant + benign[b]	**12/60**	**12/60**	**10/60**	**21/58**	**29/61**
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]**
Oral cavity, squamous cell carcinoma	0/60	0/60	0/60	2/60	1/61
Oral cavity squamous papilloma	0/60	3/60	2/60	1/60	7/61[a]
Oral cavity malignant + benign[b]	0/60	3/60	2/60	3/60	8/60
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.
[b]Incidences of benign and malignant tumors in these sites (mammary gland or oral cavity) were added herein, based on an assumption that rats assessed with malignant tumors were not also assessed with tumors.

Source: Johnson et al. (1986, 061340).

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, 086237).

D.1.2. 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:

$$P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)] \qquad \text{Equation D-1}$$

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-Weibull model in BMDS (version 1.3.2) was used for all multistage model fits.

The multistage-Weibull model is given by:

$$P(d,t) = 1 - \exp[-(q_0 - q_1 d - q_2 d^2 . \ldots - q_k d^k)\,(t - t_0)^j] \qquad \text{Equation D-2}$$

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 (Howe and Crump, 1985, 402270).

D.2. RESULTS

D.2.1. *Friedman et al.* (1995, 224307) *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 ($p = 0.47$) (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 $(mg/kg\text{-}day)^{-1}$ (Table D-3).

Table D-3. Risk estimate derived from separate and combined incidence of mammary or thyroid tumors in female F344 rats exposed to AA in drinking water

Tumor site	BMD_R (mg/kg-day)	$BMDL_R$ (mg/kg-day)	Rat cancer slope factor[a] (per mg/kg-day)
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]Rat cancer 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.

Source: Friedman et al. (1995, 224307).

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 $(mg/kg\text{-}day)^{-1}$ (Table D-3).

Despite a few early mortalities, there were no statistically significant incidences of early mortalities in female rats exposed to AA. 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 rat cancer slope factors corresponding to mammary tumors and to follicular cell thyroid tumors in female F344 rats were very similar, 0.13 versus 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 AA.

The EPA cancer guidelines (U.S. EPA, 2005, 086237) 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, 006424) *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 an approach that adds 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 AA in the drinking water

(Tegeris Laboratories, 1989, 224400) 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) (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 twofold 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 (NRC, 1994, 006424; U.S. EPA, 2005, 086237) 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 AA 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, 006424) 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 central tendency or MLEs of unit potency (i.e., risk per unit of exposure) were estimated by R/BMD_R, and the UCL on the unit risk estimated by $R/BMDL_R$.

(3) The central tendency or MLEs of unit potency (i.e., risk per unit of exposure), estimated by R/BMD_R, were summed across the multiple sites for male or 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% UCL according to the formula

$$95\%\ UCL = MLE + (1.645 \times SD) \qquad \text{Equation D-3}$$

where 1.645 is the t-statistic corresponding to a one-sided 95% CI 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-4 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-3), 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 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 0.21 $(mg/kg\text{-}day)^{-1}$, and the summed central tendency was 0.16 $(mg/kg\text{-}day)^{-1}$, about a 1.3-fold difference (Table D-4). 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 twofold higher than either of the individual risks. For these data, the two approaches yield very similar results.

Table D-4. Risk estimates derived from separate and summed dose-response modeling of mammary and thyroid tumors in female F344 rats exposed to AA in drinking water

Tumor site	BMD_R (mg/kg-day)	$BMDL_R$ (mg/kg-day)	Central tendency oral potency[a] (per mg/kg-day)	Upper bound on lifetime extra risk (per mg/kg-day)
Mammary (benign and malignant)	1.2	0.78	8.3×10^{-2}	0.14
Thyroid (adenomas and carcinomas)	1.3	0.94	7.7×10^{-2}	0.11
Risk of either mammary or thyroid tumors			0.16	0.21[b]

[a]Central tendency oral potency = R/BMD_R, where R = 0.1. The combined central tendency risk is the sum of the individual oral potencies.
[b]The rat cancer 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 rat cancer slope factor should not be used with exposures >3 mg/kg-d, because above this level, the dose-response relationship is likely to be nonlinear.

Source: Friedman et al. (1995, 224307).

D.2.2. Friedman et al. (1995, 224307) 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 TVMs.

Because male rats in the highest dose group in the Friedman et al. (1995, 224307) study showed early mortalities, models that adjusted for early mortality were fit to the data for TVMs

and thyroid follicular cell adenomas and carcinoma. Pathology reports for individual rats in the study (Tegeris Laboratories, 1989, 224400) 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 $(mg/kg\text{-}day)^{-1}$ (Table D-5).

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 $(mg/kg\text{-}day)^{-1}$ (Table D-5).

Table D-5. Risk estimates for separate and combined incidence of TVMs or thyroid tumors in male rats exposed to AA in drinking water

Incidence modeled	BMD_R[a] (mg/kg-day)	$BMDL_R$[a] (mg/kg-day)	Rat cancer slope factor (risk level/BMDL) (per mg/kg-day)
TVM	1.2	0.75	0.13
Follicular cell thyroid tumors	0.71	0.45	0.22
TVM or thyroid tumors[b]	0.70	0.30	0.33

[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, 006424) approach, the slope factor was 0.34 (see discussion below).

Source: Friedman et al. (1995, 224307).

The first recommended method in the EPA cancer guidelines for assessing total risk from multiple tumor sites (NRC, 1994, 006424; U.S. EPA, 2005, 086237) 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-6 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-5), 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-5). 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-6. Risk estimates derived from modeling separate and summed incidence of TVM and thyroid tumors in male F344 rats exposed to AA in drinking water

Tumor site	BMD_R (mg/kg-day)	$BMDL_R$ (mg/kg-day)	Central tendency oral potency[a] (per mg/kg-day)	Upper bound on lifetime extra risk (per mg/kg-day)
TVM	1.2	0.75	8.3×10^{-2}	0.14
Thyroid (adenomas and carcinomas)	0.71	0.45	0.14	0.23
Risk of either TVM or thyroid tumors			0.22	0.32[b]

[a]Central tendency oral potency = R/BMD_R, where R = 0.1. The combined central tendency risk is the sum of the individual oral potencies.
[b]The rat cancer slope factor for the combination of tumor sites is the 95% upper bound on lifetime extra risk (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 rat cancer slope factor should not be used with exposures >2 mg/kg-day, because above this level, the dose-response relationship is likely to be nonlinear.

Source: Friedman et al. (1995, 224307).

The resulting 95% UCL on the summed risk of TVM or thyroid follicular cell adenomas/carcinomas for male F344 rats was 0.32 $(mg/kg\text{-}day)^{-1}$, and the summed central tendency was 0.22 $(mg/kg\text{-}day)^{-1}$, about a 1.4-fold difference (Table D-6). The estimated risk for thyroid tumors was the more variable, contributing about 73% 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 rat extra cancer risk from continuous, lifetime oral exposure to AA is 0.3 $(mg/kg\text{-}day)^{-1}$, rounding

the summed risk for male rats above to one significant digit.[1] 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.

As in most cancer assessments, extrapolation of study data to estimate potential risks to human populations from exposure to AA 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, 086237). 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 AA. Also, while the female rats did not appear to have as strong a carcinogenic response as the male rats, it is not known which gender is more relevant for extrapolation of risk to humans.

Parameter uncertainty can be assessed through CIs 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.

D.2.3. Johnson et al. (1986, 061340) F344 Rat Tumor Modeling

Data for tumors with statistically significant increases in the Johnson et al. (1986, 061340) drinking water bioassays were modeled to derive potential PODs for an oral slope factor and IUR. For males, the tumor types were TVMs, thyroid follicular cell (adenoma/carcinoma), and adrenal pheochromocytomas. For females, the tumor types were mammary gland tumors (malignant and benign combined), thyroid follicular cell (adenoma/carcinoma), CNS tumors of glial origin, and oral cavity tumors (malignant and benign combined). The data for uterine adenocarcinomas and pituitary gland adenomas were not analyzed because the statistical significance of the elevated incidences in the high-dose group was only demonstrated after

[1] 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 a method consistent with the NRC (1994, 006424) recommendation.

Mantel-Haenszel mortality adjustment (Table D-2). The data for clitoral adenomas were not analyzed because the number of tissues examined in each group was small (≤ 5, Table D-2).

The tumor data for each sex and tumor site were fit with the multistage model to estimate the BMD and the 95% lower confidence limit on the BMD, the BMDL. Because individual animal data were not available for the time of death from the Johnson et al. (1986, 061340) bioassay, no adjustments or special modeling was done for early mortalities.

The POD results for separate modeling of the female mammary, thyroid, CNS, and oral cavity tumor incidence data are presented in Table D-7. In addition, rat cancer slope factors for the summed risks for these tumor types were calculated using the method described above, and are presented in Table D-7. Table D-8 shows the calculations for summing risks across tumor sites in the female rats.

Table D-7. Risk estimates derived from separate incidence of mammary, thyroid, CNS, or oral cavity tumors in female F344 rats exposed to AA in drinking water

Tumor site	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)	Slope factor[a] (per mg/kg-day)
Mammary (benign and malignant)	0.44	0.30	0.34
Thyroid follicular cell (adenomas and carcinomas)	2.93	1.47	0.07
CNS tumors of glial origin	1.80	1.03	0.10
Oral cavity (malignant and benign)	1.80	0.99	0.10

[a]Rat cancer slope factor is the upper bound on lifetime extra risk, calculated using $R/BMDL_R$, where R = 0.1.

Source: Johnson et al. (1986, 061340).

Table D-8. Calculation of summed risks for tumors at several sites in female F344 rats exposed to AA in drinking water in the Johnson et al. (1986, 061340) bioassay

Tumor site	BMR	Oral slope factor[a] (central tendency) (per mg/kg-day)	Oral slope factor[b] (upper bound) (per mg/kg-day)	t-statistic	SD	σ^2
Mammary	0.1	2.3×10^{-1}	3.4×10^{-1}	1.645	6.79×10^{-2}	4.61×10^{-3}
Thyroid	0.1	3.4×10^{-2}	6.8×10^{-2}	1.645	2.06×10^{-2}	4.25×10^{-4}
Cumulative variance		5.04×10^{-3} ($\Sigma\sigma^2$)				
Cumulative SD		7.10×10^{-2} ($\sqrt{\Sigma\sigma^2}$)				
Sum of central tendency risks		2.6×10^{-1} (mg/kg-day)$^{-1}$				
Upper bound on cumulative risk		3.8×10^{-1} (mg/kg-day)$^{-1}$				
Mammary	0.1	2.3×10^{-1}	3.4×10^{-1}	1.645	6.79×10^{-2}	4.61×10^{-3}
Thyroid	0.1	3.4×10^{-2}	6.8×10^{-2}	1.645	2.06×10^{-2}	4.25×10^{-4}
CNS	0.1	5.6×10^{-2}	9.7×10^{-2}	1.645	2.52×10^{-2}	6.37×10^{-4}
Cumulative variance		5.67×10^{-3} ($\Sigma\sigma^2$)				
Cumulative SD		7.53×10^{-2} ($\sqrt{\Sigma\sigma^2}$)				
Sum of central tendency risks		3.2×10^{-1} (mg/kg-day)$^{-1}$				
Upper bound on cumulative risk		4.4×10^{-1} (mg/kg-day)$^{-1}$				
Mammary	0.1	2.3×10^{-1}	3.4×10^{-1}	1.645	6.79×10^{-2}	4.61×10^{-3}
Thyroid	0.1	3.4×10^{-2}	6.8×10^{-2}	1.645	2.06×10^{-2}	4.25×10^{-4}
CNS	0.1	5.6×10^{-2}	9.7×10^{-2}	1.645	2.52×10^{-2}	6.37×10^{-4}
Oral cavity	0.1	5.6×10^{-2}	1.0×10^{-1}	1.645	2.76×10^{-2}	7.64×10^{-4}
Cumulative variance		6.44×10^{-3} ($\Sigma\sigma^2$)				
Cumulative SD		8.02×10^{-2} ($\sqrt{\Sigma\sigma^2}$)				
Sum of central tendency risks		3.7×10^{-1} (mg/kg-day)$^{-1}$				
Upper bound on cumulative risk		5.0×10^{-1} (mg/kg-day)$^{-1}$				

[a]Derived by dividing the BMR (0.1) by the BMD_{10}.
[b]Derived by dividing the BMR (0.1) by the $BMDL_{10}$.

The POD results for separate modeling of the male tunica vaginalis, thyroid, and adrenal tumor incidence data are presented in Table D-9. In addition, rat cancer slope factors for summed risks for these tumor types were calculated, and are presented in Table D-9. Table D-10 shows the calculations for summing risks across tumor sites in the male rats.

Table D-9. Risk estimates derived from separate incidence of TVM, thyroid tumors in male F344 rats exposed to AA in drinking water

Tumor site	BMD_{10} (mg/kg-day)	$BMDL_R$ (mg/kg-day)	Rat cancer slope factor[a] (per mg/kg-day)
TVM[b]	0.27	0.16	0.61
Thyroid (adenomas and carcinomas)	2.04	1.12	0.09
Adrenal pheochromocytoma	2.55	1.08	0.09

[a]Rat cancer slope factor is the upper bound on lifetime extra risk, calculated using $R/BMDL_R$, where R = 0.1.
[b]An adequate fit could not be achieved using the full dataset, however, dropping the highest dose did produce an adequate fit to the data with the1 degree polynomial model (χ^2 goodness of fit value = 0.08).

Source: Johnson et al. (1986, 061340).

Table D-10. Calculation of summed risks for tumors at several sites in male F344 rats exposed to AA in drinking water in the Johnson et al. (1986, 061340) bioassay

Tumor site	BMR	Oral slope factor[a] (central tendency) (per mg/kg-day)	Oral slope factor[b] (upper bound) (per mg/kg-day)	t-statistic	SD	σ^2
TVM	0.1	3.7×10^{-1}	6.1×10^{-1}	1.645	1.48×10^{-1}	2.18×10^{-2}
Thyroid	0.1	4.9×10^{-2}	8.9×10^{-2}	1.645	2.45×10^{-2}	5.99×10^{-4}
Cumulative variance		2.24×10^{-2} ($\Sigma\sigma^2$)				
Cumulative SD		1.50×10^{-1} ($\sqrt{\Sigma\sigma^2}$)				
Sum of central tendency risks		4.2×10^{-1} (mg/kg-day)$^{-1}$				
Upper bound on cumulative risk		6.7×10^{-1} (mg/kg-day)$^{-1}$				
TVM	0.1	3.7×10^{-1}	6.1×10^{-1}	1.645	1.48×10^{-1}	2.18×10^{-2}
Thyroid	0.1	4.9×10^{-2}	8.9×10^{-2}	1.645	2.45×10^{-2}	5.99×10^{-4}
Adrenal	0.1	3.9×10^{-2}	9.3×10^{-1}	1.645	3.24×10^{-2}	1.05×10^{-3}
Cumulative variance		2.35×10^{-2} ($\Sigma\sigma^2$)				
Cumulative SD		1.53×10^{-1} ($\sqrt{\Sigma\sigma^2}$)				
Sum of central tendency risks		4.6×10^{-1} (mg/kg-day)$^{-1}$				
Upper bound on cumulative risk		7.1×10^{-1} (mg/kg-day)$^{-1}$				

[a]Derived by dividing the BMR (0.1) by the BMD_{10}.
[b]Derived by dividing the BMR (0.1) by the $BMDL_{10}$.

D.3. DATA PRINTOUTS FOR BMD MODELING FOR THE FRIEDMAN ET AL. (1995, 224307) TUMOR DATA SETS

D.3.1. Female Rats, Malignant and Benign Mammary Tumors, AA

DATA SOURCE: Tegeris Laboratories (1989, 224400)

```
 ======================================================================
      Multistage Model. (Version: 2.5;  Date: 10/17/2005)
      Input Data File: G:\_BMDS\PCE\AA_FRIEDMAN_F.(d)
      Gnuplot Plotting File:  G:\_BMDS\PCE\AA_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

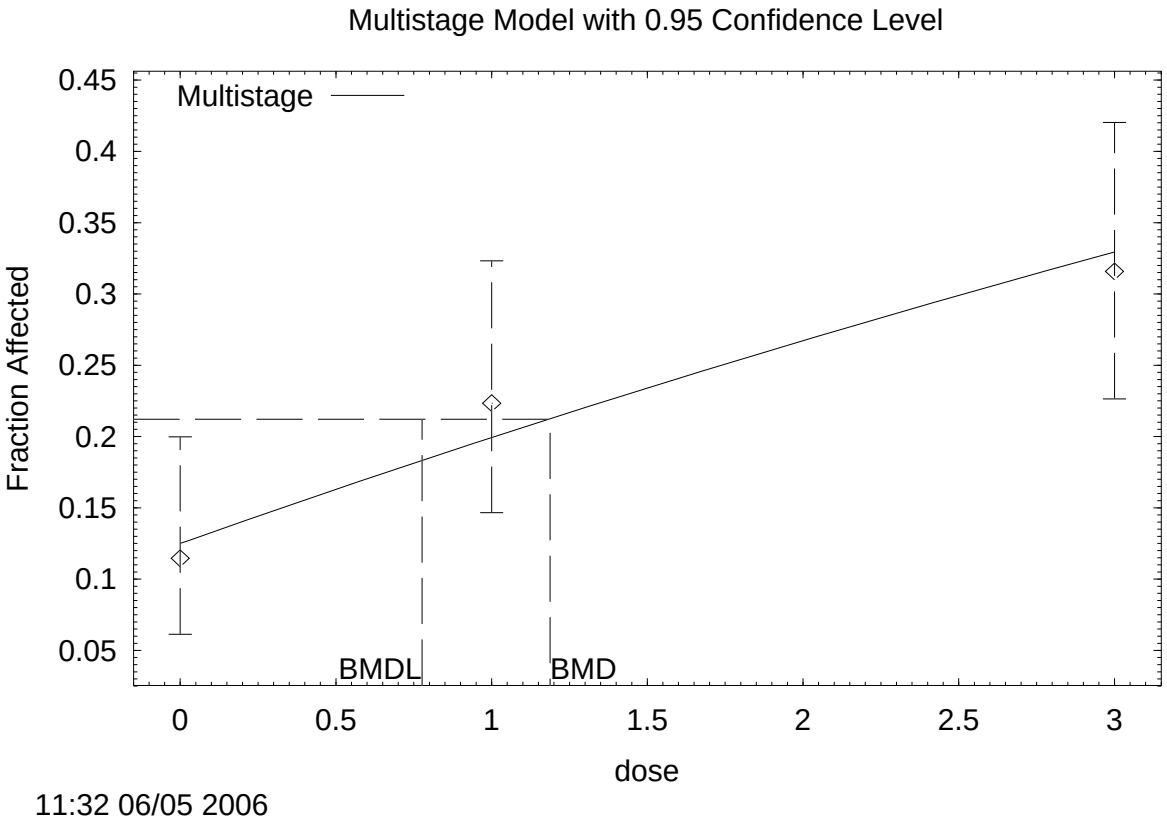
Goodness of Fit

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



Source: Friedman et al. (1995, 224307).

Figure D-1. Observed and predicted incidences for mammary gland tumors in female rats exposed to AA in drinking water for 2 years.

D.3.2. Female Rats, Thyroid Follicular Cell Adenomas or Carcinomas, AA

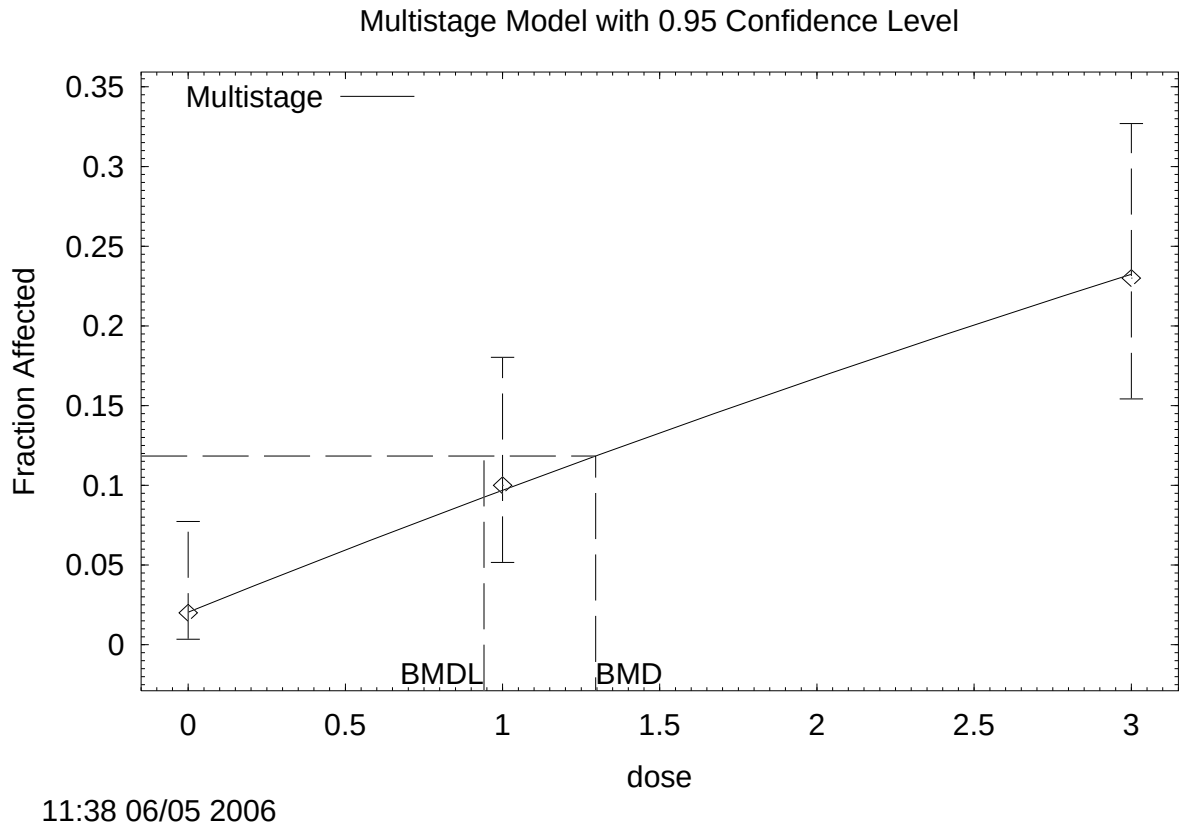
DATA SOURCE: Tegeris Laboratories (1989, 224400)

```
 ======================================================================
     Multistage Model. (Version: 2.5;  Date: 10/17/2005)
     Input Data File: G:\_BMDS\PCE\AA_FRIEDMAN_F.(d)
     Gnuplot Plotting File:  G:\_BMDS\PCE\AA_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
                                          Scaled
    Dose    Est._Prob.   Expected   Observed    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	Specified effect = 1e-005

Risk Type = Extra risk Confidence level = 0.95 BMD = 1.29585 BMDL = 0.941045	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



Source: Friedman et al. (1995, 224307).

