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Center For The Evaluation Of Risks To Human Reproduction

DRAFT

NTP-CERHR EXPERT PANEL REPORT on the REPRODUCTIVE and DEVELOPMENTAL TOXICITY of ACRYLAMIDE

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PREFACE

The preface will be added after the Expert Panel meeting.

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Note to Reader:

This report is prepared according to the Guidelines for CERHR Panel Members established by NTP/NIEHS. The guidelines are available from the CERHR web site (http://cerhr.niehs.nih.gov/). The format for Expert Panel Reports includes synopses of studies reviewed, followed by an evaluation of the Strengths/Weaknesses and Utility (Adequacy) of the study for a CERHR evaluation. Statements and conclusions made under Strengths/Weaknesses and Utility evaluations are those of the Expert Panel and are prepared according to the NTP/NIEHS guidelines. In addition, the Panel often makes comments or notes limitations in the synopses of the study. Bold, square brackets are used to enclose such statements. As discussed in the guidelines, square brackets are used to enclose key items of information not provided in a publication, limitations noted in the study, conclusions that differ from those of the authors, and conversions or analyses of data conducted by the Panel.

Abbreviations

ACGIH	American Conference of Governmental Industrial Hygienists
ABT	1-aminobenzotriazole
ANOVA	analysis of variance
AUC	area under the concentration versus time curve
BMD_{10}	benchmark dose, 10% effect level
BMDL	benchmark dose 95th percentile lower confidence limit
bw	body weight
^{14}C	carbon-14
C	Celsius
CAS RN	Chemical Abstracts Service Registry Number
CERHR	Center for the Evaluation of Risks to Human Reproduction
CFR	Code of Federal Regulations
CIIT	Chemical Industry Institute of Toxicology
cm^2	centimeter(s) squared
CNS	central nervous system
CYP	cytochrome P450
DAPI	4',6-diamidino-2-phenylindole
dB	decibel(s)
DNA	deoxyribonucleic acid
DPM	disintegrations per minute
DOE	Department of Energy
EASE	estimation and assessment of substance exposure
EPA	Environmental Protection Agency
f	female
F_0	parental generation
F_1	first filial generation
F_2	second filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FISH	fluorescence *in situ* hybridization
g	gram(s)
G_2	Second gap phase of meiosis
GD	gestation day(s)
GLP	Good Laboratory Practice
GSH	glutathione
GST	glutathione-S-transferase
h	hour(s)
HBSS	Hanks' balanced salt solution
hCG	human chorionic gonadotropin
Hg	mercury
HPLC	high performance liquid chromatography
HSDB	Hazardous Substances Data Bank
IARC	International Agency for Research on Cancer
i.p.	intraperitoneal
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
i.v.	intravenous
JIFSAN	Joint Institute for Food Safety and Applied Nutrition
K_D	receptor affinity

kg	kilogram
K_{ow}	octanol-water partition coefficient
L	liter(s)
LC	liquid chromtography
LD_{50}	lethal dose, 50% mortality
LOAEL	low observed adverse effect level
m	male
M	molar
m^3	meter(s) cubed
mg	milligram
min	minute(s)
mL	milliliter(s)
mm	millimeter(s)
mM	millimolar
mmol	millmole(s)
MS	mass spectrometry
n or no.	number
NCFST	National Center for Food Safety and Technology
ng	nanogram(s)
NICHD	National Institute of Child Health and Human Development
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NIOSH	National Institute of Occupational Safety and Health
nmol	nanomole(s)
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NS	non-significant
NTP	National Toxicology Program
OECD	Organization for Economic Co-operation and Development
OSHA	Occupational Safety and Health Administration
PBPK	physiologically based pharmacokinetic model
PBS	phosphate-buffered saline
PEL	permissible exposure limit
pmol	picomole
po	peroral
ppb	parts per billion
PND	postnatal day(s)
ppm	parts per million
RACB	reproductive assessment by continuous breeding
REL	relative exposure limit
SD	standard deviation
SEM	standard error of the mean
SOCMA	Synthetic Organic Chemical Manufacturers Association
TLV	threshold limit value
TWA	time weighted average
UDS	unscheduled DNA synthesis
WHO	World Health Organization
Δ	change
µg	microgram(s)
µm	micrometer(s)
µmol	micromole(s)

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1.0 CHEMISTRY, USE, AND HUMAN EXPOSURE

As noted in the CERHR Expert Panel Guidelines, Section 1 is initially based on secondary review sources. Primary study reports are addressed by the Expert Panel if they contain information that is highly relevant to a CERHR evaluation of developmental or reproductive toxicity or if the studies were released subsequent to the reviews. For primary study reports that the Expert Panel reviewed in detail, statements are included about the strengths, weaknesses, and adequacy of the studies for the CERHR review process.

1.1 Chemistry

1.1.1 Nomenclature

The CAS RN for acrylamide is 79-06-1. Synonyms for acrylamide include *(5)*:

2-propenamide; 2-propeneamide; acrylic amide; ethylene carboxamide; ethylenecarboxamide; propenamide; propenoic acid, amide; vinyl amide

1.1.2 Formulae and Molecular Mass

Acrylamide has a molecular mass of 71.08 and a molecular formula of C_3H_5NO *(6)*. The structure for acrylamide is shown in Figure 1.

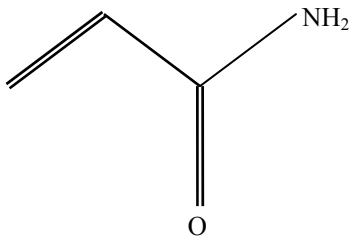


Figure 1. Structure of acrylamide

1.1.3 Chemical and Physical Properties

Acrylamide is available as an odorless, white crystalline solid or as an aqueous solution *(7)*. Physicochemical properties are listed in Table 1.

In air, 1 ppm acrylamide = 5 mg/m^3 *(7)*.

Table 1. Physicochemical Properties of Acrylamide

Property	Value
Boiling point	125ºC @ 25 mm Hg
Melting point	84.5ºC
Flammability	Non-flammable[a]
Specific gravity	1.122 g/mL
Solubility in water	2,215 g/L @ 30ºC
Vapor pressure	6.75×10^{-3} mm Hg
Stability	May polymerize violently at temperatures above melting point
Reactivity	Reacts with acids, bases, and oxidizing agents[a]
Log K_{ow}	From -1.65 to -0.67

HSDB *(6)*, European Union *(8)*
[a]Mallinckrodt *(9)*

1.1.4 Technical Products and Impurities

The solid form of acrylamide is available as a technical grade that is 97% pure and an ultra-pure grade that is 99% pure *(6)*. Concentrations of aqueous solutions range from 40 to 50%. Copper is often added at levels less than 100 ppm to inhibit polymerization. Trace impurities depend on the method of manufacture and can include water, iron, butanol, sodium sulfate, acrylic acid, sulfuric acid, acrylonitrile, 3-hydroxypropionitrile, 3-hydroxypropionamide, and tris-nitrilopropionamide *(6, 8)*.

Trade names for acrylamide include: AAM, Optimum, Amresco Acryl-40, and Acrylage 1 *(10)*.

1.2 Use and Human Exposure

1.2.1 Production Information

The two main methods of manufacturing acrylamide include the sulfuric acid method or catalytic hydration of acrylonitrile *(6, 7)*. In the sulfuric acid method, acrylamide monomer is separated from its sulfate salt using a base neutralization or an ion exchange column *(6)*. With the catalytic hydration method, acrylonitrile solution is passed over a fixed copper catalyst bed at 70–120ºC to produce a 48–52% solution *(7)*. Unreacted acrylonitrile is recycled over the catalyst bed in a continuous process. Acrylonitrile is removed by evaporation and catalyst is removed by filtration. The catalytic method has been the preferred process since the 1970s due to increased purity, no undesirable by-products, greater conversion efficiency, and elimination of a costly purification step. An enzymatic hydration process using micro-organisms to convert acrylonitrile to acrylamide can also be used to manufacture acrylamide *(11)*.

Past or current manufacturers of acrylamide include: Ciba Specialty Chemicals Corp., Cytec Industries Inc., Dow Chemical U.S.A., and Nalco Chemical Co. *(6)*. Additional manufacturers or importers may include American Cyanamid Company, BF Goodrich Co., and Cosan Chemical Corp *(10)*.

The demand for acrylamide was reported at 170 million pounds in 1999 and 205 million pounds in 2003 *(6)*. In 1997, the total output of acrylamide in the U.S. was 217 million pounds *(12)*. One

hundred million pounds of acrylamide were produced and 15 million pounds were imported in the U.S. in 1992 *(13)*.

1.2.2 Use

Acrylamide is used in scientific research, as a cement binder, and in the production of polymers and gels *(14)*. The majority of acrylamide (>90%) is used in the manufacture of polymers such as polyacrylamide. Such polymers can contain trace levels of monomer *(6, 8)*. In 1999, 60% of polyacrylamide was used in water treatment, 20% in pulp and paper production, and 10% in mineral processing *(6)*. Polyacrylamide polymers are also used in certain cosmetics, some food packaging materials such as paperboard, soil conditioning agents, plastics, and specialized grouting agents *(12, 15)*. A search of the NLM Household Products Database *(16)* revealed polyacrylamide as an ingredient in several skin lotions or creams. Acrylamide polymers or co-polymers are also used in textile industries, in electrophoretic gels, as a medium for hydroponically-grown crops, in sugar refining, and in bone cement *(14)*. Polyacrylamide is also used in crude oil production; coatings used in home appliances, building materials, and automotive parts; explosives; adhesives; printing inks; adhesive tapes; latex; herbicidal gels; and as a clarifier in food manufacturing *(8, 12)*. Polyacrylamide is also used in gelatin capsules, in the manufacture of dyes, and in co-polymers used in contact lenses *(12)*.

1.2.3 Occurrence

Acrylamide could potentially be present in food, drinking water, indoor air, or the environment as a result of anthropogenic or natural processes.

The presence of acrylamide in some types of food cooked at high temperatures was reported by the Swedish National Food Administration and researchers from Stockholm University in April, 2002 *(17)*. In a limited survey of various food types, acrylamide levels were found to be highest in starchy foods cooked by methods such as frying, grilling, and baking. There is evidence that acrylamide levels increase with higher temperature and longer cooking duration. The survey found no acrylamide in foods cooked at temperatures below 120ºC. In most surveys, acrylamide was not detected or present at low levels in unheated or boiled foods *(14, 18)*. Table 2 lists acrylamide levels detected in various food types. It was noted that the types of food analyzed included staple foodstuffs representing more than one third of consumer caloric intake in the U.S. *(19)*. Acrylamide concentrations were found to vary widely among food categories.

Table 2. Acrylamide Levels in Foods, as Reported in Friedman *(14)*

Food	Acrylamide Level (µg/kg=ppb)
Almonds, roasted	260
Asparagus, roasted	143
Baked products: bagels, breads, cakes, cookies, pretzels	70–430
Beer, malt, and whey drinks	30–70
Biscuits, crackers	30–3,200
Cereals, breakfast	30–1,346
Chocolate powder	15–90
Coffee powder	170–351
Corn chips, crisps	34–416
Crispbread	800–1,200
Fish products	30–39
Gingerbread	90–1,660
Meat and poultry products	30–64
Onion soup and dip mix	1,184
Nuts and nut butter	64–457
Peanuts, coated	140
Potato boiled	48
Potato chips, crisps	170–3,700
Potato, French fried	200–12,000
Potato, puffs, deep-fried	1,270
Snacks, other than potato	30–1,915
Soybeans, roasted	25
Sunflower seeds, roasted	66
Taco shells, cooked	559

There are some data on mechanisms of acrylamide formation in foods. Data suggest that a large portion of acrylamide in baked or fried foods is derived from heat-based reactions between the amino group of the amino acid asparagine and the carbonyl group of reducing sugars such as glucose *(14)*. A Panel assembled by JIFSAN/NCFST *(20)*, ". . . felt generally confident that free asparagine and carbohydrates (especially free reducing sugars) accounted for the majority of acrylamide in fried potato products." Foods rich in both asparagine and reducing sugars originate from plant sources such as potatoes or cereal grains but apparently not animal products such as beef, poultry, or fish *(14)*.

The presence of trace acrylamide levels in food could also result from the use of acrylamide polymers or co-polymers in food processing or food packaging materials. As noted in an IARC *(11)* review, FDA regulates the use of acrylamide polymers or co-polymers in food contact materials (21 CFR 175.105, 175.300, 177.1010, 176.180), limits residual acrylamide levels to 0.05% in resins used in food treatment (21 CFR 173.5) or as boiler water additives (21 CFR 173.310), and limits residual acrylamide levels to 0.2% in polymers added to water used to wash fruit and vegetables (21 CFR 173.315), in polymers or resins used in paper or paperboard intended for food contact (21 CFR 176.110, 176.170), or in modified starch (21 CFR 178.3520).

Trace levels of acrylamide could also occur in some drugs. The FDA requires that residual acrylamide levels not exceed 0.2% in polymers used as film formers in gelatin capsules (21 CFR 172.255) *(11)*.

Acrylamide may be present in drinking water due to the use of polyacrylamide flocculants to remove particulate contaminants. Federal regulations require that residual acrylamide levels do not exceed 0.05% when polyacrylamide is added to drinking water at 1 ppm **[1 mg/L];** public water systems must annually certify (by third party or manufacturer certification) to the State that polymer and monomer levels are within acceptable limits *(21, 22)*. Due to this regulation, acrylamide levels in drinking water are not expected to exceed 0.5 ppb **[0.5 µg/L]**.

Acrylamide is a component of cigarette smoke *(15, 17)*; therefore, smoking could potentially be a source of acrylamide in indoor air. Mainstream cigarette smoke has been measured for acrylamide content, which was 1.1-2.34 µg per cigarette (reviewed by Smith, et al. *(23)*). There are no data reporting acrylamide levels in indoor air from environmental tobacco smoke.

Acrylamide could be present in the environment as a result of direct releases or leaching of residual monomer during the use of polyacrylamide polymers in applications such as water purification or soil conditioning. During sludge conditioning processes, 92–100% of residual acrylamide was reported to leach from acrylamide polymers *(7)*. According to the TRI database, 8.7 million pounds of acrylamide were released to the environment from U.S. manufacturing and processing facilities in 2000 *(24)*.

Acrylamide released into outdoor air can react with species such as hydroxyl radicals; the half-life for the reaction occurring at room temperature was reported at 8.3 h *(8)*. Because of its high water solubility, acrylamide will likely be removed from the atmosphere by rain.

Acrylamide released to surface waters will not likely volatilize to air because of its high water solubility and low vapor pressure. Biodegradation appears to be the main process of removal from surface water. An OECD study found acrylamide to be readily biodegradable at concentrations less than 2 mg/L *(8)*. Half lives of 55–70 h were reported in fresh water under aerobic conditions *(7)*. IPCS *(7)* concluded that because acrylamide is readily biodegraded by microorganisms and because it has a high water solubility and low lipid solubility (log K_{ow} = –1.65), it is unlikely to bioconcentrate or biomagnify in food organisms.

Adsorption of acrylamide to soils or sediments is likely negligible and acrylamide is reported to be highly mobile in soils *(8)*. Acrylamide is degraded in soil with a rate dependent on soil type, pH, and temperature. Half-lives for degradation in soil were reported at 20–45 h at 25 mg/kg at 22ºC and 95 h at 500 mg/kg at 20ºC *(7, 8)*.