Figure D-2. Observed and predicted incidences for thyroid tumors in female rats exposed to AA in drinking water for 2 years.

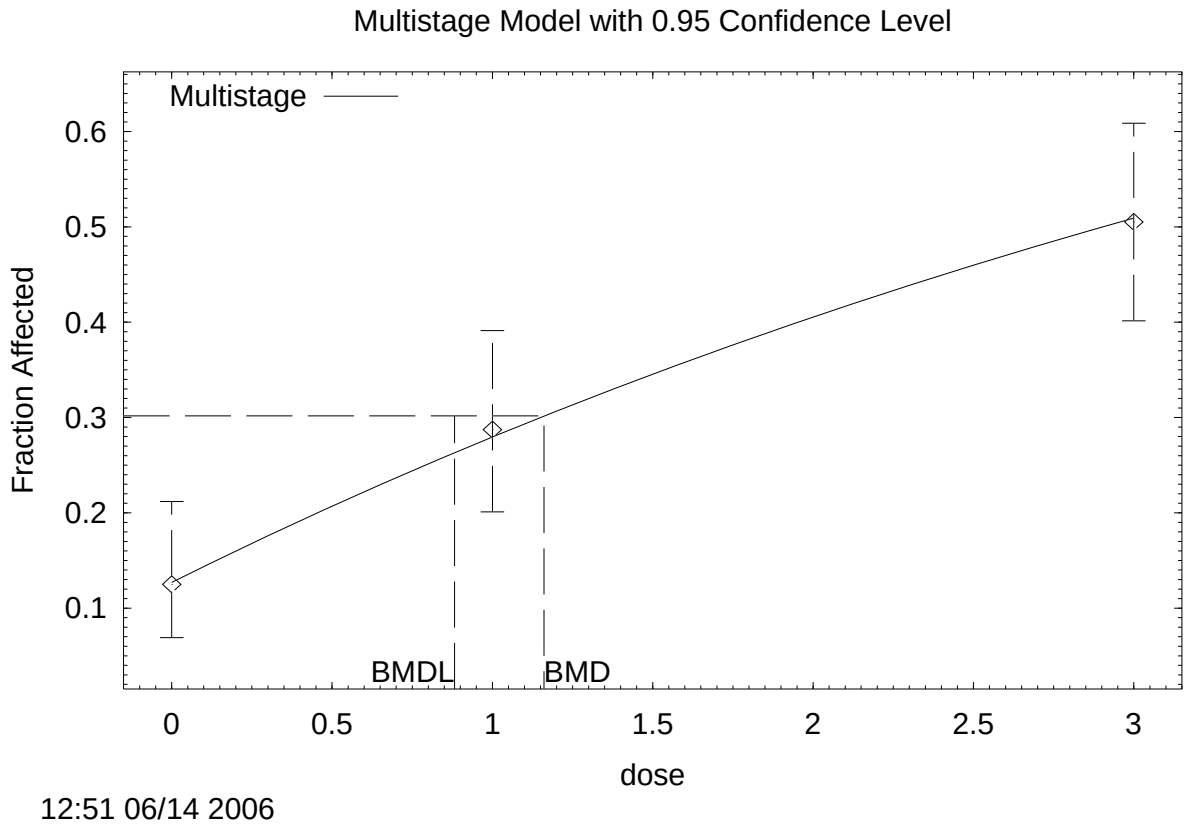
D.3.3. Female Rats, Mammary or Thyroid Follicular Cell Tumors, AA

DATA SOURCE: Tegeris Laboratories (1989, 224400)

```
 ======================================================================
     Multistage Model. (Version: 2.5;  Date: 10/17/2005)
     Input Data File: G:\_BMDS\PCE\AA_FRIEDMAN_F.(d)
     Gnuplot Plotting File:  G:\_BMDS\PCE\AA_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
                                          Scaled
    Dose    Est._Prob.   Expected   Observed    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
```



Source: Friedman et al. (1995, 224307).

Figure D-3. Observed and predicted incidences for mammary or thyroid tumors in female rats exposed to AA in drinking water for 2 years.

D.3.4. Male Rat, Tunica Vaginalis Mesothelioma, AA with Induction Time Estimated (Time Unit = Weeks)

DATA SOURCE: Tegeris Laboratories (1989, 224400)

```
 DATE: 06-07-03          TIME: 18:11:17
MULTI-WEIB (Howe and Crump, 1985, 402270)
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 RUSTON, LA  71270
(318) 255-4800

  THE  36 OBSERVATIONS AT LEVEL  1 WITH A DOSE OF  .000000

          TUMOR                  TUMOR
TIME  #         INDICATOR      TIME  #  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

****

CONFIDENCE

RISK	MLE DOSE	ON DOSE	LOWER BOUND ON RISK	UPPER BOUND ON RISK	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!

D.3.5. Male Rat, Follicular Cell Adenoma and Carcinoma, AA with No Induction Time Estimated

DATA SOURCE: Tegeris Laboratories (1983, 024400)

[NOTE FOR THE RECORD: When SRC examined the individual male rat pathology reports provided in the Tegeris Laboratories (1989, 224400) 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, 224307) 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, 224307), 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, 224400) 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 (Howe and Crump, 1985, 402270)

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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	LIMIT INTERVAL	CONFIDENCE TIME
.500000	7.190726E-02	.110089	95.0%	108.000
2.00000	.258066	.372827	95.0%	108.000

NORMAL COMPLETION!

D.3.6. Time-to-Tumor Model Results for the Combined Incidence of Thyroid Tumors or TVM in Male Rats Exposed to AA in the Drinking Water

MULTI-WEIB (Howe and Crump, 1985, 402270)
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1201 GAINES STREET
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!
```

D.4. DATA PRINTOUTS FOR BMD MODELING FOR THE JOHNSON ET AL. TUMOR DATA SETS

Tumor data sets used from Johnson et al. (1986, 061340).

D.4.1. BMD Cancer Multistage 1-Degree Polynomial Model Results for the Incidence of Mammary Tumors (Malignant and Benign) in Female F344 Rats Exposed to AA in the Drinking Water (10% extra risk)

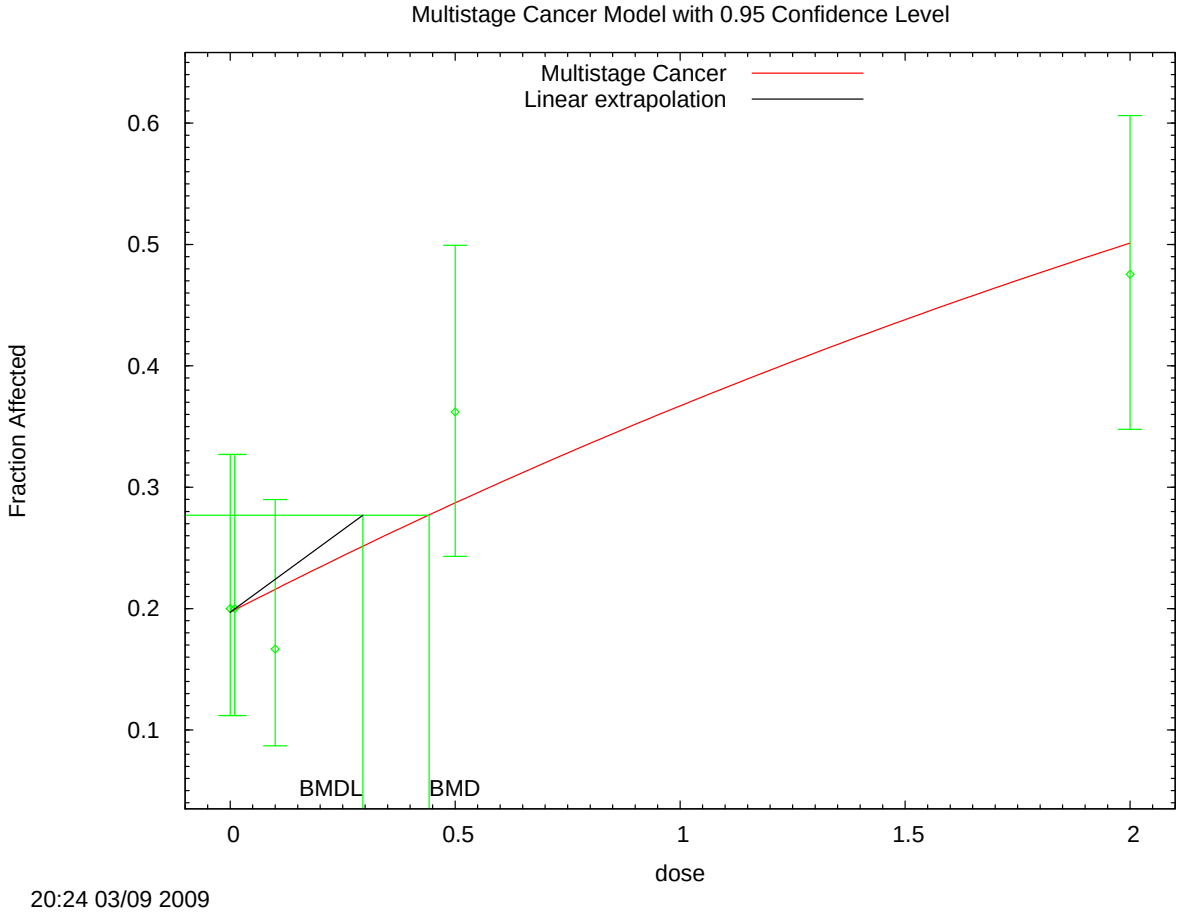


Figure D-4. BMD Cancer Multistage 1-Degree Polynomial Model Results for the Incidence of Mammary Tumors (Malignant and Benign) in Female F344 Rats Exposed to AA in the Drinking Water (10% extra risk).

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\IRIS\AA\female\mammary\1MulfemMS_.(d)
        Gnuplot Plotting File:  C:\USEPA\IRIS\AA\female\mammary\1MulfemMS_.plt
                                              Mon Mar 09 21:24:49 2009
=================================================================

BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive
```

```
  Dependent variable = incidence
  Independent variable = dose

 Total number of observations = 5
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008

****  We are sorry but Relative Function and Parameter Convergence    ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****


                  Default Initial Parameter Values
                     Background =     0.205539
                        Beta(1) =      0.21947


            Asymptotic Correlation Matrix of Parameter Estimates

              Background      Beta(1)

Background            1        -0.53

   Beta(1)        -0.53            1


                                  Parameter Estimates

                                                                  95.0% Wald Confidence
Interval
            Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper
Conf. Limit
           Background         0.196578            *               *
*
              Beta(1)         0.238242            *               *
*

       * - Indicates that this value is not calculated.


                           Analysis of Deviance Table

            Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
          Full model        -167.257         5
         Fitted model       -168.557         2       2.60026      3          0.4574
        Reduced model       -177.557         1       20.5999      4       0.0003801

                  AIC:         341.114


                                  Goodness  of  Fit
                                                                        Scaled
            Dose     Est._Prob.    Expected    Observed     Size       Residual
          ----------------------------------------------------------------------
            0.0000     0.1966        11.795    12.000          60        0.067
            0.0100     0.1985        11.909    12.000          60        0.029
```

```
    0.1000     0.2155        12.930    10.000          60       -0.920
    0.5000     0.2868        16.635    21.000          58        1.267
    2.0000     0.5011        30.567    29.000          61       -0.401

 Chi^2 = 2.62      d.f. = 3        P-value = 0.4542


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =       0.442241

            BMDL =       0.295052

            BMDU =       0.778339

Taken together, (0.295052, 0.778339) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =      0.338923
```

D.4.2. BMD Cancer Multistage 1-Degree Polynomial Model Results for the Incidence of Thyroid Follicular Cell (Adenomas and Carcinomas) in Female F344 Rats Exposed to AA in the Drinking Water (10% extra risk)

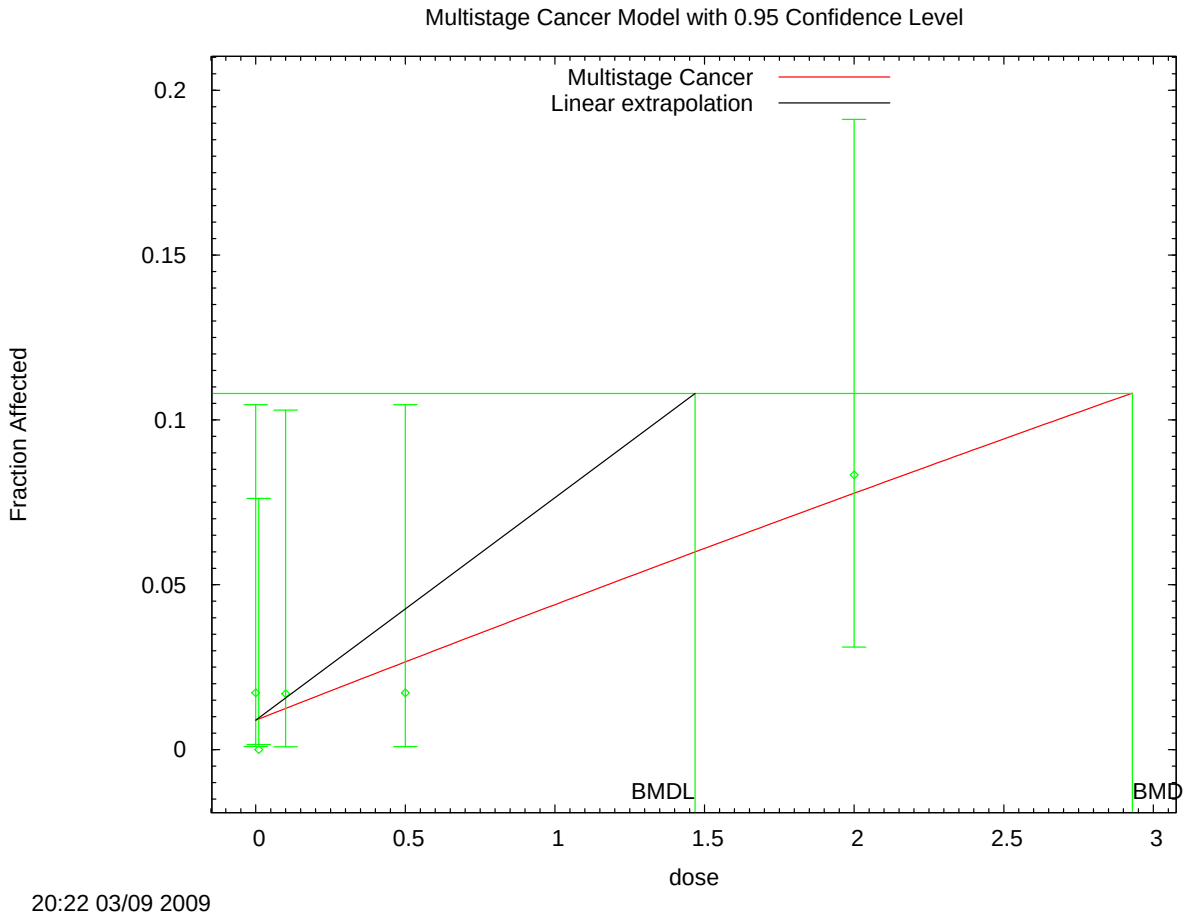


Figure D-5. BMD Cancer Multistage 1-Degree Polynomial Model Results for the incidence of thyroid follicular cell (adenomas and carcinomas) in female F344 rats exposed to AA in the drinking water (10% extra risk).

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\IRIS\AA\female\FCAC\1MulfemMS_.(d)
        Gnuplot Plotting File:  C:\USEPA\IRIS\AA\female\FCAC\1MulfemMS_.plt
                                            Mon Mar 09 21:22:28 2009
=================================================================

BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive
```

```
  Dependent variable = incidence
  Independent variable = dose

 Total number of observations = 5
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008

****  We are sorry but Relative Function and Parameter Convergence    ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****


                  Default Initial Parameter Values
                     Background =    0.0075858
                        Beta(1) =    0.0386267


            Asymptotic Correlation Matrix of Parameter Estimates

              Background      Beta(1)

Background            1        -0.54

   Beta(1)        -0.54            1


                                Parameter Estimates

                                                     95.0% Wald Confidence
Interval
         Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper
Conf. Limit
       Background       0.00893025            *               *
*
          Beta(1)        0.0359638            *               *
*

   * - Indicates that this value is not calculated.


                         Analysis of Deviance Table

          Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -32.3827         5
   Fitted model         -33.2785         2       1.79148      3          0.6168
  Reduced model         -36.7233         1       8.68109      4         0.06958

            AIC:         70.5569


                                Goodness  of  Fit
                                                                    Scaled
      Dose     Est._Prob.    Expected    Observed     Size       Residual
   ----------------------------------------------------------------------
     0.0000     0.0089         0.518     1.000          58        0.673
```

```
    0.0100     0.0093         0.548     0.000          59       -0.744
    0.1000     0.0125         0.737     1.000          59        0.309
    0.5000     0.0266         1.542     1.000          58       -0.443
    2.0000     0.0777         4.663     5.000          60        0.163

 Chi^2 = 1.32      d.f. = 3        P-value = 0.7236


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        2.92963

            BMDL =        1.46827

            BMDU =        9.25522

Taken together, (1.46827, 9.25522) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =     0.0681074
```

D.4.3. BMD Cancer Multistage 1-Degree Polynomial Model Results for the Incidence of CNS Tumors of Glial Origin in Female F344 Rats Exposed to AA in the Drinking Water (10% extra risk)

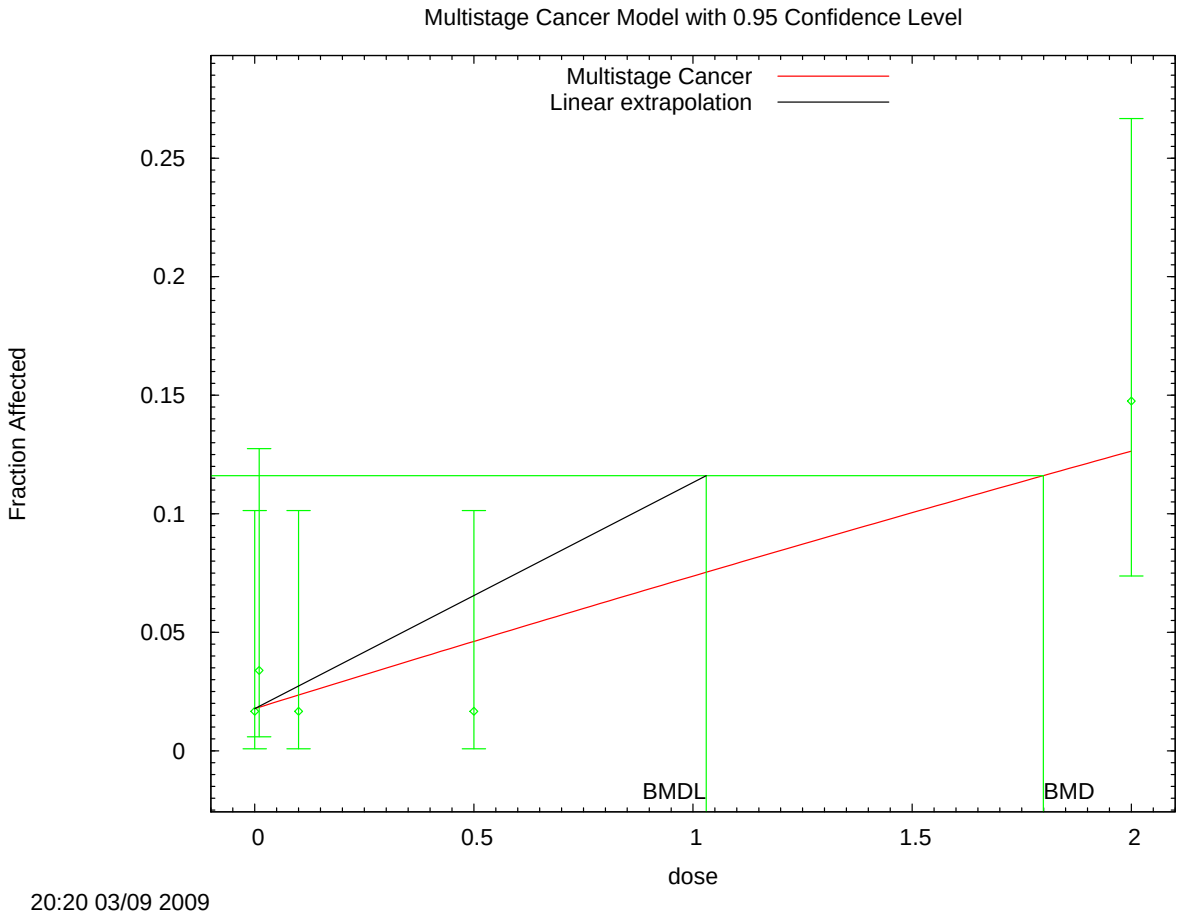


Figure D-6. BMD Cancer multistage 1-degree polynomial model results for the incidence of CNS tumors of glial origin in female F344 rats exposed to AA in the drinking water (10% extra risk).