Information on acrylamide levels in environmental samples is limited to reports published in the 1970s and 1980s *(8)*. In those reports acrylamide levels were generally low in surface or sea waters in the U.S. or U.K. (<0.2–3.4 µg/L). The recent European Union *(8)* analysis of exposures to acrylamide in drinking water as a result of treatment with polyacrylamide resin estimated a worst-case level of 0.125 µg/L. Acrylamide levels were below the detection limit (0.1–25 µg/L) in five drinking water samples from the U.S. or U.K. Treated waste waters from sewage and chemical plants can be much higher. Water control with acrylamide-containing grout can lead to water contamination; for example, 400 µg/L was measured in a sample from a drain treated in Japan. Acrylamide levels ranged from <0.2 to 1,100 µg/L in various waste or process water samples obtained primarily from the U.K.

1.2.4 Human Exposure

1.2.4.1 General population exposure

The general population can be exposed to acrylamide through oral, dermal, or inhalation routes.

As noted in Section 1.2.3, acrylamide is produced in some foods during high temperature cooking methods. A Panel assembled by the FAO/WHO *(17)* estimated exposure to acrylamide through food intake using food consumption data from Australia, Norway, the Netherlands, Sweden, and the U.S. The lower bound estimate of typical acrylamide food exposures was 0.3–0.8 μg/kg bw/day; intakes in children were estimated to be 2–3 times the adult rate when expressed as a body weight ratio. Although based on limited data, the FAO/WHO Panel stated that the data do allow for uncertainty estimates for median food exposures for Western European, Australian, and North American diets.

Additional estimates of acrylamide intake through food were reported in a review by the European Commission *(25)*. The review reports acrylamide intakes ranging between 35 and 40 μg/day (~0.5 μg/kg bw/day based on a 70 kg bw) as estimated by a Swedish group. Intakes of 0.30–1.10 μg/kg bw/day in adults and 0.30–2.1 μg/kg bw/day in 13-year-old children were estimated by a Norwegian group.

Results of initial food testing conducted by the FDA are in basic agreement with reported levels of acrylamide in foods from other nations *(18)*. The FDA will continue to estimate and update exposure estimates as new data are obtained on acrylamide food levels.

Sorgel et al. *(26)* reported breast milk acrylamide levels of 10.6–18.8 ng/mL and 3.17–4.86 ng/mL in 2 women who consumed about 1 mg and 800 μg of acrylamide, respectively, through potato chips. Based on an assumed daily consumption of 500 mL breast milk, Sorgel et al. estimated acrylamide intake in infants at 2–10 μg/day. Intake in a 3-kg baby was estimated at 0.66–3.3 μg/kg bw/day.

Strengths/Weaknesses: Industrial releases of acrylamide to surface waters are limited and unlikely to accumulate because of biodegradation. Water contamination with acrylamide will not result in bioaccumulation because the acrylamide is highly water soluble and not lipophilic. In the 1970s and 1980s, acrylamide was present in public water supplies as a result of water treatment with polyacrylamide to aid flocculation, measured. Polyacrylamide/free acrylamide content was limited to below 0.125 μg/L. Currently there are few data, but acrylamide concentrations in all drinking water samples were lower than the limits of detection. The current data for assessment of food, water, and general environmental exposures are limited and highly uneven. The most data are available for major baked, roasted, and fried food sources, most of which show low acrylamide levels of approximately 15–350 μg/kgof food. However, baked and fried carbohydrates containing asparagine and reducing sugars, especially potatoes cooked at high temperatures for prolonged periods, can produce 120–12,000 μg/kg of acrylamide. There is the possibility of acrylamide uptake by food from container coatings, but limits on free acrylamide in the polyacrylamide used in coatings are likely to minimize the levels of food contamination. There are no data to show the extent of contamination. Exposures by ingestion of contaminated food have been extrapolated from the limited data on food content by making assumptions about the quantities of food items eaten by various population subgroups. These estimates must be considered very rough and approximate. Uptake from cosmetics, consumer products, some gardening products, paper and pulp products, coatings, and textiles is possible because of contact with polyacrylamide containing free acrylamide, but such exposures have not been characterized.

Uptake is unlikely to exceed trace levels because of individual product limits on acrylamide content. Although acrylamide has been measured in cigarette smoke, there are no data on indoor exposures from environmental tobacco smoke. While the major routes of intake have been identified and indications of the ranges of exposure are available, there are insufficient statistical data to indicate the probability of exposure at various levels. For example, the highest concentrations appear to occur in some fried foods, but there have been no reported market basket survey data. Breast milk samples from only two pregnant women have been tested after consumption of potato chips to obtain an indication of uptake by a nursing infant.

Utility/Adequacy for CERHR Evaluative Process: The data for general population exposures are too limited and anecdotal to provide more than indications of possible exposures of importance. Risk assessments to estimate dose have been conducted by several agencies using a range of assumptions. Given the limited data to guide these calculations, the significance of these estimates is uncertain.

In estimating dermal exposure through contact with consumer products, the European Union *(8)* considered exposure patterns of cosmetic use, soil-conditioning gardening product use, and contact with paper and pulp products, coatings, and textiles that contain polyacrylamide. It was concluded that the only relevant consumer exposures resulted from sporadic use of soil conditioners (5 µg/use) and potential daily exposure to cosmetics (67 µg/day). Using assumptions for residual acrylamide levels (0.01%), 75% absorption, and 70 kg bw, a worst-case exposure level of 1 µg/kg bw/day was estimated. The European Union noted that the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Customers recommended reducing residual acrylamide levels in cosmetics. Based on those recommendations, the European Union estimated that use of new cosmetics would result in exposure levels that are 200–1,000 fold lower. Residual acrylamide levels in cosmetics sold in the U.S. are unknown.

Drinking water consumption was assumed by the European Union *(8)* to be the only significant source of human environmental exposure to acrylamide. Such exposure can be estimated by assuming that drinking water contains the maximum concentration of acrylamide (0.5 µg/L in the U.S., see Section 1.2.3), an intake rate of 2 L/day, and a body weight of 70 kg *(8)*. Based on these assumptions, the estimated exposure for adults would be 0.014 µg/kg bw/day in the U.S. The European Union *(8)* also estimated local human exposures that could potentially result following sewer repairs using acrylamide grouts. A value of 0.11 µg/kg bw/day was estimated for small scale repairs. Using acrylamide levels measured in surface or ground water following an incident during tunnel construction in Sweden and assuming that the contaminated water would be used for drinking water, a worst case exposure was estimated at 2,620 µg/kg bw/day. **[CERHR notes that the incident involved unintended leaking of a grouting agent into a nearby waterway and that this event represents an unlikely exposure scenario because the use of acrylamide grouts has been phased out]**.

Cigarette smoke is another source of acrylamide exposure. The acrylamide content in mainstream cigarette smoke has been measured at 1.1–2.34 µg per cigarette *(23)*. Levels of acrylamide-hemoglobin adducts in cigarette smokers are discussed in Section 1.2.4.3

1.2.4.2 Occupational exposures

Occupational exposure to acrylamide could occur during the manufacture of acrylamide monomers or polymers, during polyacrylamide use, in the preparation of polyacrylamide gels, and during the use of polyacrylamide grouts *(8)*. Exposure is a function of the quantity of free acrylamide present. Historically, under uncontrolled manufacturing conditions, exposures have been very high, such as 1–3 mg/m^3 in China *(27)*. During the use of polyacrylamide, where the

exposure comes from the residual acrylamide in the solid polymer, exposures are low. Exposure to acrylamide is possible for workers in a wide range of industries that use polyacrylamide: paper and pulp, construction, foundry, oil drilling, textiles, cosmetics, food processing, plastics, mining, and agricultural occupations. However, the amount of free acrylamide is limited to 0.1% in the polymer, which sharply limits the level of exposure the the monomer in situations where there is contact. Workers could be exposed by inhaling dusts or vapors and through dermal contact with monomers or polymers. In the National Occupational Exposure Survey, the National Institute of Occupational Safety and Health (NIOSH) estimated that 10,651 workers, 721 of them female, were exposed to acrylamide in 1981–83 *(28)*. Researchers or technicians who prepare polyacrylamide gels may also experience variable and intermittent exposures to acrylamide.

The American Council of Governmental Industrial Hygienists (ACGIH) established a time weighted average (TWA) threshold limit value (TLV) of 0.03 mg/m^3 for acrylamide based on central nervous system (CNS) effects, dermatitis, and carcinogenicity (observed only in experimental animal studies) *(29, 30)*. A skin notation was included because limited data demonstrated toxicity following absorption of acrylamide through intact skin of humans and animals. NIOSH *(31)* also established a TWA relative exposure limit (REL) of 0.03 mg/m^3, with a notation for dermal absorption for acrylamide. The Occupational Safety and Health Administration (OSHA) permissible exposure level (PEL) for acrylamide is 0.3 mg/m^3 *(32)*. **[The documentation for these standards may be useful to estimate exposures, but the limits themselves are not.]**

The European Union *(8)* summarized workplace exposures to acrylamide, values are listed in **Table 3**. Acrylamide exposures were highest during monomer production, with geometric means ranging from 0.09 to 0.13 mg/m^3. Polyacrylamide production had lower exposures, with geometric means from 0.01 to0.02 mg/m^3. Later stages of polymer production represent less risk for exposure because the excess acrylamide monomer becomes trapped in the polymer matrix. Use of acrylamide grout for sewer sealing applications enatils more opportunity for exposure because the free monomer is used to make the grout on site. In addition, the European Union review noted that some of the values were obtained for production work processes that have since been automated, which would have reduced exposure. Some of the measurements were taken during accidents or prior to installation of engineering controls. In addition, respiratory protection was used in some cases and actual respiratory exposures would be lower. The only U.S. data reported in Table 3 were for use of acrylamide grout in small-scale sewer repair operations. Those data were collected in two surveys conducted in 1986 and 1987 *(8)*.

Table 3. Workplace Inhalation Exposures to Acrylamide, European Union *(8)*[c]

Industry	Country	No. Samples	Arithmetic Mean (mg/m^3)	Geometric Mean (mg/m^3)	Range (mg/m^3)
Acrylamide manufacture	UK	11	0.18	0.09	0.05–0.34
	Germany[a]	44	0.01	No data	<0.001–0.022
	Netherlands[a]	87	0.17	0.13	<0.05–1.3
Polyacrylamide manufacture	UK[a]	422	0.05	No data	0.01–0.77
	UK[a]	10	0.03	0.02	0.001–0.08
	UK[a]	4	0.01	0.01	0.01
	Germany[a]	No data	No data	No data	all <0.03
	Germany[a]	23	0.03	0.02	<0.001–0.099
Electrophoresis gels	UK[b] (manufacture)	4	0.03	0.006	0.002–0.012
	UK (use)	2	0.04	NA	<0.005–0.067
Polyacrylamide use	EASE model	NA	NA	NA	0.0001–0.003
	UK[a]	No data	No data	No data	All <0.015
	Netherlands	NA	NA	NA	<0.001–0.012
Large-scale acrylamide grout use (i.e, tunnel work)	Sweden	9	0.018	0.01	0.005–0.076
Small-scale acrylamide grout use (i.e., sewer repair)	U.S.	5	0.047	0.029	0.008–0.12

[a]Personal samples. [b]Values measured within an air-fed pressure suit. [c]Information was obtained from Table 4.16 in the European Union report. Much of the information in this table is historical. See text for explanation. NA=Non-applicable. EASE=estimation and assessment of substance exposure.

Some U.S. occupational exposure data were reported by IARC *(11)* and values for personal exposures are summarized in Table 4. All of the data are more than 10 years old. As seen in the European data, monomer production had the highest exposures, with geometric mean values ranging from 0.065 to 0.085 mg/m^3 in the 1980s. In the 1970s levels were higher, approximately 0.1–0.9 mg/m^3. The overall trend in exposures in most U.S. chemical production facilities has been downward since the 1970s because of improvements in engineering controls and personal protective equipment. Polymer production levels in the 1980s had geometric mean values of 0.023–0.031 mg/m^3. Average exposures varied by job with utility workers having the highest geometric mean, 0.116 mg/m^3. It is not known if the monitored workers used personal protection or if engineering controls were utilized. It is likely that the use of all types of controls has increased. Neither Table 3 nor 4 consider exposure that could occur through the dermal route. Since the addition of skin notations to the TLV for acrylamide, it is likely that protective equipment currently used in most production workplaces where there may be skin contact.

Table 4. Workplace Inhalation Exposures to Acrylamide in the U.S., IARC *(11)*

Operation/ Job Description	Sample Type	No. Samples	Air Concentrations (mg/m^3)		Year(s) Measured
			Mean	Range	
Monomer production					
Reactor operator	Personal 4-h	1	0.48	NA	1971–1975
Dryer operator	Personal 4-h	1	0.52	NA	
Packing	Personal 4-h	2	0.64	0.52–0.76	
Control room	Personal 8-h	NR	NR	0.1–0.4	
Bagging room	Personal 8-h	NR	NR	0.1–0.9	
Processing	Personal 8-h	NR	NR	0.1–0.4	
Monomer and polymer production					
Monomer operators	Personal	19	0.065 GM	0.001–0.227	1984-1985
Polymer operators	Personal	27	0.031 GM	0.001–0.181	
Monomer material handlers	Personal	4	0.085 GM	0.017–0.260	
Polymer material handlers	Personal	4	0.023 GM	0.018–0.035	
Maintenance	Personal	14	0.013 GM	0.001–0.132	
Utility operators	Personal	4	0.116 GM	0.004–0.392	
Continuous monomer production	Personal TWA	NR	NR	0.1–0.6	1957–1970
Sewer Line Repair					
Grouting operation (2 sites)	Personal	12	0.010	0.003–0.02	1990
Grouting operation	Personal	2	0.005	0.002–0.007	1985
Grouting operation	Personal	6	0.10	0.008–0.36	1988
Coal plant					
Static thickening of coal waste	Personal	2	NR	<0.001	1992

NR=Not reported. NA=Non-applicable. GM=Geometric mean

Dermal contact with acrylamide powder, solution, or vapor condensation may be a significant source of worker exposure. The European Union *(8)* estimated dermal exposures to workers. Using an unvalidated method of measuring acrylamide levels on cotton liners worn within protective gloves, mean and high-end exposures were estimated, respectively, at 0.004 and 0.01 mg/cm^2 **[skin assumed]**/day during acrylamide manufacture and at 0.0004–0.01 and 0.08 mg/cm^2/day during polymer production. Dermal exposure during polymer use was predicted at from 1×10^{-5} to 1×10^{-4} mg/cm^2/day using the EASE model, which has large uncertainties for dermal exposures. For small-scale use of acrylamide grouts, the European Union used a dermal

exposure value of 5 mg/h in their risk characterization. They noted that additional dermal measurements included an acrylamide value of 2.49 mg/glove (4.98 mg/working shift total). However, it is clear neither how much of this acrylamide would have been absorbed, nor whether a worker could have tolerated this much acrylamide on his or her skin for 8 h. If all of the acrylamide were absorbed, the exposure would lead to very high internal doses. **[The very limited U.S. data are consistent with the European data; because EASE estimates are not precise, the Expert Panel believes it appropriate to assume the European values apply to U.S. workers]**.

A 2002 study by Pantusa et al. *(33*) measured air acrylamide exposures in personnel from biomedical research laboratories in Houston. Personal short-term air samples (15 minutes) were taken while subjects weighed crystalline acrylamide or poured liquid acrylamide for the preparation of polyacrylamide gels. Personal long-term air exposures were measured during the entire period when exposure to acrylamide was possible and TWAs were calculated. Twenty subjects used crystalline acrylamide while nine subjects used solutions. Latex gloves were worn by all subjects and five individuals using crystalline acrylamide wore dust masks. Fifteen subjects working with crystalline acrylamide and six working with acrylamide solution wore lab coats. Short term exposures exceeded the detection limit in all but three subjects using crystalline acrylamide and one subject using solution. Short-term exposures ranged from <0.00056 mg/m^3 to 0.022 mg/m^3 in users of crystalline acrylamide and from <0.0002 to 0.014 mg/m^3 in users of acrylamide solutions. TWA exposures ranged from 0.00007 to 0.020 mg/m^3 in subjects using crystalline acrylamide and from 0.00009 to 0.0028 mg/m^3 in subjects using solutions.