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\IRIS\AA\female\CNS\1MulfemMS_.(d)
        Gnuplot Plotting File:  C:\USEPA\IRIS\AA\female\CNS\1MulfemMS_.plt
                                              Mon Mar 09 21:20:09 2009
=================================================================

BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive
```

```
  Dependent variable = incidence
  Independent variable = dose

 Total number of observations = 5
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008

****  We are sorry but Relative Function and Parameter Convergence    ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****


                  Default Initial Parameter Values
                     Background =    0.0124331
                        Beta(1) =     0.069725


            Asymptotic Correlation Matrix of Parameter Estimates

              Background      Beta(1)

Background            1        -0.52

   Beta(1)        -0.52            1


                                Parameter Estimates

                                                      95.0% Wald Confidence
Interval
         Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper
Conf. Limit
       Background        0.0178358            *               *
*
          Beta(1)        0.0585541            *               *
*

   * - Indicates that this value is not calculated.


                         Analysis of Deviance Table

          Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model          -49.516         5
   Fitted model         -50.7987         2       2.56534      3          0.4636
  Reduced model         -56.5743         1       14.1166      4        0.006932

            AIC:         105.597


                                Goodness  of  Fit
                                                                    Scaled
      Dose     Est._Prob.    Expected    Observed     Size       Residual
   ----------------------------------------------------------------------
     0.0000     0.0178         1.070     1.000          60       -0.068
```

0.0100	0.0184	1.086	2.000	59	0.885
0.1000	0.0236	1.414	1.000	60	-0.352
0.5000	0.0462	2.770	1.000	60	-1.089
2.0000	0.1264	7.709	9.000	61	0.497

Chi^2 = 2.35 d.f. = 3 P-value = 0.5038

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.79937

BMDL = 1.03024

BMDU = 4.00245

Taken together, (1.03024, 4.00245) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0970644

D.4.4. BMD Cancer Multistage 1-Degree Polynomial Model Results for the Incidence of Oral Cavity Tumors (Malignant and Benign) in Female F344 Rats Exposed to AA in the Drinking Water (10% extra risk)

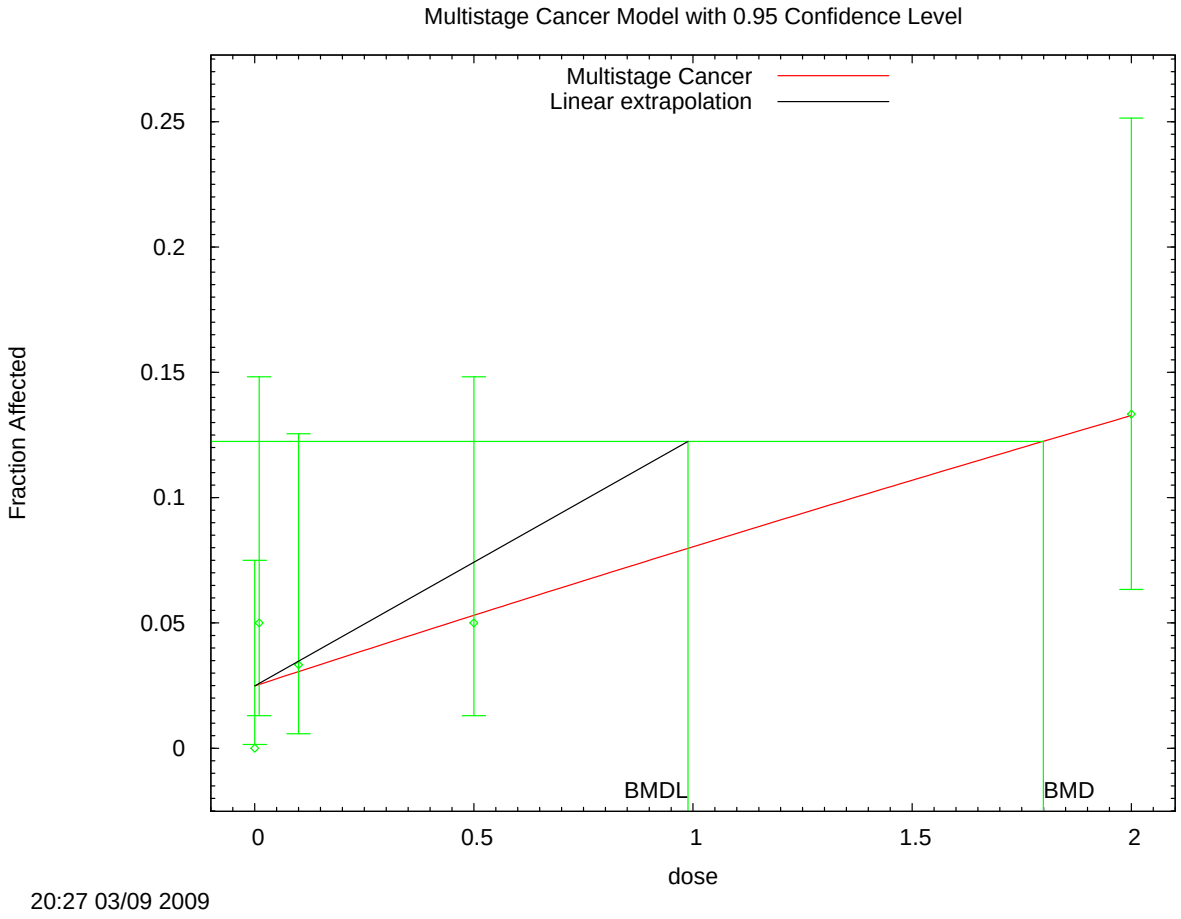


Figure D-7. BMD cancer multistage 1-degree polynomial model results for the incidence of oral cavity tumors (malignant and benign) in female F344 rats exposed to AA in the drinking water (10% extra risk).

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\IRIS\AA\female\oral\1MulfemMS_.(d)
        Gnuplot Plotting File:  C:\USEPA\IRIS\AA\female\oral\1MulfemMS_.plt
                                              Mon Mar 09 21:27:04 2009
=================================================================

BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive
```

```
  Dependent variable = incidence
  Independent variable = dose

 Total number of observations = 5
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008

****  We are sorry but Relative Function and Parameter Convergence    ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****


                  Default Initial Parameter Values
                     Background =    0.0250102
                        Beta(1) =    0.0586007


            Asymptotic Correlation Matrix of Parameter Estimates

              Background      Beta(1)

Background            1        -0.55

   Beta(1)        -0.55            1


                                  Parameter Estimates

                                                                  95.0% Wald Confidence
Interval
            Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper
Conf. Limit
          Background        0.0249031            *               *
*
             Beta(1)        0.0585561            *               *
*

       * - Indicates that this value is not calculated.


                           Analysis of Deviance Table

            Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
          Full model         -56.151         5
         Fitted model        -58.2474         2       4.19284      3          0.2414
        Reduced model        -62.4646         1       12.6273      4         0.01325

                 AIC:         120.495


                                  Goodness  of  Fit
                                                                    Scaled
          Dose     Est._Prob.    Expected    Observed     Size       Residual
        ---------------------------------------------------------------------
```

0.0000	0.0249	1.494	0.000	60	-1.238
0.0100	0.0255	1.528	3.000	60	1.206
0.1000	0.0306	1.836	2.000	60	0.123
0.5000	0.0530	3.182	3.000	60	-0.105
2.0000	0.1327	7.960	8.000	60	0.015

Chi^2 = 3.01 d.f. = 3 P-value = 0.3897

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.79931

BMDL = 0.988695

BMDU = 4.74373

Taken together, (0.988695, 4.74373) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.101143

D.4.5. BMD Cancer Multistage 1-Degree Polynomial Model Results for the Incidence of Uterus Adenocarcinomas in Female F344 Rats Exposed to AA in the Drinking Water (10% extra risk)

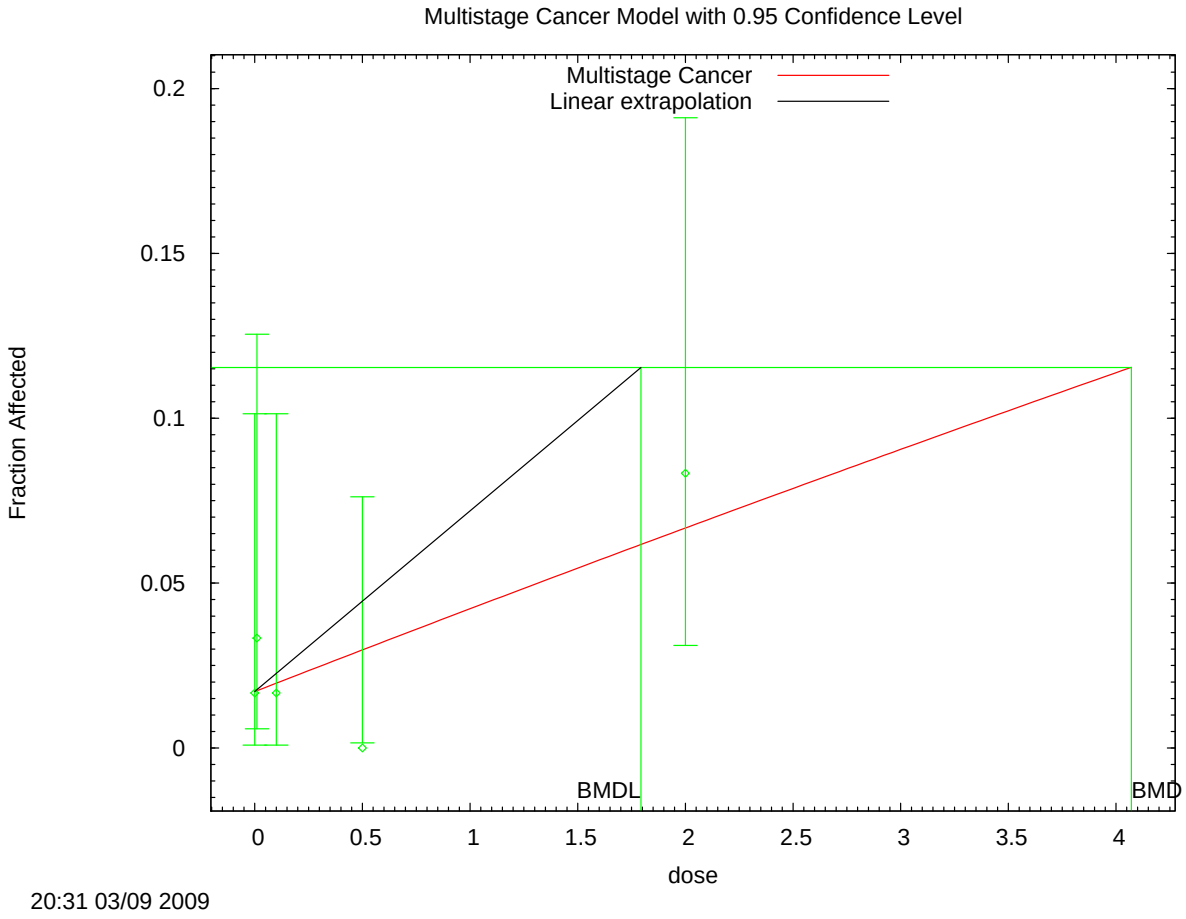


Figure D-8. BMD cancer multistage 1-degree polynomial model results for the incidence of uterus adenocarcinomas in female F344 rats exposed to AA in the drinking water (10% extra risk).

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\IRIS\AA\female\uterus\1MulfemMS_.(d)
        Gnuplot Plotting File:  C:\USEPA\IRIS\AA\female\uterus\1MulfemMS_.plt
                                              Mon Mar 09 21:31:38 2009
=================================================================

BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive
```

```
  Dependent variable = incidence
  Independent variable = dose

 Total number of observations = 5
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008

****  We are sorry but Relative Function and Parameter Convergence    ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****


                  Default Initial Parameter Values
                     Background =    0.0136297
                        Beta(1) =    0.0329157


            Asymptotic Correlation Matrix of Parameter Estimates

              Background      Beta(1)

Background            1        -0.51

   Beta(1)        -0.51            1


                                 Parameter Estimates

                                                                  95.0% Wald Confidence
Interval
            Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper
Conf. Limit
          Background        0.0171456            *               *
*
             Beta(1)        0.0258801            *               *
*

      * - Indicates that this value is not calculated.


                          Analysis of Deviance Table

           Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
        Full model        -36.1508         5
      Fitted model        -38.4257         2       4.54986      3          0.2079
     Reduced model        -40.3921         1       8.48273      4         0.07541

            AIC:         80.8514


                                 Goodness  of  Fit
                                                                      Scaled
        Dose     Est._Prob.    Expected    Observed     Size       Residual
      ---------------------------------------------------------------------
```

```
    0.0000     0.0171         1.029     1.000          60       -0.029
    0.0100     0.0174         1.044     2.000          60        0.944
    0.1000     0.0197         1.181     1.000          60       -0.168
    0.5000     0.0298         1.757     0.000          59       -1.346
    2.0000     0.0667         4.003     5.000          60        0.516

 Chi^2 = 3.00      d.f. = 3        P-value = 0.3921


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =        4.07111

             BMDL =        1.79347

             BMDU =        27.8943

Taken together, (1.79347, 27.8943) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =     0.0557578
```

D.4.6. BMD Cancer Multistage 1-Degree Polynomial Model Results for the Incidence of Clitoral Adenomas (Benign) in Female F344 Rats Exposed to AA in the Drinking Water (10% extra risk)

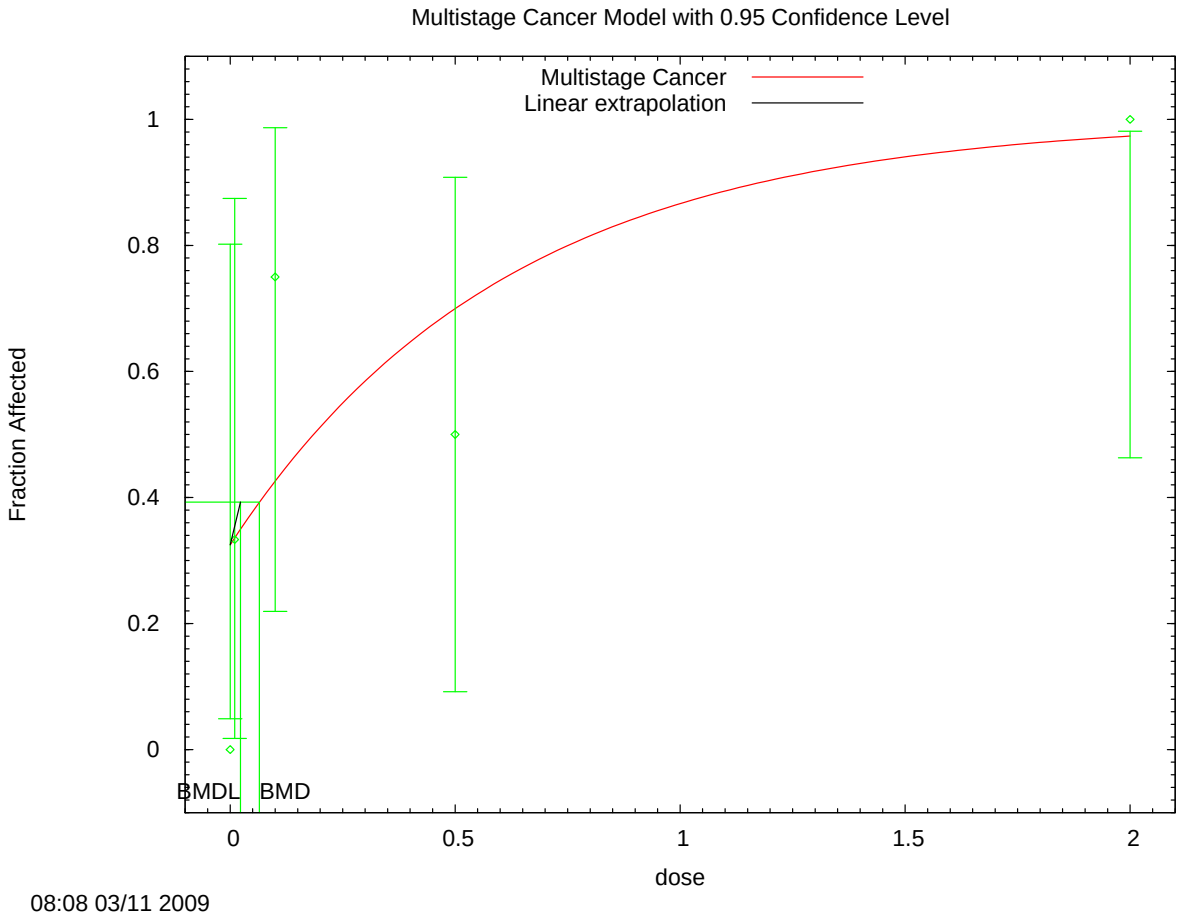


Figure D-9. BMD cancer multistage 1-degree polynomial model results for the incidence of clitoral adenomas (benign) in female F344 rats exposed to AA in the drinking water (10% extra risk).

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\IRIS\AA\female\clitoral\1MulfemMS_.(d)
        Gnuplot Plotting File:  C:\USEPA\IRIS\AA\female\clitoral\1MulfemMS_.plt
                                          Wed Mar 11 09:08:19 2009
=================================================================

BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive
```

```
  Dependent variable = incidence
  Independent variable = dose

 Total number of observations = 5
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008

****  We are sorry but Relative Function and Parameter Convergence    ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****


                  Default Initial Parameter Values
                     Background =           0
                        Beta(1) = 5.10063e+019


            Asymptotic Correlation Matrix of Parameter Estimates

              Background      Beta(1)

Background            1        -0.47

   Beta(1)        -0.47            1


                                  Parameter Estimates

                                                                    95.0% Wald Confidence
Interval
            Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper
Conf. Limit
          Background         0.325271            *               *
*
             Beta(1)          1.62417            *               *
*

       * - Indicates that this value is not calculated.


                           Analysis of Deviance Table

            Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
          Full model        -6.93147         5
         Fitted model       -9.06517         2        4.2674      3           0.234
        Reduced model       -12.0285         1        10.194      4         0.03728

                AIC:         22.1303


                                  Goodness  of  Fit
                                                                      Scaled
          Dose     Est._Prob.    Expected    Observed     Size       Residual
        ---------------------------------------------------------------------
```