Strengths/Weaknesses: There are few current data for occupational exposures. In many of the historical situations, both inhalation and dermal routes of exposure were important. There are very few estimates of the degree of dermal contact; dermal exposure is difficult to estimate. The data from the Pantusa et al. study are somewhat useful for laboratory workers and show that these workers have very low exposures, although the number of observations is small. It is not clear how relevant the European Union data on 1980s production worker exposures are.

Utility (Adequacy) for CERHR Evaluative Process: The exposure data are inadequate for estimating current exposures. There are too few observations and the data are generally not current.

1.2.4.3 Exposures based on adduct formation

As discussed in Section 2.1.2, acrylamide and its metabolite, glycidamide, both can form covalent bonds with hemoglobin. The hemoglobin adduct products are being investigated as biomarkers of acrylamide exposure. Studies in rats have demonstrated dose-related increases in acrylamide adduct formation *(19*).

Calleman *(27*) reported a study conducted to establish relationships between total exposures, diagnostic indicators, and toxic effects in Chinese workers. The study examined biomarkers of acrylamide exposure and neurologic effects in 41 workers employed at a plant that produced acrylamide monomers and co-polymers. Ten people from the same city were also examined and used to determine control values. Mean levels of biomarkers are summarized in Table 5, according to job categories. Levels of acrylamide valine adducts ranged from 300 to 34,000 pmol/g globin in exposed workers and were directly proportional to glycidamide adducts. Levels of acrylamide adducts and acrylamide area under the concentration versus time curve (AUC) over the active lifetime of workers correlated most highly with neurotoxicity. Correlation was also noted with urinary mercapturic acid S-(2-carboxyethyl)cysteine but that biomarker is nonspecific because it also reflects acrylonitrile exposure. Plasma acrylamide levels correlated

poorly with neurologic symptoms. Based on toxicokinetic parameters extrapolated from measurements in rats and adduct formation in a suicide victim, a first-order elimination rate of 0.15 h^{-1} was estimated for humans and used in a model to convert hemoglobin adduct levels to mg/kg bw/day concentrations.

Table 5. Biomarkers of Acrylamide Exposures in Chinese Workers, Calleman *(27)*[a]

Job category	Plasma acrylamide (μmol/L)	S-(2-carboxyethyl)cysteine (μmol/24 h)	Acrylamide adduct (pmol/g globin)	Lifetime acrylamide AUC (mM*h)
Controls	0.92	3.0±1.8	0	0
Packaging	2.2	93±72	3,900±2,500	8.9±9.1
Polymerization	1.3	58±75	7,700±3,400	10.0±5.8
Ambulatory	2.0	53±35	9,500±7,300	11.3±9.8
Synthesis	1.8±0.8	64±46[b]	13,400±9,800	19.2±10.6

[a]Values presented as means±SD; however, SD were not listed for some values
[b]This value was reported as 643 μmol in the text of the study

A JIFSAN/NCFST *(19)* panel noted limitations regarding the estimation of human exposure levels to acrylamide based on adduct levels. First, the estimates represent exposures occurring over 120 days, the life of a human red blood cell. Second, adduct formation depends on numerous factors such as absorption of acrylamide and rate of metabolic removal. These factors limit the utility of adducts for predicting an individual's exposure. However, comparisons of adduct levels across exposure groups can give useful relative differences in exposure magnitude where the differences are large. Additionally, because toxicokinetics and metabolic factors vary among species, extrapolation of data from rodents may not result in an accurate description of acrylamide and glycidamide kinetics in humans.

Although estimation of exposure based on adduct formation is uncertain, some values are presented in this section for comparative purposes. Two recent original publications *(34, 35)* and values reported in a JIFSAN/NCFST *(19)* review are presented below.

In a study conducted at a German university, Schettgen et al. *(35)* measured hemoglobin adduct concentrations in 63 male and 9 female German subjects (ages 19–59 years) who were not exposed to acrylamide in the workplace. The subjects were first divided into groups of smokers and nonsmokers, based on the detection of N-cyanoethylvaline, an acrylonitrile adduct that is a specific and sensitive marker for cigarette smoke. Levels of N-2-carbamoylethylvaline, the acrylamide adduct, were reported separately for the 47 smokers and 25 nonsmokers, and those values are outlined in Table 6. The study authors noted that their values for acrylamide adducts, <12–50 pmol/g globin in nonsmokers and 13–294 pmol/g globin in smokers, were within the ranges reported in other studies, which were 20–70 pmol/g in nonsmokers and 116 pmol/g in smokers. Based on adduct levels, considerations discussed by Calleman *(27)*, and an elimination rate of 0.15 h^{-1}, Schettgen et al. *(35)* estimated an average acrylamide intake of 0.85 μg/kg bw/day in nonsmokers, and estimated that the value in smokers was about 4 times higher. These investigators also examined the relationships between cigarettes smoked per day and acrylamide adducts; they estimated 6.1 pmol/g globin was formed per cigarette, which compared well with the 8.5 pmol/g globin per cigarette observed by Fennel *(36)*. Mainstream cigarette smoke has been measured for acrylamide content, which was 1.1–2.34 μg per cigarette *(23)*. In combination with the estimates of adducts, there are approximately 3–8 pmol/g globin formed per μg of

acrylamide in cigarette smoke. This adduct formation represents pure inhalation exposure and may be helpful for calibrating other inhalation exposure data.

Strengths/Weaknesses: The adduct data have the advantage that they integrate all sources of exposure. Insofar as the exposures can be isolated, their relative magnitude can be estimated. Adduct levels in two groups of Germans *(34, 35*) and one group of Swedish unexposed workers *(37*) were 10–70 pmol/g globin. These adducts are most likely coming from drinking water and dietary exposures, and possibly from environmental tobacco smoke exposures. Adduct levels in smokers were higher by about 4-fold in nearly all cases. Workers with occupational exposures measured by Calleman *(27*) had adduct levels that were orders of magnitude higher. The toxicokinetics of acrylamide in subjects will not be identical, so some variability in between-subject differences is expected. Additionally, how representative the German and Swedish background levels are of other locations is unknown, although the levels were of the same order of magnitude. More importantly, we cannot determine how much of the variation in adduct levels resulted from differences in exposures or differences in toxicokinetics, but the range in background levels can provide an indication of the range in acrylamide from water and dietary sources, if not the magnitude of intake.

Utility (Adequacy) for CERHR Evaluative Process: The hemoglobin adduct data from Schettgen, et al. *(34, 35*) provide an estimate of the background level of adducts from drinking water, cosmetics, and dietary sources for nonsmokers. If the smoking rates of the smokers are known, then the rate of adduct formation by an inhalation exposure can be estimated. The occupational dermal exposures can be estimated if the inhalation exposures are known. Given that the adduct levels in smokers are much lower than those in occupationally exposed humans, the occupational inhalation exposures must be much higher than smoking expsosures.

Table 6. Levels of Acrylamide Adduct, N-2-Carbamoylethylvaline, in Non-Occupationally Exposed German Subjects, Schettgen et al. *(35*).

Measurement	N-2-Carbamoylethylvaline levels in pmol/g globin (μg/L blood)	
	Nonsmokers	Smokers
Range	<12–50 (NE–1.4)	13–294 (NE–8.0)
Median	21 (0.6)	85 (2.3)
95th Percentile	46 (1.3)	159 (4.3)

NE=Not estimated

In another publication, Schettgen et al. *(34*) measured levels of N-2-carbamoylethylvaline in workers exposed to ethylene and propylene oxide, but apparently not to acrylamide. The range of N-2-carbamoylethylvaline levels was <11–50 pmol/g globin in 24 nonsmokers and 16–294 pmol/g globin in 38 smokers.

A JIFSAN/NCFST review *(19*) summarized some values of acrylamide adducts measured in occupationally and non-occupationally-exposed individuals and those values are outlined in Table 7. Although quantitation of acrylamide exposure is uncertain, the values suggest that acrylamide exposures in some workers greatly exceed general population exposures. Values also show that cigarette smoking leads to a greater intake of acrylamide than dietary and other background sources.

Table 7. Acrylamide Adduct Levels Presented in Review by JIFSAN/NCFST *(19)*

Study Description	Subjects	Acrylamide Adduct Levels (pmol/g globin) [a]
Workers in a Chinese acrylamide and polyacrylamide manufacturing plant	Exposed workers	300–34,000
Comparison in smokers, nonsmokers, and lab workers using acrylamide to prepare polyacrylamide gels	Lab workers Smokers Nonsmoking controls	54 116 31
Comparison in workers with and without occupational exposure	Occupational exposure No occupational exposure	27–1,854 30
Tunnel workers exposed to acrylamide and N-methylacrylamide through grouting material	"Normal background range" in workers Remaining workers	20–70 ≤17,000

[a]Values were converted to pmol and the remaining units were reported as presented in the review. It is assumed that g globin was the denominator for all these values, although it was not specified in some cases.

1.3 Utility of Data

Overall there are very few data on the magnitude of current exposures. The available data do not represent a population sample, are often more than 10 years old, and some samples include so few data that they would be more appropriately considered anecdotal observations. Adduct data indicate that acrylamide exposures from dietary (including drinking water) and cosmetic sources are modest compared to exposures from smoking. Occupational exposures with substantial direct contact are much larger than exposures from smoking and diet. However, market basket sampling and other broadly based measurements are necessary to describe the mean and variance of dietary exposures, and industrial hygiene surveys are necessary to determine occupational exposures. More importantly, population data are needed to define the upper tail of the exposure distribution.

1.4 Summary of Human Exposure Data

Humans may be potentially exposed to acrylamide by ingesting contaminated food or drink, through dermal contact with contaminated materials, and by inhaling vapors or particles. Data available to characterize each of these routes are very limited.

Most of the exposure data are available for acrylamide present in food cooked at high temperatures. Starchy foods, expecially potato preparations, fried, grilled, or baked, had the highest levels, ranging from 120 to 12,000 μg/kg; other cooked foods were lower, ranging from 15 to 350 μg/kg. Estimates by several national and international groups indicated adult intakes in the range of 0.3–1.1 μg/kg bw/day and children's rates 2–3 times higher. A single test with two nursing women who ingested about 1 mg of acrylamide in potato chips showed breast milk to contain 3–19 ng/mL of acrylamide. Based on these data and a daily ingestion of 500 mL of milk, a 3-kg baby was estimated to have an intake rate of 0.7–3 μg/kg bw/day.

Industries producing or using large amounts of acrylamide may release significant quantities into the environment. Contamination of drinking water by industrial emissions into domestic water supplies is not a general problem because acrylamide is rapidly biodegraded. Acrylamide also does not undergo bioaccumulation in the aquatic food chain because it is highly water soluble and

not lipophilic. These features do not eliminate the possibility of a local pollution problem, but there are no data to indicate one exists near any major sources. Drinking water is commonly treated with polyacrylamide resins to remove suspended particulates. This practice was estimated to produce acrylamide concentrations much lower than 0.125 μg/L because of limits on the amount of free acrylamide in the polymer used, <0.05% w/w. The estimated exposure is 0.01 μg/kg bw/day from drinking 2 L/day. A similar argument can be made for minimal contamination of food or drugs by acrylamide leaking from container coatings (limited to <0.05% w/w) or gelatin capsules (limited to <0.2% w/w) that contain polyacrylamide. Thus, only trace exposures are likely from these sources.

Dermal contact with cosmetics, consumer products, some gardening products, paper and pulp products, coatings, and textiles is possible because the polyacrylamide used in these products may contain some free acrylamide, <0.01% w/w. However, the amount of acrylamide available from these sources is likely to be very small as a result of the restrictions on free acrylamide in the polymer.

Historically, occupational inhalation and dermal exposures have been substantial in the major producers and users of acrylamide. These exposure scenarios include production of the acrylamide monomer (0.09–0.13 mg/m^3) and polymer (0.01–0.02 mg/m^3), and in the use of acrylamide grout as a waste water system sealant (0.01–0.05 mg/m^3). One investigator (31) has estimated that skin absorption predominated in production exposures in China. Skin exposures and uptake are unknown and difficult to measure. Recent measurements of inhalation exposures during the laboratory preparation of polyacrylamide gels from acrylamide crystals or solutions reflect very low air levels, 0.00007–0.02 mg/m^3.

Cigarette smoke contains acrylamide, 1–2 μg/cigarette. This amount of acrylamide gives smokers an exposure that measurably increases their hemoglobin adducts, 3–4 fold higher than nonsmokers with no occupational exposure. Side-stream smoke has not been measured, but probably also contains acrylamide, which will lead to indoor environmental tobacco smoke exposures for nonsmokers. This source of acrylamide exposure has not been measured.

The relative intake of acrylamide from dietary sources, cigarette smoking, and occupational exposures can be estimated from measurements of acrylamide adducts on hemoglobin. Subjects without occupational exposures had a median adduct level of 21, range <12–50 pmol/g globin in nonsmokers and a median 85, range 13–294 pmol/g globin in smokers, consistent with values from 2 other studies. Based on those data, Schettgen et al. *(35*) estimated an intake of 0.85 μg/kg bw/day from dietary sources. Given a median adduct level about four times higher in smokers, smoking appears to be a much more important source of acrylamide exposure than the daily dietary intake based on the limited German and Swedish data. Adduct level ranges from occupational exposures have tended to cover a much wider range, going up to levels several orders of magnitude higher than in smokers, depending on the job activities. This finding is also consistent with the limited inhalation exposure data. While dermal exposures in occupational settings have been reported to be high enough to cause skin damage, the magnitude of human intake by skin absorption has not been measured and estimates are very rough.

2.0 GENERAL TOXICOLOGY AND BIOLOGIC EFFECTS

As noted in the CERHR Expert Panel Guidelines, Section 2 of this report is initially based on secondary review sources. Primary studies are addressed by the Expert Panel if they contain information that is highly relevant to a CERHR evaluation of developmental or reproductive toxicity or if the studies were released subsequent to the reviews. For primary study reports that the Expert Panel reviewed in detail, statements are included about the strengths, weaknesses, and adequacy of the studies for the CERHR review process.

2.1 Toxicokinetics and Metabolism

2.1.1 Absorption

There are no known quantitative data on acrylamide absorption in humans. However, symptoms observed in poisoning cases or occupational exposures indicate that acrylamide is absorbed through oral, dermal, and inhalation exposure routes *(7, 8*).

A pilot study conducted by a German group demonstrated that acrylamide is absorbed from food *(26*). Urinary acrylamide levels were measured in 9 healthy male volunteers (age 18–52 years) before and within 8 h after they ate up to 500 g of commercially available potato chips or crisp bread. A newly-developed liquid chromatography/tandem mass spectrometry (LC/MS/MS) method was used to quantitate acrylamide levels. Before eating, urinary acrylamide levels could only be estimated in 3 subjects and ranged from 0.659 to 2.04 ng/mL or from 0.338 to 1.084 µg excreted (quantification level reported at 1 ng/mL). Within 8 h after eating potato chips or crisp bread, acrylamide was detected in the urine of 8 subjects at levels ranging from 1.97 to 6.31 ng/mL or from 0.482 to 5.702 µg excreted. **[There was no mention of dietary restrictions prior to the study, the amount of acrylamide in food samples was not reported, and acrylamide intake was not estimated.]** Acrylamide half-lives were measured using urinary excretion data in two subjects and those values are reported in Section 2.2.4. In addition, Sorgel et al. *(26*) measured acrylamide levels in human milk and conducted an *in vitro* placental transfer study, as discussed in Section 2.1.2.