0.0000	0.3253	0.651	0.000	2	-0.982
0.0100	0.3361	1.008	1.000	3	-0.010
0.1000	0.4264	1.706	3.000	4	1.309
0.5000	0.7005	2.802	2.000	4	-0.875
2.0000	0.9738	4.869	5.000	5	0.367

Chi^2 = 3.58 d.f. = 3 P-value = 0.3109

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0648702

BMDL = 0.022986

BMDU = 0.29871

Taken together, (0.022986, 0.29871) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 4.35048

D.4.7. BMD Cancer Multistage 1-Degree Polynomial Model Results for the Incidence of Pituitary Gland Adenomas in Female F344 Rats Exposed to AA in the Drinking Water (10% extra risk)

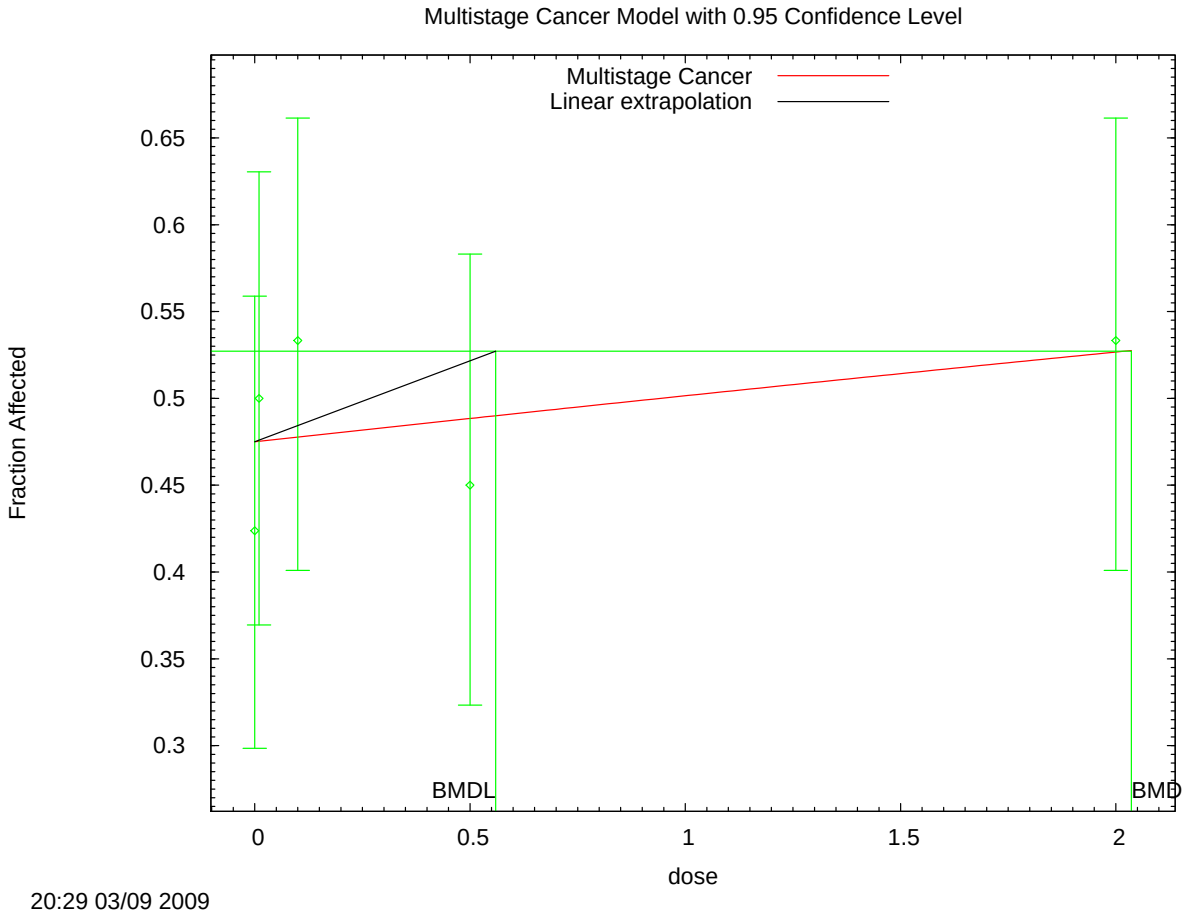


Figure D-10. BMD cancer multistage 1-degree polynomial model results for the incidence of pituitary gland adenomas in female F344 rats exposed to AA in the drinking water (10% extra risk).

```
        ================================================================
                Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
                Input Data File: C:\USEPA\IRIS\AA\female\pituitary\1MulfemMS_.(d)
                Gnuplot Plotting File:
C:\USEPA\IRIS\AA\female\pituitary\1MulfemMS_.plt
                                                    Mon Mar 09 21:29:12 2009
        ================================================================

        BMDS Model Run
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

          The form of the probability function is:

          P[response] = background + (1-background)*[1-EXP(
                       -beta1*dose^1)]
```

The parameter betas are restricted to be positive

Dependent variable = incidence
Independent variable = dose

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

**** We are sorry but Relative Function and Parameter Convergence ****
**** are currently unavailable in this model. Please keep checking ****
**** the web sight for model updates which will eventually ****
**** incorporate these convergence criterion. Default values used. ****

Default Initial Parameter Values
Background = 0.47609
Beta(1) = 0.0514418

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.55
Beta(1)	-0.55	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.474642	*	*	*
Beta(1)	0.0517505	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-205.995	5			
Fitted model	-206.935	2	1.88098	3	0.5975
Reduced model	-207.169	1	2.34909	4	0.6718

AIC: 417.87

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual

```
 ------------------------------------------------------------------------
   0.0000     0.4746        28.004    25.000          59       -0.783
   0.0100     0.4749        28.495    30.000          60        0.389
   0.1000     0.4774        28.641    32.000          60        0.868
   0.5000     0.4881        29.284    27.000          60       -0.590
   2.0000     0.5263        31.578    32.000          60        0.109

 Chi^2 = 1.88      d.f. = 3        P-value = 0.5981


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =       2.03593

            BMDL =      0.559619


BMDU did not converge for BMR = 0.100000
BMDU calculation failed
            BMDU = Inf
```

D.4.8. BMD Cancer Multistage 1-Degree Polynomial Model Results for the Incidence of Tunica Vaginalis Mesothelioma in Male F344 Rats Exposed to AA in the Drinking Water (all doses included) (10% extra risk)

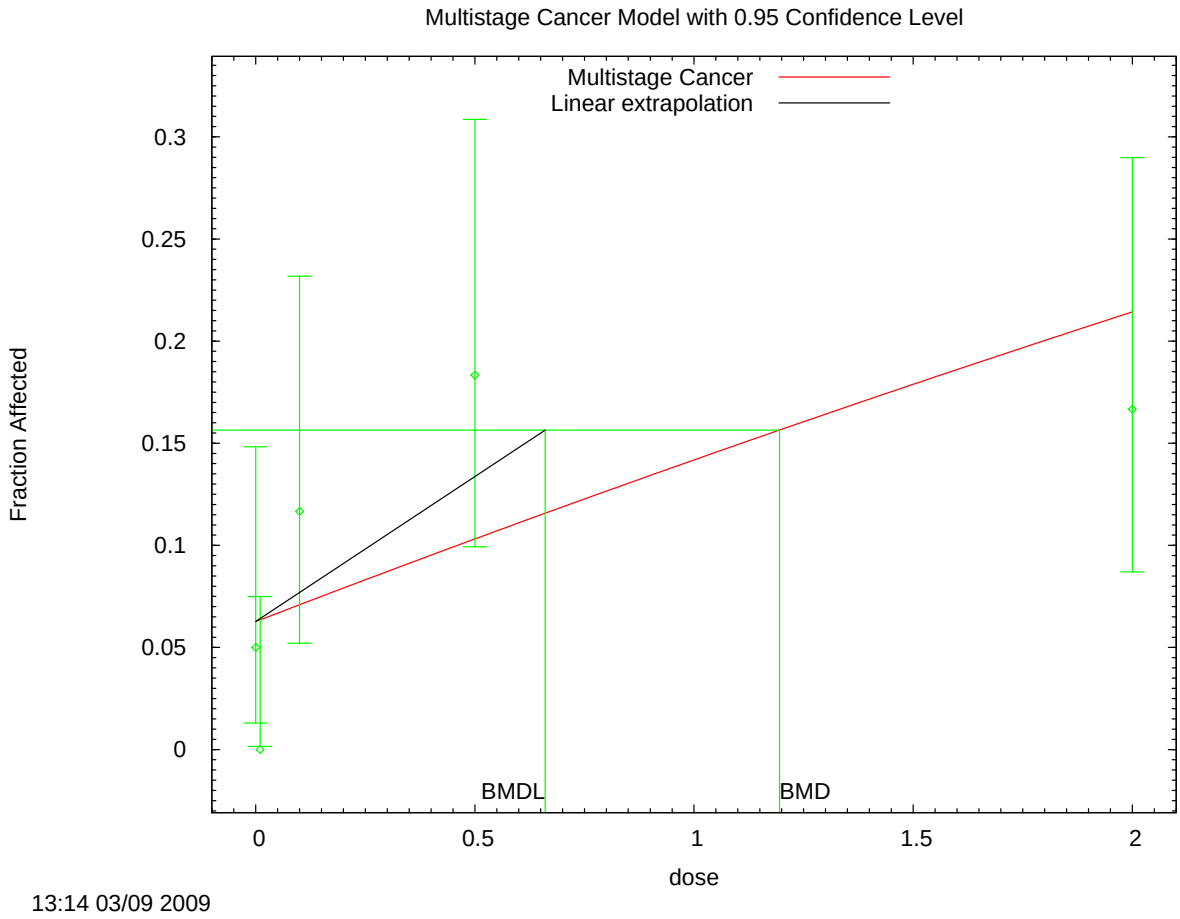


Figure D-11. BMD cancer multistage 1-degree polynomial model results for the incidence of tunica vaginalis mesothelioma in male F344 rats exposed to AA in the drinking water (all doses included) (10% extra risk).

```
        ================================================================
                Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
                Input Data File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.(d)
                Gnuplot Plotting File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.plt
                                                    Mon Mar 09 14:14:47 2009
        ================================================================

        BMDS Model Run
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

          The form of the probability function is:

          P[response] = background + (1-background)*[1-EXP(
                       -beta1*dose^1)]
```

The parameter betas are restricted to be positive

Dependent variable = incidence
Independent variable = dose

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

**** We are sorry but Relative Function and Parameter Convergence ****
**** are currently unavailable in this model. Please keep checking ****
**** the web sight for model updates which will eventually ****
**** incorporate these convergence criterion. Default values used. ****

Default Initial Parameter Values
Background = 0.0755454
Beta(1) = 0.0641515

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.61
Beta(1)	-0.61	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0626677	*	*	*
Beta(1)	0.0881625	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-89.143	5			
Fitted model	-96.1436	2	14.0011	3	0.002904
Reduced model	-99.7038	1	21.1215	4	0.0002996
AIC:	196.287				

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual

0.0000	0.0627	3.760	3.000	60	-0.405
0.0100	0.0635	3.810	0.000	60	-2.017
0.1000	0.0709	4.254	7.000	60	1.381
0.5000	0.1031	6.185	11.000	60	2.044
2.0000	0.2142	12.852	10.000	60	-0.897

Chi^2 = 11.12 d.f. = 3 P-value = 0.0111

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.19507

BMDL = 0.660363

BMDU = 3.50031

Taken together, (0.660363, 3.50031) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.151432

D.4.9. BMD Cancer Multistage 2-Degree Polynomial Model Results for the Incidence of Tunica Vaginalis Mesothelioma in Male F344 Rats Exposed to AA in the Drinking Water (all doses included) (10% extra risk)

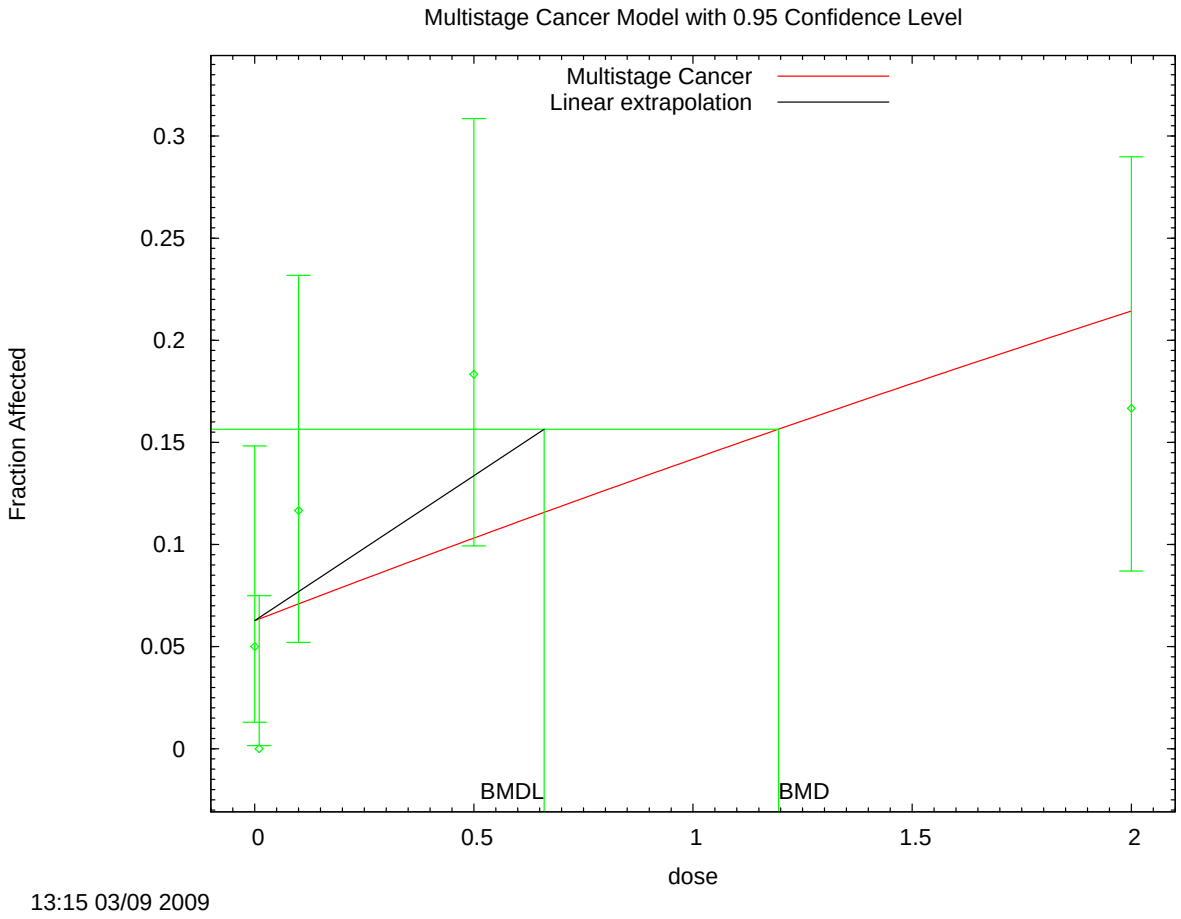


Figure D-12. BMD cancer multistage 2-degree polynomial model results for the incidence of tunica vaginalis mesothelioma in male F344 rats exposed to AA in the drinking water (all doses included) (10% extra risk).

```
        ================================================================
                Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
                Input Data File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.(d)
                Gnuplot Plotting File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.plt
                                                    Mon Mar 09 14:15:41 2009
        ================================================================

        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 = incidence
   Independent variable = dose

  Total number of observations = 5
  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: 2.22045e-016
  Parameter Convergence has been set to: 1.49012e-008

 ****  We are sorry but Relative Function and Parameter Convergence    ****
 ****  are currently unavailable in this model.  Please keep checking  ****
 ****  the web sight for model updates which will eventually           ****
 ****  incorporate these convergence criterion.  Default values used.  ****


                  Default Initial Parameter Values
                     Background =    0.0755454
                        Beta(1) =    0.0641515
                        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        -0.61

     Beta(1)        -0.61            1


                                Parameter Estimates

                                                                    95.0% Wald Confidence
Interval
          Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper
Conf. Limit
        Background        0.0626677            *               *
*
           Beta(1)        0.0881624            *               *
*
           Beta(2)                0            *               *
*

  * - Indicates that this value is not calculated.


                         Analysis of Deviance Table

            Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
        Full model         -89.143         5
      Fitted model        -96.1436         2       14.0011      3        0.002904
```

Reduced model	-99.7038	1	21.1215	4	0.0002996

AIC: 196.287

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0627	3.760	3.000	60	-0.405
0.0100	0.0635	3.810	0.000	60	-2.017
0.1000	0.0709	4.254	7.000	60	1.381
0.5000	0.1031	6.185	11.000	60	2.044
2.0000	0.2142	12.851	10.000	60	-0.897

Chi^2 = 11.12 d.f. = 3 P-value = 0.0111

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.19507

BMDL = 0.660363

BMDU = 3.50031

Taken together, (0.660363, 3.50031) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.151432

D.4.10. BMD Cancer Multistage 3-Degree Polynomial Model Results for the Incidence of Tunica Vaginalis Mesothelioma in Male F344 Rats Exposed to AA in the Drinking Water (all doses included) (10% extra risk)

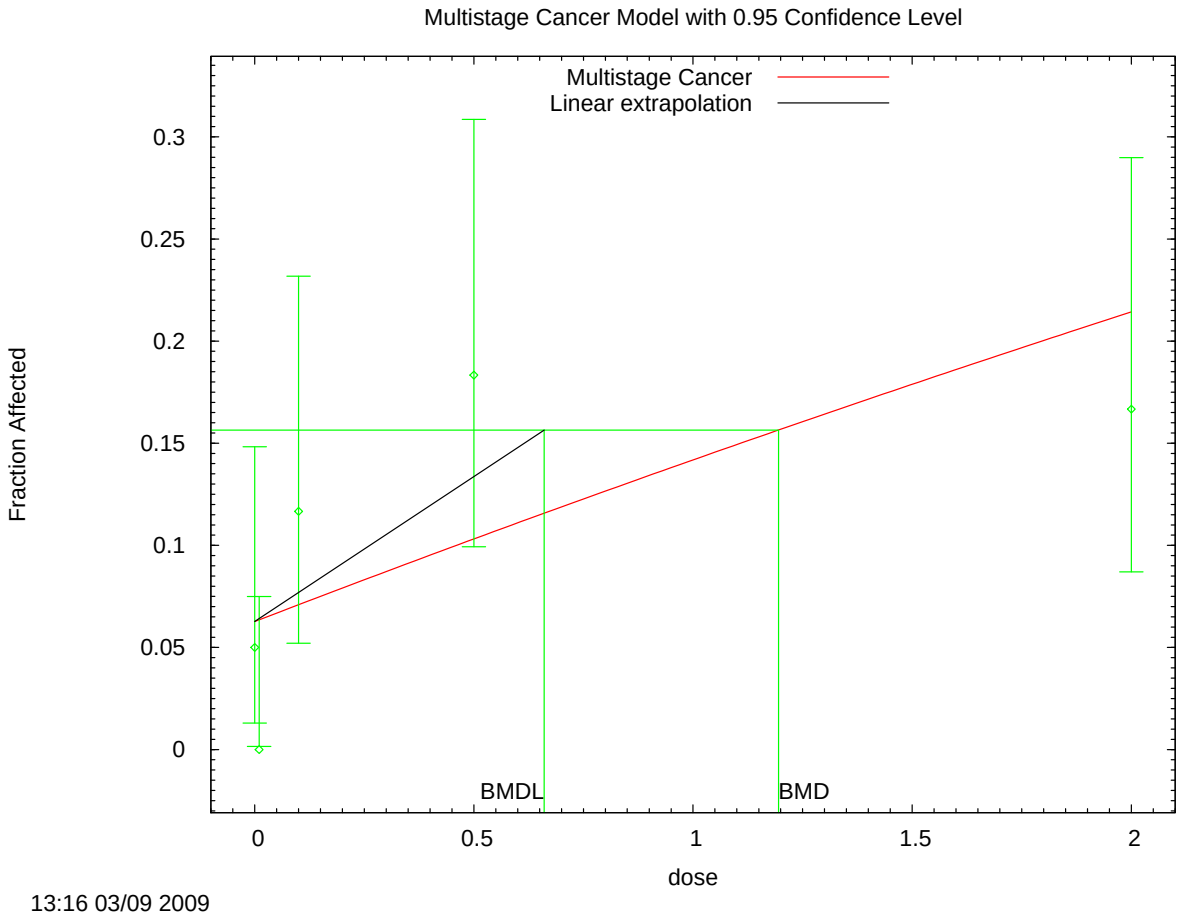


Figure D-13. BMD cancer multistage 3-degree polynomial model results for the incidence of tunica vaginalis mesothelioma in male F344 rats exposed to AA in the drinking water (all doses included) (10% extra risk).

```
        ================================================================
                Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
                Input Data File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.(d)
                Gnuplot Plotting File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.plt
                                                    Mon Mar 09 14:16:36 2009
        ================================================================

        BMDS Model Run
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

          The form of the probability function is:

          P[response] = background + (1-background)*[1-EXP(
                       -beta1*dose^1-beta2*dose^2-beta3*dose^3)]
```

```
   The parameter betas are restricted to be positive


   Dependent variable = incidence
   Independent variable = dose

  Total number of observations = 5
  Total number of records with missing values = 0
  Total number of parameters in model = 4
  Total number of specified parameters = 0
  Degree of polynomial = 3


  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 2.22045e-016
  Parameter Convergence has been set to: 1.49012e-008

 ****  We are sorry but Relative Function and Parameter Convergence    ****
 ****  are currently unavailable in this model.  Please keep checking  ****
 ****  the web sight for model updates which will eventually           ****
 ****  incorporate these convergence criterion.  Default values used.  ****



                  Default Initial Parameter Values
                     Background =    0.0755454
                        Beta(1) =    0.0641515
                        Beta(2) =            0
                        Beta(3) =            0


            Asymptotic Correlation Matrix of Parameter Estimates

            ( *** The model parameter(s)  -Beta(2)    -Beta(3)
                  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        -0.61

   Beta(1)        -0.61            1


                                Parameter Estimates

                                                                95.0% Wald Confidence
Interval
         Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper
Conf. Limit
       Background        0.0626677            *               *
*
          Beta(1)        0.0881624            *               *
*
          Beta(2)                0            *               *
*
          Beta(3)                0            *               *
*

  * - Indicates that this value is not calculated.


                          Analysis of Deviance Table
```

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-89.143	5			
Fitted model	-96.1436	2	14.0011	3	0.002904
Reduced model	-99.7038	1	21.1215	4	0.0002996

AIC: 196.287

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0627	3.760	3.000	60	-0.405
0.0100	0.0635	3.810	0.000	60	-2.017
0.1000	0.0709	4.254	7.000	60	1.381
0.5000	0.1031	6.185	11.000	60	2.044
2.0000	0.2142	12.851	10.000	60	-0.897

Chi^2 = 11.12 d.f. = 3 P-value = 0.0111

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.19507

BMDL = 0.660363

BMDU = 3.50031

Taken together, (0.660363, 3.50031) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.151432

D.4.11. BMD Cancer Multistage 4-Degree Polynomial Model Results for the Incidence of Tunica Vaginalis Mesothelioma in Male F344 Rats Exposed to AA in the Drinking Water (all doses included) (10% extra risk)

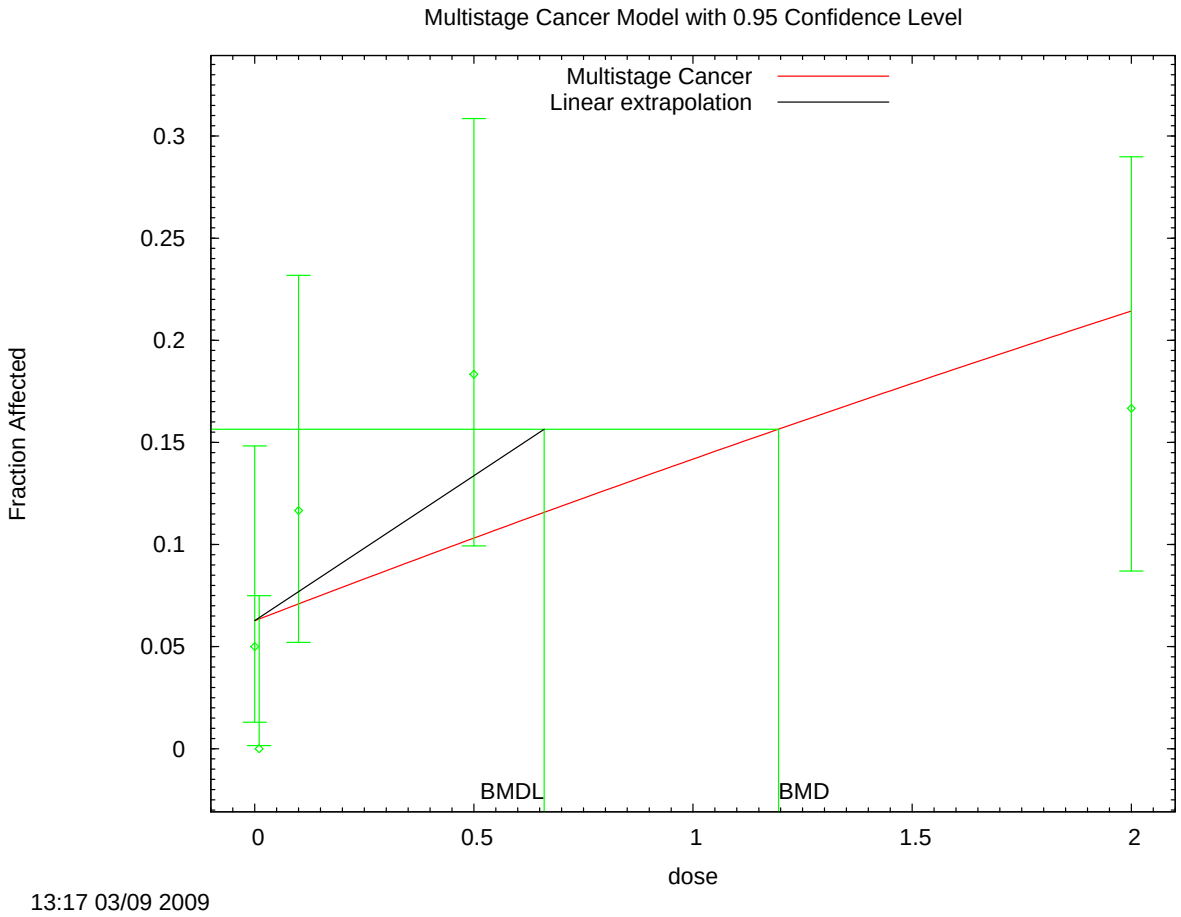


Figure D-14. BMD cancer multistage 4-degree polynomial model results for the incidence of tunica vaginalis mesothelioma in male F344 rats exposed to AA in the drinking water (all doses included) (10% extra risk).

```
        ================================================================
                Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
                Input Data File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.(d)
                Gnuplot Plotting File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.plt
                                                    Mon Mar 09 14:17:33 2009
        ================================================================

        BMDS Model Run
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

          The form of the probability function is:

          P[response] = background + (1-background)*[1-EXP(
                       -beta1*dose^1-beta2*dose^2-beta3*dose^3-beta4*dose^4)]
```

```
   The parameter betas are restricted to be positive


   Dependent variable = incidence
   Independent variable = dose

  Total number of observations = 5
  Total number of records with missing values = 0
  Total number of parameters in model = 5
  Total number of specified parameters = 0
  Degree of polynomial = 4


  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 2.22045e-016
  Parameter Convergence has been set to: 1.49012e-008

 ****  We are sorry but Relative Function and Parameter Convergence    ****
 ****  are currently unavailable in this model.  Please keep checking  ****
 ****  the web sight for model updates which will eventually           ****
 ****  incorporate these convergence criterion.  Default values used.  ****



                    Default Initial Parameter Values
                       Background =    0.0755454
                          Beta(1) =    0.0641515
                          Beta(2) =            0
                          Beta(3) =            0
                          Beta(4) =            0


              Asymptotic Correlation Matrix of Parameter Estimates

              ( *** The model parameter(s)  -Beta(2)    -Beta(3)    -Beta(4)
                    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        -0.61

     Beta(1)        -0.61            1


                                  Parameter Estimates

                                                                95.0% Wald Confidence
Interval
          Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper
Conf. Limit
        Background        0.0626677            *               *
*
           Beta(1)        0.0881624            *               *
*
           Beta(2)                0            *               *
*
           Beta(3)                0            *               *
*
           Beta(4)                0            *               *
*

  * - Indicates that this value is not calculated.
```

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-89.143	5			
Fitted model	-96.1436	2	14.0011	3	0.002904
Reduced model	-99.7038	1	21.1215	4	0.0002996

AIC: 196.287

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0627	3.760	3.000	60	-0.405
0.0100	0.0635	3.810	0.000	60	-2.017
0.1000	0.0709	4.254	7.000	60	1.381
0.5000	0.1031	6.185	11.000	60	2.044
2.0000	0.2142	12.851	10.000	60	-0.897

Chi^2 = 11.12 d.f. = 3 P-value = 0.0111

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.19507

BMDL = 0.660363

BMDU = 3.50031

Taken together, (0.660363, 3.50031) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.151432

D.4.12. BMD Cancer Multistage 1-Degree Polynomial Model Results for the Incidence of Tunica Vaginalis Mesothelioma in Male F344 Rats Exposed to AA in the Drinking Water (Highest dose dropped) (10% extra risk)

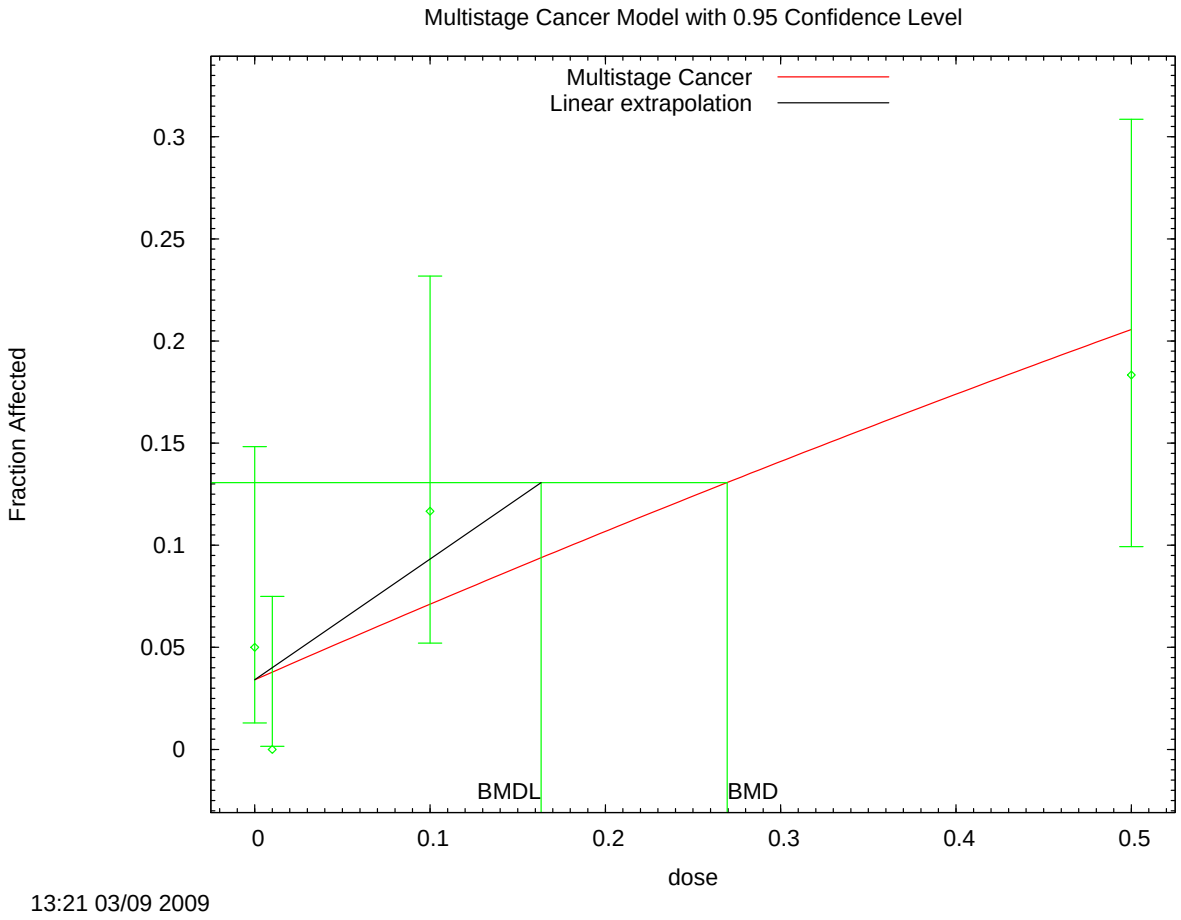


Figure D-15. BMD cancer multistage 1-degree polynomial model results for the incidence of tunica vaginalis mesothelioma in male F344 rats exposed to AA in the drinking water (Highest dose dropped) (10% extra risk).

```
        ================================================================
                Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
                Input Data File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.(d)
                Gnuplot Plotting File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.plt
                                                  Mon Mar 09 14:21:13 2009
        ================================================================

        BMDS Model Run
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

          The form of the probability function is:

          P[response] = background + (1-background)*[1-EXP(
```

```
                    -beta1*dose^1)]

          The parameter betas are restricted to be positive


          Dependent variable = incidence
          Independent variable = dose

        Total number of observations = 4
        Total number of records with missing values = 0
        Total number of parameters in model = 2
        Total number of specified parameters = 0
        Degree of polynomial = 1


        Maximum number of iterations = 250
        Relative Function Convergence has been set to: 2.22045e-016
        Parameter Convergence has been set to: 1.49012e-008

       ****  We are sorry but Relative Function and Parameter Convergence    ****
       ****  are currently unavailable in this model.  Please keep checking  ****
       ****  the web sight for model updates which will eventually           ****
       ****  incorporate these convergence criterion.  Default values used.  ****



                        Default Initial Parameter Values
                           Background =    0.0423896
                              Beta(1) =     0.335431


                  Asymptotic Correlation Matrix of Parameter Estimates

                    Background      Beta(1)

       Background            1         -0.6

          Beta(1)         -0.6            1


                                       Parameter Estimates

                                                                  95.0% Wald Confidence
Interval
             Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper
Conf. Limit
           Background        0.0340742            *               *
*
              Beta(1)         0.390914            *               *
*

       * - Indicates that this value is not calculated.


                               Analysis of Deviance Table

              Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
            Full model        -62.1094         4
           Fitted model       -65.5208         2        6.8229      2         0.03299
          Reduced model       -71.2117         1       18.2046      3       0.0003991

                    AIC:        135.042


                                       Goodness  of  Fit
```

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0341	2.044	3.000	60	0.680
0.0100	0.0378	2.271	0.000	60	-1.536
0.1000	0.0711	4.266	7.000	60	1.373
0.5000	0.2056	12.334	11.000	60	-0.426

Chi^2 = 4.89 d.f. = 2 P-value = 0.0867

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.269523

BMDL = 0.163397

BMDU = 0.578796

Taken together, (0.163397, 0.578796) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.612008

D.4.13. BMD Cancer Multistage 2-Degree Polynomial Model Results for the Incidence of Tunica Vaginalis Mesothelioma in Male F344 Rats Exposed to AA in the Drinking Water (Highest dose dropped) (10% extra risk)