Strengths/Weaknesses: The greatest strength of the study by Sorgel et al. *(26*) is the attempt to obtain toxicokinetic data in humans exposed to acrylamide in food. Qualitative data are presented indicating that ingestion of acrylamide-containing food results in increased levels of the chemical in urine and milk. This pilot study was limited by its small sample (9 men and 2 lactating women) and qualitative nature. The study also is hampered by the fact that intake was not determined and by the measurement of acrylamide in the urine rather than in blood plasma or serum. Excretion of acrylamide in urine accounts for only a small percentage of absorbed material and does not account for the large amount that was metabolized. The report suffers from inadequate, sketchy descriptions of the methodology used, which makes the validity of the reported data questionable.

Utility (Adequacy) for CERHR Evaluation Process: The data are not useful for a quantitative assessment of acrylamide absorption.

Rapid and complete uptake of acrylamide from the gastrointestinal tract of rats was evident by elimination rates that were similar following gavage and i.v. dosing *(6, 11, 38*). Rapid absorption from the gastrointestinal tract was also demonstrated in studies with mice, dogs, and miniature pigs *(8, 39*).

Animal studies indicate absorption through the dermal route. In rats, 25% of 2 or 50 mg/kg bw doses were absorbed within 24 h; 26% of a 0.5% aqueous solution was absorbed within 24 h with 35% remaining in the skin *(38)*. Another review reported 14–61% absorption of a dermally applied dose in rats *(39)*. In studies comparing oral and dermal administration of ^{14}C-acrylamide in mice, less label was found in internal organs with dermal than with oral dosing *(6)*; based on average ratios for crude binding to proteins, RNA, and DNA, Calleman *(27)* estimated that uptake through dermal exposure was about 35% of uptake through oral exposure. Studies in rabbits demonstrated that a 10–30% aqueous solution of acrylamide was rapidly absorbed through skin *(6, 7)*. *In vitro* skin absorption studies demonstrated 42–93% absorption in rats, 94% absorption in pigs, and 27–33% absorption in humans *(39)*. Variability of dermal absorption in rats was confirmed in 4 animals treated topically with 162 mg/kg acrylamide that absorbed 14, 15, 27 and 30% of the administered dose *(40)*.

[The Expert Panel notes that information comparing *in vitro* dermal absorption in rats, pigs, and humans was obtained from three different studies. Direct comparison of the three studies, which may have been conducted by different methods, may not be appropriate.]

An unspecified amount of acrylamide was reported to be absorbed in mice and rats following a 6-h inhalation exposure to 3 ppm acrylamide *(39)*.

Strengths/Weaknesses: The secondary sources disagree on whether acrylamide absorption is complete. Some such as Calleman and the NIOH/NIOSH document *(38)* reach such a conclusion but others such as the JIFSAN/NCFST workshop suggest that such a conclusion cannot be reached on the basis of the Miller study, which itself does not come to such a conclusion. The workshop further pointed out that in another study by Barber et al. *(41)* there was a difference in absorption between oral and i.p. administration of acrylamide, suggesting that absorption from the gut is incomplete although the different dose levels used, 20 and 50 mg/kg, might account for some of the discrepancy.

Utility (Adequacy) for CERHR Evaluation Process: The data indicate that absorption following oral administration in rats is rapid. Different studies showing differences in absorption following dermal applications cannot be directly compared. There may be more than a two-fold difference among individual animals in dermal absorption.

2.1.2 Distribution

Studies in rats, pigs, and dogs demonstrate that acrylamide absorbed through any exposure route is rapidly distributed throughout the body *(38)*. Higher concentrations have been found in the liver and kidney of rats than in other tissues *(6)*. Multiple dosing with 0.05 or 30 mg/kg bw for 13 days did not greatly alter distribution in most tissues, with the exception of red blood cells, plasma, and testes *(38)*. Acrylamide and its metabolite glycidamide form adducts with sulfhydryl groups of hemoglobin and about 12% of the radiolabel is found in the red blood cells of rats dosed with radiolabeled acrylamide *(27)*. These adducts persist in red blood cells with a half-life of 10.5 days, reported in several reviews *(6, 38, 39)*. Calleman *(27)* noted that the half-life estimate of 10.5 days is most likely incorrect: the estimate mistakenly assumed first-order kinetics for elimination because calculations did not consider weight gain of the animals. In contrast to red blood cells, plasma levels of acrylamide or metabolites drop quickly *(6)*. In testis, fat, liver, plasma, and kidney, there is a delay in reaching peak levels due to an initial absorption phase of ^{14}C-labeled chemical *(6, 38)*. The high lipid content of the testis may account for the delay in absorption *(42)*. Acrylamide and its metabolites were not found to bioconcentrate in neural tissues *(38)*. While the formation of acrylamide DNA adducts is unlikely *in vivo* and has not been detected, glycidamide was observed to form adducts with DNA in rats *(19, 27)*.

One human study was reviewed in detail because it measured transfer of acrylamide across the placenta and into milk.

Sorgel et al. *(26)* measured the *in vitro* transfer of acrylamide in three human placentae. Acrylamide was added to a tissue culture maternal perfusate at 1 µg/mL and levels of acrylamide were measured in maternal and fetal perfusate by LC/MS at 1, 3, 5, 9, 15, 25, and 30 minutes during perfusion. Average placental penetration of acrylamide was 18.2–23.2% during 5 through 30 minutes of perfusion. There was considerable variability with individual placental transfer percentages ranging from 8.9 to 49.6.

In the milk transfer study conducted by Sorgel et al. *(26)*, acrylamide levels in milk were measured in 2 mothers (age 24 and 33 years) prior to and at 1, 3, 4, and 8 h after consuming food contaminated with acrylamide. The mothers did not consume food that could be contaminated with acrylamide for 10 h prior to the study. During the study, 1 mother ate 100 g of self-prepared potato chips, resulting in an estimated acrylamide intake of 1 mg. The second mother ate 100 g of commercially available potato chips, resulting in an estimated intake of 800 µg acrylamide. Prior to consuming the chips, milk acrylamide levels were below the quantification level (5 ng/mL by LC/MS) in both mothers. In the first mother, acrylamide was detected in the milk at 3 and 4 h post-dosing at 10.6 and 18.8 ng/mL, respectively. Acrylamide levels in milk from the second mother were measured at 4.86 and 3.17 ng/mL at 4 and 8 h post dosing, respectively. Acrylamide levels at all other time points were below the quantification limit. Sorgel et al. *(26)* estimated infant exposure levels based on acrylamide levels in milk and those estimates are discussed in Section 1.2.4.1.

Strenths/Weaknesses: The placental transfer studies of Sorgel et al. *(26)* used only three placentae, with considerably variability in the transfer results. While the authors attributed this variability to individual characteristics of the subjects, it is not possible from the paper to know whether there were technical problems in the placental perfusion preparations. The physical functional condition of the perfused placentas was not ascertained before or after the study. The data on transfer in milk have the advantage of including estimates of acrylamide intake and milk measurements before and after ingestion of potato chips.

Utility (Adequacy) for CERHR Evaluation Process: The Panel has no confidence that the placental perfusion studies accurately represent *in vivo* placental transfer of acrylamide. The milk studies are useful in estimating potential exposure of nursing infants after maternal consumption of acrylamide-containing foods.