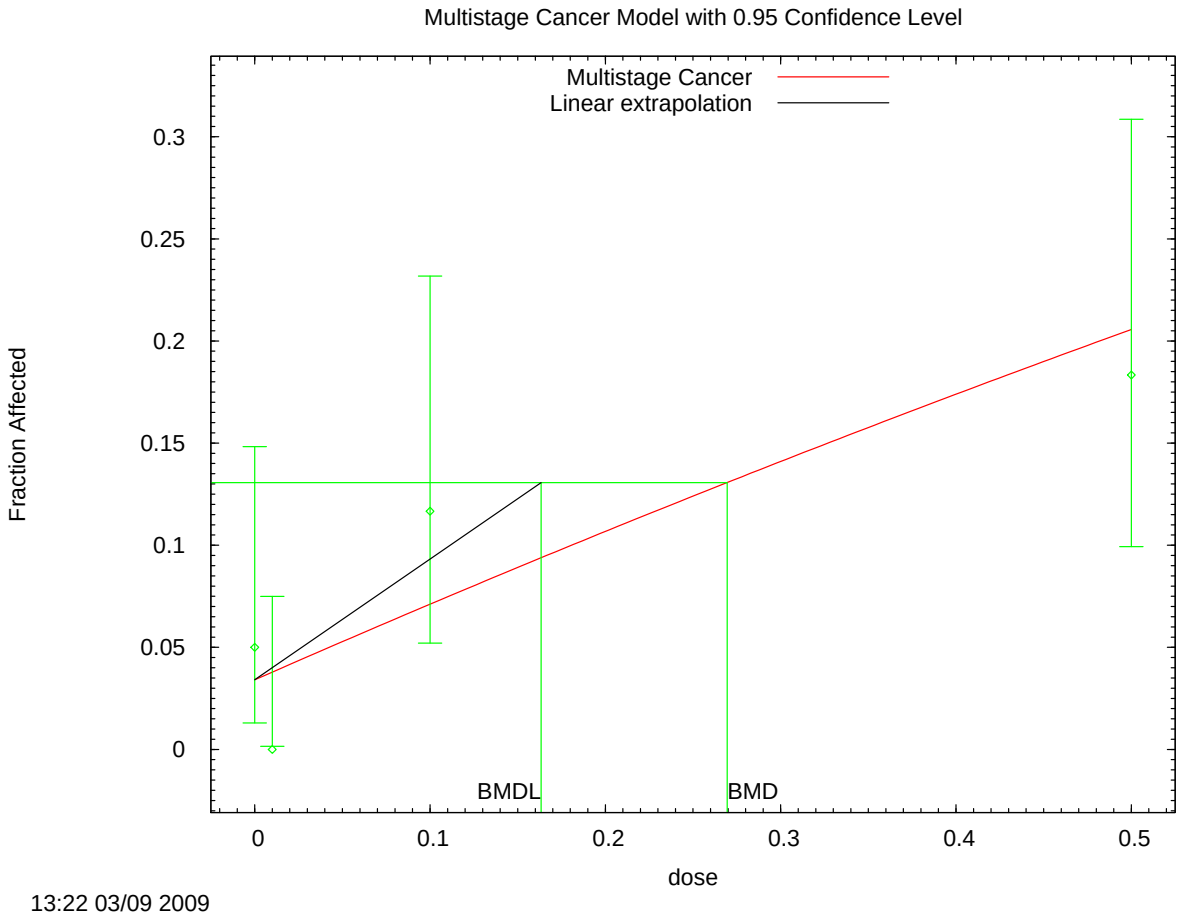


Figure D-16. BMD cancer multistage 2-degree polynomial model results for the incidence of tunica vaginalis mesothelioma in male F344 rats exposed to AA in the drinking water (Highest dose dropped) (10% extra risk).

```
        ================================================================
                Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
                Input Data File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.(d)
                Gnuplot Plotting File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.plt
                                                  Mon Mar 09 14:22:01 2009
        ================================================================

        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 = incidence
Independent variable = dose

Total number of observations = 4
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: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

**** We are sorry but Relative Function and Parameter Convergence ****
**** are currently unavailable in this model. Please keep checking ****
**** the web sight for model updates which will eventually ****
**** incorporate these convergence criterion. Default values used. ****

Default Initial Parameter Values
Background = 0.0423896
Beta(1) = 0.335431
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	-0.6
Beta(1)	-0.6	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0340742	*	*	*
Beta(1)	0.390914	*	*	*
Beta(2)	0	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-62.1094	4			

Fitted model	-65.5208	2	6.8229	2	0.03299
Reduced model	-71.2117	1	18.2046	3	0.0003991

AIC: 135.042

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0341	2.044	3.000	60	0.680
0.0100	0.0378	2.271	0.000	60	-1.536
0.1000	0.0711	4.266	7.000	60	1.373
0.5000	0.2056	12.334	11.000	60	-0.426

Chi^2 = 4.89 d.f. = 2 P-value = 0.0867

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.269523

BMDL = 0.163397

BMDU = 0.578796

Taken together, (0.163397, 0.578796) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.612008

D.4.14. BMD Cancer Multistage 3-Degree Polynomial Model Results for the Incidence of Tunica Vaginalis Mesothelioma in Male F344 Rats Exposed to AA in the Drinking Water (Highest dose dropped) (10% extra risk)

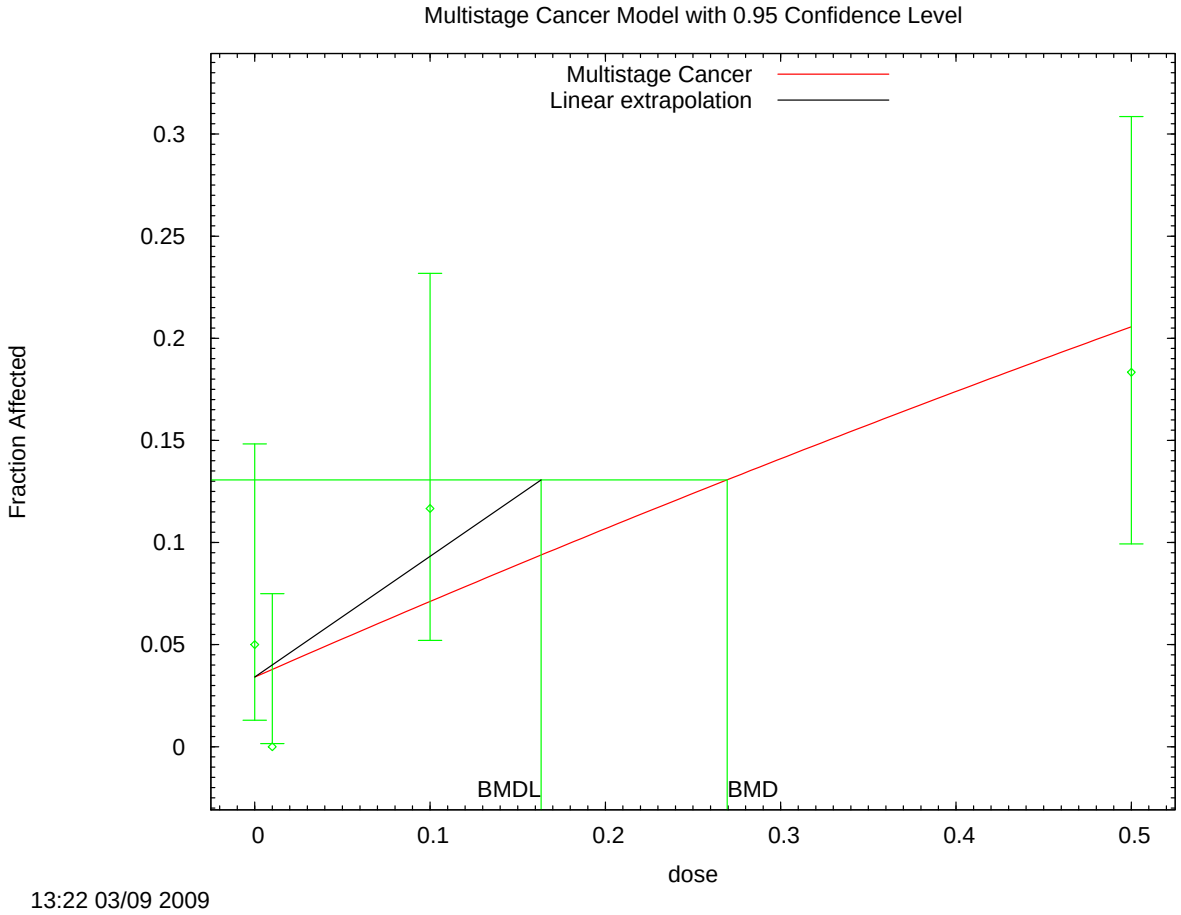


Figure D-17. BMD cancer multistage 3-degree polynomial model results for the incidence of tunica vaginalis mesothelioma in male F344 rats exposed to AA in the drinking water (Highest dose dropped) (10% extra risk).

```
        ================================================================
                Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
                Input Data File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.(d)
                Gnuplot Plotting File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.plt
                                                    Mon Mar 09 14:22:39 2009
        ================================================================

        BMDS Model Run
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

          The form of the probability function is:

          P[response] = background + (1-background)*[1-EXP(
                       -beta1*dose^1-beta2*dose^2-beta3*dose^3)]
```

The parameter betas are restricted to be positive

Dependent variable = incidence
Independent variable = dose

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 4
Total number of specified parameters = 0
Degree of polynomial = 3

Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

**** We are sorry but Relative Function and Parameter Convergence ****
**** are currently unavailable in this model. Please keep checking ****
**** the web sight for model updates which will eventually ****
**** incorporate these convergence criterion. Default values used. ****

Default Initial Parameter Values
Background = 0.0423896
Beta(1) = 0.335431
Beta(2) = 0
Beta(3) = 0

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(2) -Beta(3)
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	-0.6
Beta(1)	-0.6	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0340742	*	*	*
Beta(1)	0.390914	*	*	*
Beta(2)	0	*	*	*
Beta(3)	0	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-62.1094	4			
Fitted model	-65.5208	2	6.8229	2	0.03299
Reduced model	-71.2117	1	18.2046	3	0.0003991

AIC: 135.042

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0341	2.044	3.000	60	0.680
0.0100	0.0378	2.271	0.000	60	-1.536
0.1000	0.0711	4.266	7.000	60	1.373
0.5000	0.2056	12.334	11.000	60	-0.426

Chi^2 = 4.89 d.f. = 2 P-value = 0.0867

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.269523

BMDL = 0.163397

BMDU = 0.578796

Taken together, (0.163397, 0.578796) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.612008

D.4.15. BMD Cancer Multistage 1-Degree Polynomial Model Results for the Incidence of Tunica Vaginalis Mesothelioma in Male F344 Rats Exposed to AA in the Drinking Water (Two highest dose dropped) (10% extra risk)

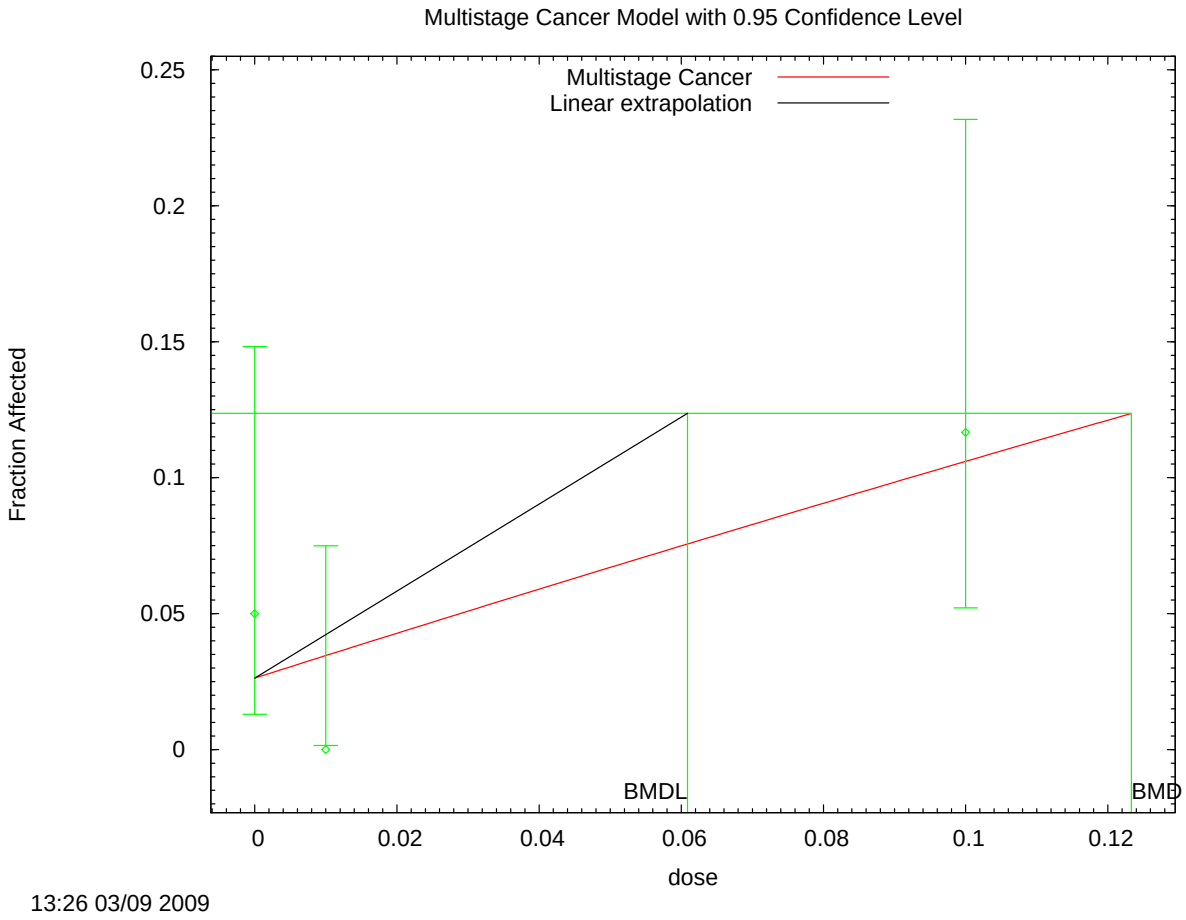


Figure D-18. BMD cancer multistage 1-degree polynomial model results for the incidence of tunica vaginalis mesothelioma in male F344 rats exposed to AA in the drinking water (Two highest dose dropped) (10% extra risk).

```
        ================================================================
                Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
                Input Data File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.(d)
                Gnuplot Plotting File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.plt
                                                  Mon Mar 09 14:26:35 2009
        ================================================================

        BMDS Model Run
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

          The form of the probability function is:

          P[response] = background + (1-background)*[1-EXP(
```

```
                    -beta1*dose^1)]

          The parameter betas are restricted to be positive


          Dependent variable = incidence
          Independent variable = dose

        Total number of observations = 3
        Total number of records with missing values = 0
        Total number of parameters in model = 2
        Total number of specified parameters = 0
        Degree of polynomial = 1


        Maximum number of iterations = 250
        Relative Function Convergence has been set to: 2.22045e-016
        Parameter Convergence has been set to: 1.49012e-008

       ****  We are sorry but Relative Function and Parameter Convergence    ****
       ****  are currently unavailable in this model.  Please keep checking  ****
       ****  the web sight for model updates which will eventually           ****
       ****  incorporate these convergence criterion.  Default values used.  ****



                        Default Initial Parameter Values
                           Background =     0.022083
                              Beta(1) =     0.985041


                  Asymptotic Correlation Matrix of Parameter Estimates

                    Background      Beta(1)

       Background            1        -0.59

          Beta(1)        -0.59            1


                                       Parameter Estimates

                                                                    95.0% Wald Confidence
Interval
             Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper
Conf. Limit
           Background        0.0263158            *               *
*
              Beta(1)         0.854491            *               *
*

       * - Indicates that this value is not calculated.


                               Analysis of Deviance Table

              Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
            Full model        -33.5247         3
           Fitted model       -36.1941         2       5.33864      1         0.02086
          Reduced model       -38.6206         1       10.1918      2        0.006122

                    AIC:         76.3881


                                       Goodness  of  Fit
```

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0263	1.579	3.000	60	1.146
0.0100	0.0346	2.076	0.000	60	-1.466
0.1000	0.1061	6.364	7.000	60	0.267

Chi^2 = 3.54 d.f. = 1 P-value = 0.0601

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.123302

BMDL = 0.0608676

BMDU = 0.519103

Taken together, (0.0608676, 0.519103) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 1.64291

D.4.16. BMD Cancer Multistage 2-Degree Polynomial Model Results for the Incidence of Tunica Vaginalis Mesothelioma in Male F344 Rats Exposed to AA in the Drinking Water (Two highest dose dropped) (10% extra risk)

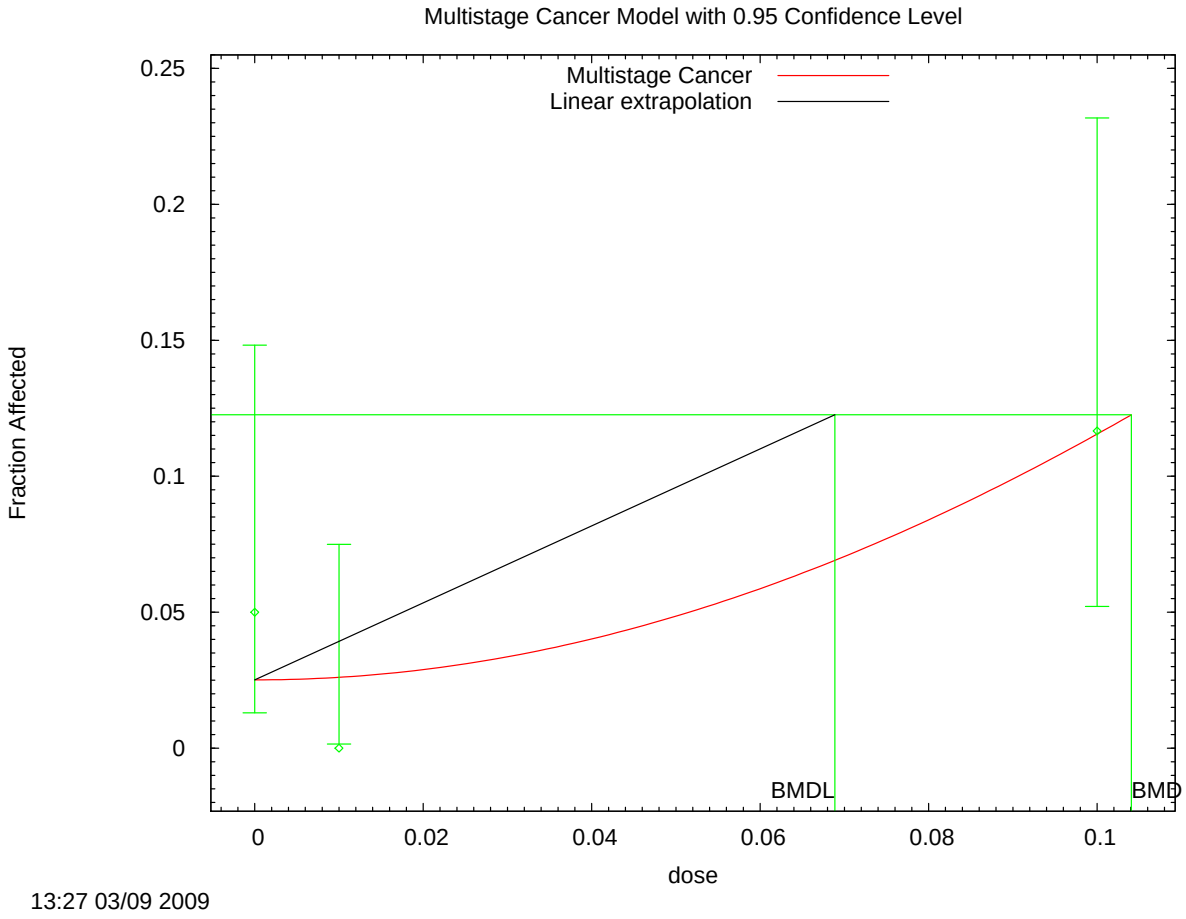


Figure D-19. BMD cancer multistage 2-degree polynomial model results for the incidence of tunica vaginalis mesothelioma in male F344 rats exposed to AA in the drinking water (Two highest dose dropped) (10% extra risk).

```
        ================================================================
                Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
                Input Data File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.(d)
                Gnuplot Plotting File:
C:\USEPA\IRIS\AA\males\tunicavaginalismesothelioma\1MulmalMS_.plt
                                                  Mon Mar 09 14:27:23 2009
        ================================================================

        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 = incidence
          Independent variable = dose

        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: 2.22045e-016
        Parameter Convergence has been set to: 1.49012e-008

       ****  We are sorry but Relative Function and Parameter Convergence    ****
       ****  are currently unavailable in this model.  Please keep checking  ****
       ****  the web sight for model updates which will eventually           ****
       ****  incorporate these convergence criterion.  Default values used.  ****



                           Default Initial Parameter Values
                              Background =    0.0249685
                                 Beta(1) =            0
                                 Beta(2) =      9.85045


                     Asymptotic Correlation Matrix of Parameter Estimates

                     ( *** The model parameter(s)  -Beta(1)
                           have been estimated at a boundary point, or have been
specified by the user,
                           and do not appear in the correlation matrix )

                       Background      Beta(2)

       Background            1        -0.56

          Beta(2)        -0.56            1


                                       Parameter Estimates

                                                                    95.0% Wald Confidence
Interval
               Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper
Conf. Limit
            Background        0.0251256            *               *
*
               Beta(1)                0            *               *
*
               Beta(2)          9.72992            *               *
*

       * - Indicates that this value is not calculated.


                                Analysis of Deviance Table
```

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-33.5247	3			
Fitted model	-35.7015	2	4.35343	1	0.03693
Reduced model	-38.6206	1	10.1918	2	0.006122

AIC: 75.4029

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0251	1.508	3.000	60	1.231
0.0100	0.0261	1.564	0.000	60	-1.267
0.1000	0.1155	6.931	7.000	60	0.028

Chi^2 = 3.12 d.f. = 1 P-value = 0.0772

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.10406

BMDL = 0.0688637

BMDU = 0.324378

Taken together, (0.0688637, 0.324378) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 1.45214

D.4.17. BMD Cancer Multistage 1-Degree Polynomial Model Results for the Incidence of Thyroid Follicular Cell Adenoma in Male F344 Rats Exposed to AA in the Drinking Water (10% extra risk)

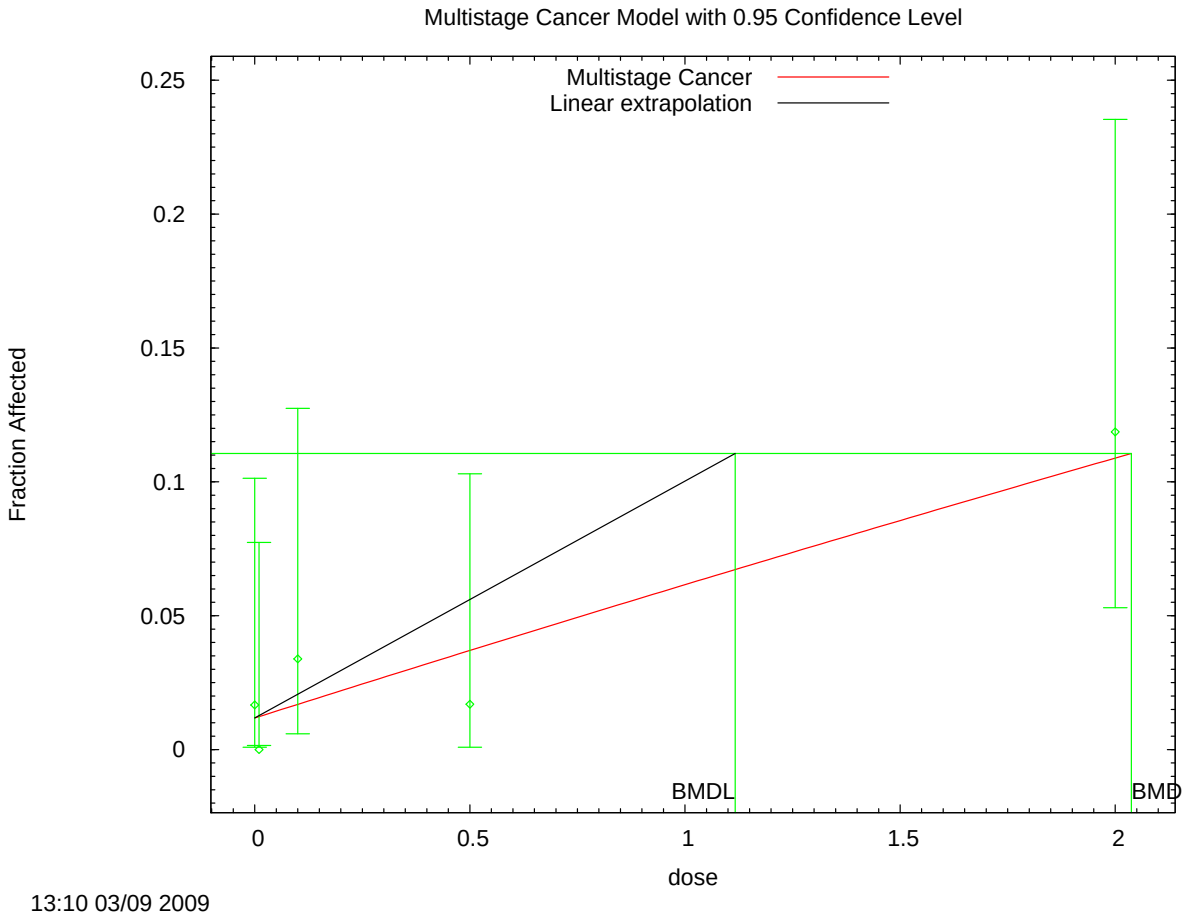


Figure D-20. BMD cancer multistage 1-degree polynomial model results for the incidence of thyroid follicular cell adenoma in male F344 rats exposed to AA in the drinking water (10% extra risk).

```
        ================================================================
                Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
                Input Data File:
C:\USEPA\IRIS\AA\males\follicularcelladenoma\1MulmalMS_.(d)
                Gnuplot Plotting File:
C:\USEPA\IRIS\AA\males\follicularcelladenoma\1MulmalMS_.plt
                                                    Mon Mar 09 14:10:33 2009
        ================================================================

        BMDS Model Run
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

          The form of the probability function is:

          P[response] = background + (1-background)*[1-EXP(
                       -beta1*dose^1)]
```

The parameter betas are restricted to be positive

Dependent variable = incidence
Independent variable = dose

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

**** We are sorry but Relative Function and Parameter Convergence ****
**** are currently unavailable in this model. Please keep checking ****
**** the web sight for model updates which will eventually ****
**** incorporate these convergence criterion. Default values used. ****

Default Initial Parameter Values
Background = 0.00953434
Beta(1) = 0.0562379

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.54
Beta(1)	-0.54	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0117608	*	*	*
Beta(1)	0.0517152	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-40.3781	5			
Fitted model	-41.9922	2	3.22812	3	0.3578
Reduced model	-46.9722	1	13.1881	4	0.01039

AIC: 87.9844

Goodness of Fit

Scaled

Dose	Est._Prob.	Expected	Observed	Size	Residual
0.0000	0.0118	0.706	1.000	60	0.352
0.0100	0.0123	0.712	0.000	58	-0.849
0.1000	0.0169	0.995	2.000	59	1.017
0.5000	0.0370	2.182	1.000	59	-0.816
2.0000	0.1089	6.423	7.000	59	0.241

Chi^2 = 2.60 d.f. = 3 P-value = 0.4572

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.03732

BMDL = 1.11679

BMDU = 4.98748

Taken together, (1.11679, 4.98748) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0895426

D.4.18. BMD Cancer Multistage 1-Degree Polynomial Model Results for the Incidence of Adrenal Pheochromocytoma in Male F344 Rats Exposed to AA in the Drinking Water (10% extra risk)

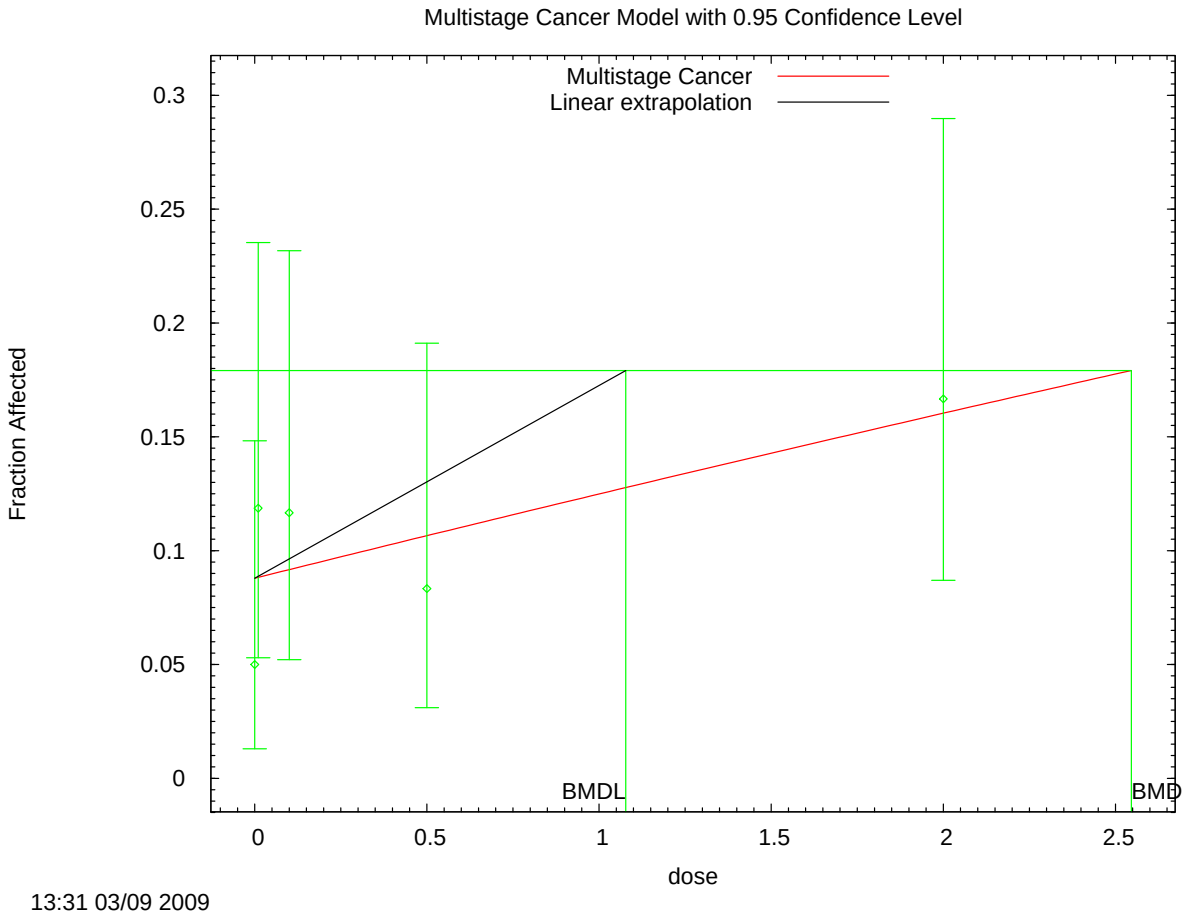


Figure D-21. BMD cancer multistage 1-degree polynomial model results for the incidence of adrenal pheochromocytoma in male F344 rats exposed to AA in the drinking water (10% extra risk).

```
        ======================================================================
                Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
                Input Data File:
C:\USEPA\IRIS\AA\males\adrenalpheochromocytoma\1MulmalMS_.(d)
                Gnuplot Plotting File:
C:\USEPA\IRIS\AA\males\adrenalpheochromocytoma\1MulmalMS_.plt
                                                    Mon Mar 09 14:31:23 2009
        ======================================================================

        BMDS Model Run
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

          The form of the probability function is:

          P[response] = background + (1-background)*[1-EXP(
                       -beta1*dose^1)]
```

The parameter betas are restricted to be positive

Dependent variable = incidence
Independent variable = dose

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

**** We are sorry but Relative Function and Parameter Convergence ****
**** are currently unavailable in this model. Please keep checking ****
**** the web sight for model updates which will eventually ****
**** incorporate these convergence criterion. Default values used. ****

Default Initial Parameter Values
Background = 0.087802
Beta(1) = 0.0427132

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.55
Beta(1)	-0.55	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0879228	*	*	*
Beta(1)	0.0413823	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-99.2572	5			
Fitted model	-100.594	2	2.67284	3	0.4449
Reduced model	-101.734	1	4.95289	4	0.2922

AIC: 205.187

Goodness of Fit

Scaled

Dose	Est._Prob.	Expected	Observed	Size	Residual
0.0000	0.0879	5.275	3.000	60	-1.037
0.0100	0.0883	5.210	7.000	59	0.821
0.1000	0.0917	5.501	7.000	60	0.670
0.5000	0.1066	6.396	5.000	60	-0.584
2.0000	0.1604	9.622	10.000	60	0.133

Chi^2 = 2.56 d.f. = 3 P-value = 0.4647

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.54603

BMDL = 1.0776

BMDU did not converge for BMR = 0.100000
BMDU calculation failed
BMDU = 1.57654e+068

APPENDIX E. DERIVATION OF IN VIVO SECOND ORDER RATE CONSTANTS AND THE ADDUCT FORMATION SIMULATION MODEL

E.1. BACKGROUND

Two methods have been used to determine the value of blood AUC s for AA and GA following administration: (1) calculation directly from measured blood AA and GA time course data; or (2) estimation based on concentrations of AAVal or GAVal Hb adducts measured in red blood cells. As an example, blood AA and GA-AUCs in humans have been estimated by dividing the measured adduct levels by a second order rate constant for the formation of the adduct (Fennell et al., (2005, 224299):

$$Dose = \frac{1}{k} x \frac{RHb}{Hb} \qquad \text{Equation E-1}$$

where k is the second order Hb adduct formation rate constant, expressed in units of L/g globin/hour, [RHb] is the adduct concentration, and [Hb] the concentration of Hb.

This second order Hb adduct formation rate constant has typically been derived in vitro using red blood cell Hb rather than whole blood (Fennell et al., 2005, 224299; Tareke et al., 2006, 224387; Törnqvist et al., 2008, 224428). The accuracy of AUCs calculated using the in vitro derived rate constant depends directly upon the accuracy of this estimate for the actual formation rates in vivo. The results are also sensitive to processes involved in the "loss or elimination" of adducts over time—red blood cell turnover, chemical loss of adduct, and body weight dependent increases in blood volume. Equations to account for these losses in adducts over time are available (Fennell et al., 2005, 224299; Walker et al., 2007, 224527); however, for exposures of 1 day or less, the influence of these processes on adduct levels and AUCs estimated from them can be ignored (Fennell et al., 2005, 224299; Walker et al., 2007, 224527).

Until recently there were insufficient in vivo data to derive either animal or human in vivo Hb adduct formation or elimination rates. Human data remains unavailable at this time, however, Tareke et al. (2006, 224387) reported AAVal and GAVal adduct levels in rats and mice exposed to single doses of AA or GA as well as serum level time course data (Doerge et al., 2005, 224348; Doerge et al., 2005, 224355). Tareke et al. (2006, 224387) also measured AAVal and GAVal adduct levels in rats exposed to AA in drinking water for up to 49 days, as well as the loss rate of both AA and GA adducts for up to 63 days (on control water) after a 21-day AA in drinking-water exposure. Together, these data are sufficient to derive in vivo adduct

formation and loss rates for the AAVal and GAVal adducts for use in estimating the blood AA and GA-AUCs from a drinking water exposure based on Hb adduct levels.

E.2. DEVELOPMENT OF IN VIVO ADDUCT FORMATION RATE CONSTANTS

The derivation of in vivo adduct formation rates requires three critical types of data from a single study: (1) the administered dose; (2) time course serum levels; and (3) time course adduct levels (including sufficient post dosing sample times to determine elimination rates) if longer than one day of exposure.

E.2.1. In Vivo Adduct Formation Rate Constants for Rats

The only studies meeting the above data requirement to derive rat in vivo rate constants are those from Doerge et al. (2005, 224355) and Tareke et al. (2006, 224387). Table E-1 lists the raw serum AUCs that were available in numerical tables from Doerge et al. (2005, 224355) and the levels of Hb that were taken from bar chart compilations in Tareke et al. (2006, 224387).

Table E-1. Serum AUC data from Doerge et al. (2005, 224355) (Tables 1 and 3) and Hb adduct level data from Tareke et al. (2006, 224387) (Figure 3) for a 0.1 mg/kg single dose of AA in male and female F344 rats.

			Data extracted from Tareke et al. (2006, 224387) (Figure 3)		Data from Doerge et al. (2005, 224355) (Tables 1 and 3)	
Type of adduct	Dosed compound	sex-route	Hb adduct level (pmole/g globin)	(SD)[1]	AUC (μM-hr)	(SD)[2]
AAVal	AA	M-control	9	4	0	0
AAVal	AA	M-Diet	19.5	4	1.8	0.23
AAVal	AA	M-gavage	20	6	2.4	0.51
AAVal	AA	M-IV	46.5	4	4.1	0
AAVal	AA	F-control	12	4	0	0
AAVal	AA	F-Diet	23	4	1.5	0.15
AAVal	AA	F-gavage	29	14	4.5	0.31
AAVal	AA	F-IV	49.5	4	4.6	0
GAVal	AA	M-control	32.5	12	0	0
GAVal	AA	M-IV	36	4	0.58	0
GAVal	AA	M-gavage	64	20	1.3	0.2
GAVal	AA	M-Diet	98.5	20	1.9	0.35
GAVal	GA	M-IV	140	26	2.8	0
GAVal	GA	M-gavage	123	16	2.8	0.46
GAVal	AA	F-control	45	16	0	0
GAVal	AA	F-IV	48.5	16	0.6	0
GAVal	AA	F-Diet	102	18	1.5	0.2
GAVal	AA	F-gavage	131	66	4.4	0.46
GAVal	GA	F-IV	177	28	3.3	0
GAVal	GA	F-gavage	198	40	3.8	0.5

[a]SD is estimated from error bars in Tareke et al. (2006, 224387) (Figure 3).
[b] No standard deviations were reported for the IV AUC measurements.

A regression of the Hb adduct levels against the AUCs resulted in a slope that represents the in vivo second order adduct formation rate constant for AAVal or GAVal in units of pmoles/g globin per μM AA-hr or μM GA-hr, respectively. These units can be converted to (L/g globin/hr)$\times 10^{-6}$ with the values of the slopes listed in Table E-2. For comparison, a compilation

of in vitro and in vivo adduct formation rate constants is presented in Table 5-5 in the main body of the Toxicological Review.

Table E-2. Regression of Hb adduct levels to AUC to derive in vivo second-order rate constants for adduct formation (i.e., the slope of the regression line)

Gender	AAVal Slope (SE) [L/g globin/h]×10^{-6}	GAVal Slope (SE) [L/g globin/h]×10^{-6}
Male	8.9 (2.2)	38.4 (3.9)
Female	5.9 (2.5)	30.6 (8.7)
Both	7.0 (1.5)	33.8 (4.9)

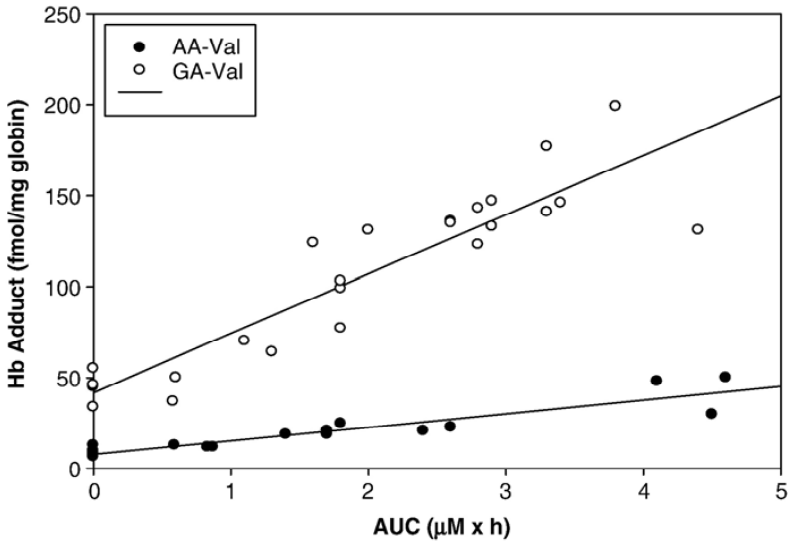
The second order AAVal adduct formation rate based on in vivo data for male, female, or both genders combined are 8.9, 5.9, and 7.0 (L/g globin/hr)×10^{-6}, respectively. The GAVal adduct formation rates for male, female, or both genders combined are 38.4, 30.6, and 33.8 (L/g globin/hr)×10^{-6}, respectively.

Tareke et al. (2006, 224387) did not report gender specific slopes, but they did report a slope of 7.5 for AAVal (both genders), and 34 for GAVal (both genders). The GAVal slope for both genders from Tareke et al. (2006, 224387) of 34 is the same as the rounded up value derived by EPA. The higher AAVal formation rate of 7.5 from Tareke et al. (2006, 224387) for the combined male and female rat data compared with the value of 7.0 from EPA's analysis could have resulted from removal of outlier levels of AAVal in the female gavage dose group. As seen in Table E-1, the nearly threefold higher standard deviation for the AA Val adduct levels in the gavage female group compared to all other male and female dose groups suggests that there may have been an outlier. The relatively low AAVal mean level of 29 for the female gavage group compared to the male value of 20, is also suspect considering that the female AUC is nearly twice the value of the males.

With little biological rationale to support gender differences in the rat for the formation of hemoglobin adducts, and with the EPA analysis of the reported Doerge et al. (2005, 224355) and Tareke et al. (2006, 224387) data closely reproducing the rates for both genders reported by Tareke et al. (2006, 224387), EPA chose to use the AAVal adduct formation rate of 7.5 (L/g globin/hr)×10^{-6} and the GAVal adduct formation rate of 34.0 (L/g globin/hr)×10^{-6} in a model simulation that estimated the blood concentrations needed to generate the levels of Hb adducts reported by Tareke et al. (2006, 224387) from the rats exposed to AA in the drinking water in the Doerge et al. (2005, 224344) study. These estimates were then used to derive the AUC and the conversion factors for AA-AUC and GA-AUC in male or female rats for a given external exposure to AA.

E.2.1.1. In Vivo Adduct Formation Rate Constants for Humans

No human in vivo data sets are available with values for all three of the variables needed to derive an in vivo second order human adduct formation rate constant. The best surrogate for a human in vivo rate is the in vivo rate that Tareke et al. (2006, 224387) generated from a linear regression of all the available in vivo adduct level versus AUC data for male and female mice and rats from the single dose studies of Doerge et el. (2005, 224348; 2005, 224355) and Tareke et al. (2006, 224387). Figure E-1 is the scatter plot and results of the regression reproduced from Tareke et al. (2006, 224387).



Source: Figure 4 in Tareke et al. (2006, 224387).

Figure E-1. Correlation of Hb adducts for AA and GA with the respective serum AUC in F344 rats and B6C3F1 mice exposed to single dose gavage administration of AA (0.1 mg/kg bw) or and equimolar gavage dose of GA. Note: Individual data points shown represent group mean Hb adducts and AUC values for male and female mice and rats.

The regression results for AAVal are:

$$\text{AAVal (pmoles/g globin)} = 7.5\ \text{AA-AUC (µM-h)} + 8\ (r2 = 0.88, p<0.001) \quad \text{Equation E-2}$$

and for GAVal:

$$\text{GAVal (pmoles/g globin)} = 32.5\ \text{GA-AUC (µM-h)} + 41.5\ (r2 = 0.83, p<0.001) \quad \text{Equation E-3}$$

The resulting in vivo adduct formation rate constants for AAVal of 7.5 (L/g globin/hr)×10^{-6}, and for GAVal of 32.5 (L/g globin/hr)×10^{-6}, were used in conjunction with the Fennell et al. ((2005, 224299) data to derive an AA-AUC or GA-AUC/AA mg/kg bw-day conversion factor to convert the rat AUC_{BMLD5} (as the POD) to a human equivalent administered dose.

The above in vivo adduct rate constants from the pooled data are not gender specific. As noted in the discussion of the rat adduct formation rates, there is little biological rationale for a gender difference in hemoglobin adduct formation rates. Hartmann et al. (2008, 224480) also provide some support for the lack of a gender difference in humans, having not observed a gender-related difference in internal exposure and metabolism of AA in a study of a nonsmoking general population especially designed for an even distribution of age and gender.

E.3. DEVELOPMENT OF THE ADDUCT FORMATION MODEL TO ESTIMATE THE RAT AUCS AT THE POD

E.3.1. Summary

To derive a value for the rat AA-AUC or GA-AUC per dose of AA administered in a drinking water study, EPA developed a simple dynamic model using ordinary differential equations (ODE) that simulated the formation AAVal and GAVal to estimate the blood concentrations needed to reach the adduct levels reported by Tareke et al. (2006, 224387) from the Doerge et al. (2005, 224344) rat drinking water exposure. The model was parameterized with the rat in vivo adduct formation rate constants derived by EPA (discussed above), and the adduct elimination rate constants reported by Tareke et al. (2006, 224387). The model simulations resulted in the following AUC conversion factors:

AA-AUC in μM-hr/mg AA/kg bw-day = 27.4 (for males) and 29.7 (for females) Equation E-4
GA-AUC in μM-hr/mg AA/kg bw-day = 16.2 (for males) and 29.2 (for females) Equation E-5

These values were used to estimate the rat internal AA-AUC following exposure at the $BMDL_5$ (from the Friedman et al. (1995, 224307) study data) for neurotoxicity and the HED_{BMDL} as the POD to derive the RfD, and for the GA-AUC following exposure to the rat BMDL (from the Johnson et al. (1986, 061340) data) for increased risk of tumors, and the applicable HED_{BMDL} as the POD to derive the oral slope factor.

Different conversion factors could be used to convert an external dose into an internal AUC due to different in vitro and in vivo adduct formation rate constants. The main body of the Toxicological Review presents the choices in Tables 5-6 (for AA-AUC) and 5-7 (for GA-AUC).

E.3.2. Background

Tereke et al. (2006, 224387) reported the AAVal and GAVal Hb adduct level time course in male and female rats exposed chronically by drinking water to AA. Tereke also measured the loss rate of both AA and GA adducts after the end of AA exposure. Separately, Doerge et al. (2005, 224344) reported blood AA and GA concentrations at several time points over the duration of the 49-day study. The data from both studies were obtained by digitization from original graphs using Data Thief software.

One can directly estimate the AUC for a given drinking water dose if there are sufficient blood level time course data. Alternately, one can derive blood AA-AUC and GA-AUC from time course data on Hb adduct levels and in vivo formation and loss rates for the AAVal and GAVal adducts.

The clearance of both AA and GA are sufficiently high to result in blood AA and GA levels that fluctuate significantly throughout the day during an oral gavage, drinking water or other repeated dose studies. Tereke et al. (2006, 224387) and Doerge et al. (2005, 224344), however, do not report when blood samples were taken to measure the adducts or AA and GA levels. Although the experimental blood AA and GA data appear to represent a steady state, the timing of the blood samples taken for analysis of AA and GA will have a significant impact on any estimate of the AUC. If blood sampling were to be conducted during an active period of drinking, blood concentrations and presumed exposure would be high, the inverse being true if sampling was conducted just after a sleeping or non-drinking period. Because the adduct half-life is between 10 and 13 days (Tareke et al., 2006, 224387) adduct measurements are not subject to this uncertainty.

The blood AA and GA concentration data reported by Doerge et al. (2005, 224344) and Tereke et al. (2006, 224387) at "various" but otherwise unspecified times, were therefore assumed not to be reliable measures of overall blood AA or GA exposure. The alternate method of using adduct levels and formation and loss rate constants was preferable for estimating the AUCs from a drinking water exposure, and to develop conversion factors for use in estimating the AUC from the rat study PODs.

E.3.3. Dynamic Model Equations

A simulation model of the formation and removal rates of AAVal and GAVal Hb adducts in rats as a function of Hb and AA or GA concentrations was developed in acslX. The amount of adduct (AADDUCT, μmoles) at any time post exposure was calculated as the integral of the balance between the formation and removal rates:

$$AADDUCT = \int_0^t KHGB1 \times RBCHGB \times VRBC \times BLCONC - KHGBD \times AADDUCT$$ Equation E-6

where KHGB1 (L/d-g Hb) is the second order rate constant for the formation of the adduct, RBCHGB (grams Hb/L in red blood cells) is the concentration of Hb in red blood cells, VRBC is the volume of red blood cells (L), BLCONC is the concentration of AA or GA in the blood (μM), and KHGBD (-day) is the first order rate constant for loss of AAVal or GAVal adducts. A unit analysis was conducted to verify the code, which was found to be accurate.

An acslX table function was adapted for use with single blood concentrations representative of daily blood AA and GA exposures expected over the course of each day.

E.3.4. Model Parameters

Some parameters are used in the model to derive the values used in the above equation, including body weight (0.25 kg), hematocrit (0.45), and fraction of body weight that is blood (0.06). These values were taken from Walker et al. (2007, 224527).

The choice of values for the second order adduct formation rate constants were discussed in section E.2 above, and are 7.5 (L/g globin/hr)×10^{-6} for AAVal formation, and 34.0 (L/g globin/hr)×10^{-6} for GAVal formation.

The elimination rate constants are based on direct measurement of declining adduct levels in male and female rats dosed for 21 days with AA in the drinking water then removed from exposure, and sampled at various time points during a 63 day follow up period on control water. Tereke et al. (2006, 224387) report half-lives of 12 days for elimination of AAVal adducts in either females or males, and 11 and 13 days for elimination of GA Val in male and female rats, respectively. Tereke et al. (2006, 224387) note that these rates are essentially the rate for red blood cell turnover since the lifetime of a red blood cell in rats is reported to be 60 days (Törnqvist et al., 2002, 597198), and a half-life of 12 days corresponds to nearly a complete turnover of adducts in 5 half-lives (i.e., only $[1/2]^5$ or ~3% of the cells would remain).

One would expect red blood cell turnover to be independent of whether the adduct was AA or GA, and independent of sex. EPA, however, used the gender specific half lives to calculate the values of the elimination rates used in the model since they were based on direct measurements of adduct losses in the test animal and exposure regimen of interest, and they represented all known and unknown (e.g., adduct instability) processes leading to adduct loss some of which may be have gender differences. In either case, there is no gender in half-life for AAVal, and the small difference between an average half-life of 12 days for GAVal or the gender specific half-lives of 11 or 13 days had little impact on the final reference values.

A half-life (in days) can be converted to an elimination rate, k_e, in hr^{-1} as follows:

$$k_e(hr^{-1}) = \frac{\ln(2)}{half-life * 24\,hr/day}$$ Equation E-7

For example, a half life of 12 can be converted to an elimination rate of 2.4 x 10^{-3} (hr^{-1}).

$$k_e = \frac{\ln(2)}{12d * 24\,hr/day} = 2.4x10^{-3}(hr^{-1}) \qquad \text{Equation E-8}$$

Parameter estimation tools in acslX were used to optimize the value of the AA or GA blood concentration. Scripts (.m files) were written for each simulation, as for all optimizations, and can be found in the model workspace.

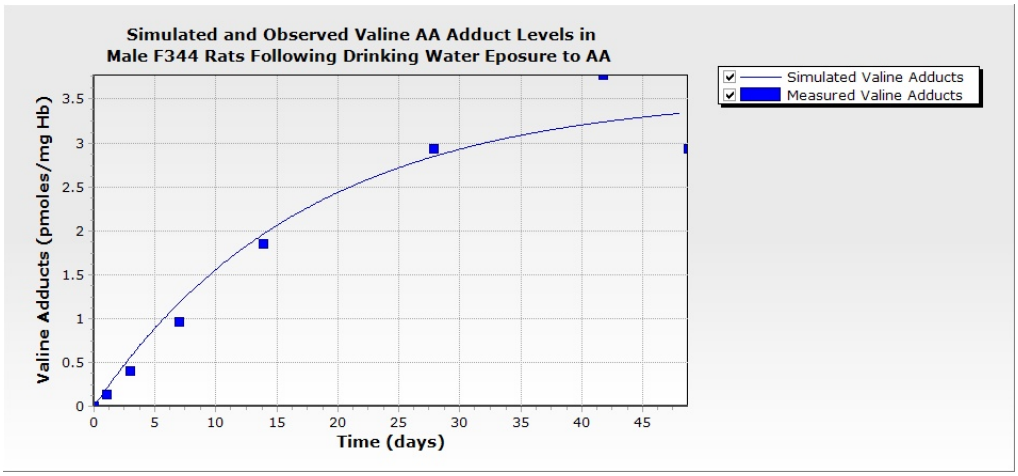
E.3.5. Results

The model generated values for AA and GA-AUCs per unit dose in the exposed rats of the Doerge et al. (2005, 224344) drinking water study data by estimating the daily average blood concentration that would be needed to optimally fit the reported AAVal and GAVal adduct levels (Tareke et al., 2006, 224387), while holding the adduct formation and elimination rates constant. The Nelder-Meade parameter optimization routine in acslX was used to optimize the average blood concentration against the adduct data.

Parameter values for AAVal and GAVal adduct formation and loss rates and the resulting model estimates of the AA-AUC or GA-AUC per mg AA/kg bw-day are presented in Table E-3. There was good correspondence between measured and modeled AAVal and GAVal adduct levels using the fixed rate constants. Figures E-2 and E-3 provide the fits for the estimates of the male rat AA and GA-AUC per dose conversion factors that were used in the assessment to estimate the internal dose in the rat at the PODs for the noncancer and cancer effects.

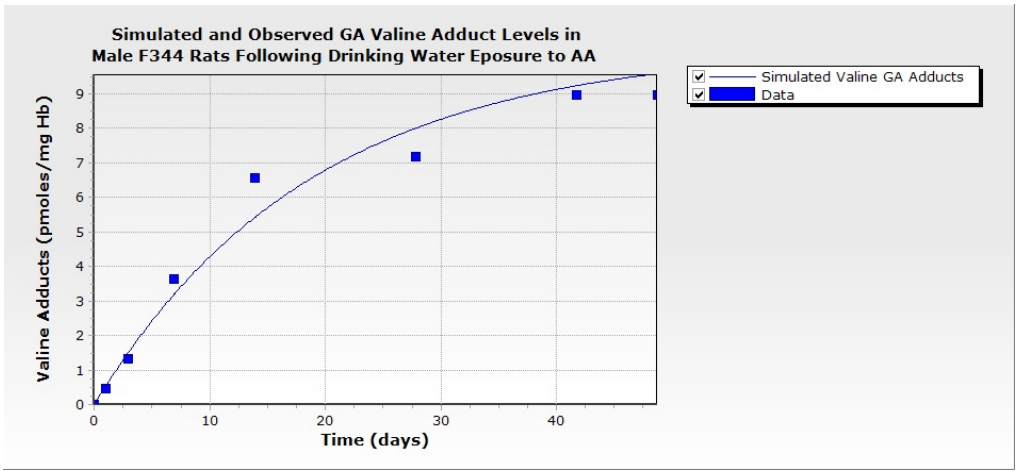
Table E-3. Estimated AUCs for AA and GA per exposure to AA based on hemoglobin adduct data and in vivo adduct formation rates from Tareke et al. (2006, 224387) for both male and female rats exposed to AA in drinking water (1 mg/kg-day) for 49 days (Doerge et al., 2005, 224344).

Compound	Gender	In vivo		
		Formation rate (L/hr-g Hb)	Elimination rate (1/hr)	Predicted AUC [µM-h/(mg AA/kg-day)]
AA	Female	7.5×10^{-6}	2.4×10^{-3}	29.7
	Male	7.5×10^{-6}	2.4×10^{-3}	27.4
GA	Female	3.40×10^{-5}	2.6×10^{-3}	29.2
	Male	3.40×10^{-5}	2.2×10^{-3}	16.2



Source: Tareke et al. (2006, 224387).

Figure E-2. Simulation fit to male rat AAVal adduct data.



Source: Tareke et al. (2006, 224387).

Figure E-3. Simulation fit to male rat GAVal adduct data (Tareke et al., 2006, 224387).

E.3.6. Uncertainties and Data Gaps

The most important uncertainty in this analysis is the use of in vivo formation rates derived from studies with single doses, rather than exposures via the drinking water. Any errors in the values of the in vivo rate constants would be proportional to the difference between the assumed and actual blood AA and GA-AUCs from the drinking water exposure. This error could

be reduced if blood sampling times and drinking behavior (time, volume) were known for the Doerge et al. (2005, 224344) drinking water study.

APPENDIX F. ALTERNATE RFC BASED ON HUMAN EPIDEMIOLOGY DATA

An RfC can be derived from the limited Calleman et al. (1994, 202900) data for comparison purposes, and to highlight the uncertainties in the results due to these data to prompt improvements in the design of future studies.

Briefly, Calleman et al. (1994, 202900) performed a cross-sectional analysis of Hb adduct formation and neurological effects in a group of 41 factory workers (34 males and 7 females, aged 18–42 years) who were exposed to AA (and acrylonitrile, from which AA is formed) for 1 month to 11.5 years (mean 3 years) during the production of AA in a factory in China. AA 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 a referent group 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.

A neurotoxicity index, with a maximal score of 50, was used to express severity of peripheral neuropathy (Table F-1); 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 Hb adduct formed by the reaction of AA with AAVal.

Table F-1. 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
ENMG 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 1–3 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, 202900).

Group mean biomarker levels and neurotoxicity indices are presented in Table F-2 for controls and the work locations of packaging, polymerization, ambulatory, and synthesis. The average neurotoxicity index scores, as well as the averages of the Hb 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 Hb 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 F-2. Group means ± SD of biomarkers in different categories of workers

	Free AA[a] (µmol/L)	Merc. ac.[b] (µmol/24 hrs)	AAVal[c] (nmol/g globin)	ANVal[d] (nmol/g globin)	$AccD_{AA}$[e] (mM/hr)	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 AA.
[b]Urinary mercapturic acid.
[c]Hb adduct between N-terminal valine and AA.
[d]Hb adduct between N-terminal valine and acrylonitrile.
[e]Predicted cumulative in vivo AA dose (based on rates of AA-Hb adduct formation in human globin hydrolysates and mean AA exposure concentrations measured in areas of polymerization and synthesis by station sampling). See Section 3.1 and Bergmark et al. (1993, 224424) for additional information.
[f]Neurotoxicity index.

Source: Calleman et al. (1994, 202900).

The data for AAVal neurotoxicity index (NIn) in Table F-2 are amenable to BMD analysis as presented in Figure F-1.

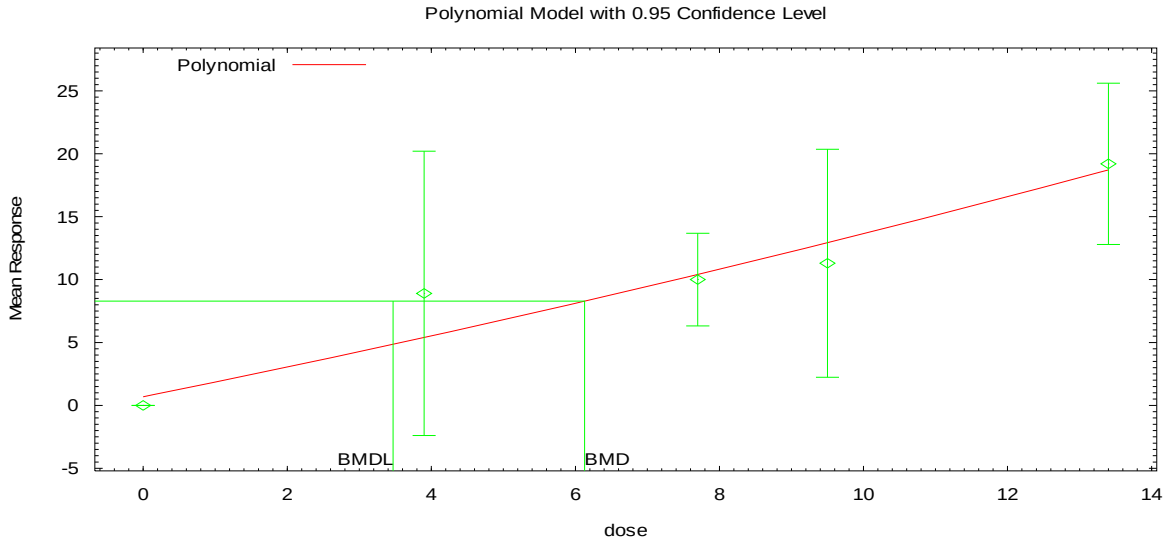


Figure F-1. BMD analysis for Calleman et al. (1994, 202900) data.

Using a BMR level of 1 SD (i.e., neurotoxicity index = 6.9), from overall mean response (neurotoxicity index = 9.9), the BMD is 6.1 nmol AAVal/g globin and the BMDL (as the POD) is 3.5 nmol AAVal/g globin. Under the assumption that this BMDL represents a steady state

level, the daily increment in adducts can be calculated using the equations presented in Fennell et al. (2005, 224299) as follows:

$$Daily\ \ adduct\ \ increment\ \ = \frac{2}{RBC\ \ lifespan} \times steady\ \ state\ \ adduct\ \ level$$

$$Daily\ \ adduct\ \ increment\ \ = \frac{2}{120} \times \frac{3.5\,nmol\ \ AAVal}{g\ \ globin} = \frac{0.058\ nmol\ \ AAVal}{g\ \ globin} \quad \text{Equation F-1}$$

To convert this daily increment in AAVal to a daily intake of AA (in mg AA/kg bw), the following equation is used for the value of 74.7 nmol AAVal/g globin/mmol AA/kg bw reported in Fennell et al. (2005, 224299), and a factor of 71.08 mg AA/mmoles AA to convert the daily intake of AA to mg/kg bw:

$$Daily\ Intake\ of\ \ AA = Daily\ increment\ of\ \ AAVal \div \frac{74.7\,nmol\ AAVal}{\frac{g\ globin}{mM\ \ AA/kg\,bw}} \times \frac{71.08\,mg\ AA}{mM\ \ AA}$$

$$Daily\ Intake\ of\ \ AA = \frac{0.05833\,nmol\ AA-Val}{g\ globin} \div \frac{74.7\,nmol\ AAVal}{\frac{g\ globin}{mM\ \ AA/kg\,bw}} \times \frac{71.08\,mg\ AA}{mM\ \ AA}$$

$$= \frac{0.056\,mg\ \ AA}{kg\ bw} \quad \text{Equation F-2}$$

The air concentration needed, to result in the daily intake of 0.056 mg AA/kg bw[1] for a 70 kg person who breathes 20 m^3/day of air, would be 0.19 mg/m^3.

$$Air\ Concentration_{BMDL-Daily\ Intake} = 0.056\,mg/kg\ \ \ bw\ \times\ 70\,kg \div \frac{day}{20\,m^3} = 0.19\,mg/m^3 \quad \text{Equation F-3}$$

This POD for a continuous inhalation exposure of 0.19 mg/m^3 is divided by a total UF of 100: 10 for consideration of intraspecies variation (UF_H: human variability), and 10 for extrapolating duration from a subchronic exposure to a chronic exposure (UF_S).

Total UF = 100

= 1 (UF_A) × 10 (UF_H) × 10 (UF_S) × 1 (UF_D) Equation F-4

A UF 1 was selected for interspecies extrapolation because this RFC is based on human data.

[1] Derivation of the daily intake of AA (Equation F-2) results in an actual value of 0.05550333 mg AA/kg bw, which was rounded to 0.056 mg AA/kg bw for presentation purposes.

A UF of 10 was used to account for interindividual variability in toxicokinetics and toxicodynamics to protect potentially sensitive populations and lifestages (UF_H).

A UF of 10 was used to extrapolate from a subchronic exposure duration to a chronic exposure duration (UF_S) because the POD was derived from a subchronic exposure to humans (i.e., the $UF_S = 10$).

A UF to account for database deficiency is not necessary for this derivation (i.e., $UF_D = 1$) because the 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 AA based on the Calleman et al. (1994, 202900) human data is calculated as follows:

$$\begin{aligned} \text{RfC} &= \text{Air Concentration}_{\text{BMDL – Daily Intake}} \div \text{UF} \\ &= 0.19\ \text{mg/m}^3 \div 100 \\ &= 0.002\ \text{mg/m}^3 \text{ (rounded to one significant digit)} \end{aligned} \qquad \text{Equation F-5}$$

F.1.1. Limitations in the Calleman et al. (1994, 202900) Data and Uncertainties in the Results

The human data from the Calleman et al. (1994, 202900) study (described in detail in Section 4.1) are limited due to a small number of subjects (n = 41), the narrow representation of the general public (i.e., workers), and by a number of potentially confounding factors including concurrent exposure to another neurotoxin (acrylonitrile), aggregate exposure via both dermal and inhalation routes, a composite index of neurotoxicity, and control groups of different size and composition. There are also no other human inhalation toxicity studies to support or challenge the reproducibility or validity of the Calleman et al. (1994, 202900) study results.