Another study evaluated acrylamide-specific hemoglobin adducts (*N*-2-carbamoylethylvaline and *N*-cyanoethylvaline) in the blood of 10 nonsmoking women and 1 smoking woman a few hours prior to childbirth and in umbilical cord blood of their 11 neonates *(43)*. The highest concentration of adducts were found in the blood of the smoking woman and her neonate, and these samples were the only ones with detectable *N*-cyanoethylvaline, which is specific for cigarette smoking. The maternal and neonatal levels of *N*-2-carbamoylethylvaline were correlated in the nonsmokers (Pearson r=0.859 **[*P*=0.0015]**). Based on the maternal adduct concentrations, the authors estimated an average daily intake of acrylamide for nonsmoking pregnant women at 0.85 µg/kg bw. Taking into consideration the relative lifespans of maternal and fetal erythrocytes, the authors estimated that fetal acrylamide internal dose on a weight adjusted basis would be at least equal to that of the mother.

Strenths/Weaknesses: The paper by Schettgen et al. clearly demonstrates a hemoglobin adduct of acrylamide, namely *N*-2-carbamoylethylvaline, in the cord blood of newborn humans. The study has strengths in the number of humans studied *(14)* and a good statistical relationship between levels in the mothers and in the cord bloods. The data indicate that there is a fair degree of parity between the levels observed in the mothers and the levels in their offspring. Although there was only one mother who smoked, her data support the conclusions of other studies with respect to higher levels of acrylamide adducts in smokers. Weaknesses include the lack of information on exposure and the reliance on another study for exposure estimates, but these estimates may not be too far off. The extension of the calculations based on the size of the neonates and the half-life of fetal erythrocytes is difficult to understand.

Utility (Adequacy) for CERHR Evaluation Process: These data are very useful in estimating acrylamide placental transfer of acrylamide to term human fetuses. The study provides confirmation of existing experimental animal data on placental transfer of acrylamide and its metabolic products. The experimental animal and human data make reasonable the estimate that exposure of the near-term fetus is similar to maternal exposure.

A number of original animal studies were reviewed in detail by CERHR because they examined maternal-fetal toxicokinetics or distribution within testes.

Edwards *(44)* gave a single 100 mg/kg i.v. dose of acrylamide **[purity unstated]** in water to pregnant Porton strain rats on gestation day (GD) 14 (n=4) or 21 (n=2) **[plug day not specified]**. One hour after dosing, rats were decapitated and fetuses removed. Fetuses were homogenized and extracted in 0.1% tris in methanol. Acrylamide in the extract was measured spectrophotometrically. Fetuses sampled on GD 14 gave mean acrylamide concentrations (± SEM) of 1.41 ± 0.03 μmol/g. The two litters sampled on GD 21 gave acrylamide concentrations of 1.43 and 1.41 μmol/g. The authors cite a study showing that 1 h after male rats were given acrylamide 100 mg/kg i.v., mean blood acrylamide concentration (± SEM) was 1.28 ± 0.04 μmol/mL. The author concludes that this finding "indicates that the placenta does not act as a barrier to acrylamide."

Strenths/Weaknesses: A strength of this study is its use of the Porton strain of rats, permitting evaluation of a different strain than is used by other investigators. It is a weakness that this study used only one acrylamide dose, which was extremely high compared to anticipated human exposures, and the number of litters sampled was very low. Adult values were obtained from a previous study in males, rather than from the dams in the current study. In addition, the spectrophotometric detection of acrylamide may lack the necessary specificity.

Utility (Adequacy) for CERHR Evaluation Process: These data are most useful in estimating comparable placental transfer of acrylamide in the Porton rat compared to other strains.

In an industry-funded study, Marlowe et al. *(45)* administered a single ^{14}C-acrylamide dose (116–121 mg/kg bw) **[purity unstated]** by gavage to male Swiss-Webster male or female mice on GD 0.5 or 17.5 (plug day considered GD 0.5 at noon) **[number of animals treated was not specified]**. Male mice were anesthetized and frozen at various time points between 0.33 to 9 h and 1, 3, and 9 days following treatment. Pregnant mice were anesthetized and frozen 3 or 4 h following treatment. The mice were later sectioned and exposed to x-ray film. Autoradiography results indicated that absorption was nearly complete within 3 h and radioactivity was widely distributed. Distribution was similar in the male and pregnant female mice. In male mice, radioactivity was detected in testis parenchyma at 1 h, in the seminiferous tubules and head of epididymis at 9 h, and only in tail of epididymis and crypts of glans penis epithelium at 9 days.

The authors noted that the movement of acrylamide through the testis paralleled that of spermatids. Radioactivity in GD 13.5 fetuses was fairly evenly distributed with possibly higher levels in the CNS compared to maternal mice. At 17.5 days, the fetal distribution pattern closely resembled that of adult rats; radioactivity in fetuses was concentrated in kidney, bladder, liver, intestinal contents, and forestomach mucosa. An intense accumulation of radioactivity was noted in fetal skin on GD 17.5.

Strenths/Weaknesses: A strength of this study is the tracking of distribution over time and the use of an alternative method for evaluating tissue distribution of a chemical. The semiquantitative nature of the results is a weakness of the study. In addition, the results shown were claimed to be representative of all animals studied, but the number of animals studied was not given in the paper. The study used only a single acrylamide dose, which was high compared to anticipated human exposures. A limitation of the study is that it speaks only to the distribution of a mixture of unchanged acrylamide and its metabolites.

Utility (Adequacy) for CERHR Evaluation Process: This study provides useful semiquantitative information on distribution, and confirms placental transfer as shown in other studies by other techniques. There are relatively high levels of acrylamide/metabolites in the testis. The high radioactivity in the intestinal contents 9 days after the acrylamide dose suggests eneterohepatic cycling of acrylamide metabolites.

In a study conducted at the FDA, Ikeda et al. *(46)* examined maternal-fetal distribution of acrylamide in pregnant beagle dogs and Hormel miniature pigs. The dog is noted to have a four-layer endothelial-chorial placenta and the pig has a six-layer epithelial-chorial placenta. A single i.v. dose of 5 mg/kg bw acrylamide (reagent grade)/^{14}C-acrylamide (radiochemical purity of ≥95%) was administered to dogs on GD 60 and pigs on GD 109. Dogs and pigs were anesthetized 110 minutes following treatment and fetuses were separated at 2 h. Tissue radioactivity levels were measured in a total of 6 dog litters with 33 fetuses and 7 pig litters with 45 fetuses. Radioactivity was widely distributed in maternal and fetal tissues of both species. In dogs, the placental distribution factor was 17.7%, indicating that 82.3% of radioactivity passed through the placenta. Blood–brain distribution factors were insignificant in maternal (5.9%) and fetal (0%) dogs. A placental distribution factor of 31% in pigs indicated that 69% of the radioactivity passed through the placenta. An insignificant blood–brain distribution (4%) was noted in maternal pigs, while radioactivity levels were higher in brain versus blood of fetal pigs. The authors concluded that both fetal dogs and pigs lack blood–brain barriers and their brains would therefore be exposed to acrylamide present in their circulation.

Strenths/Weaknesses: The 1985 paper by Ikeda et al. *(46)* is very important with respect to uptake by dog and pig fetuses. The data are generally very good compared to the data underlying many other papers. The important conclusion regarding the possible vulnerability of the brain to exposure is warranted. Although the dose is still high (5 mg/kg) compared to anticipated human exposure under most circumstances, it is lower than in many other studies. The evaluation of total radioactivity restricts the interpretation to a mixture of unchanged acrylamide and its metabolites.

Utility (Adequacy) for CERHR Evaluation Process: The general conclusion of this study is valid and useful. The observation of placental transfer in these animals as in laboratory rodents supports the conclusion that placental transfer in humans is likely to occur in a similar manner.

In another FDA study, Ikeda et al. *(47)* sought to determine intra-litter distribution of acrylamide in four animal species with different placenta types. The species examined were Osborne-Mendel

rats (single-layer hemoendothelial placenta), New Zealand White rabbit (single-layer hemoendothelial placenta), beagle dogs (four-layer endothelial-chorial placenta), and Hormel miniature pigs (six-layer epithelial-chorial placenta). Animals were given an i.v. dose of ^{14}C-acrylamide (≥95% radiochemical purity)/reagent grade acrylamide late in gestation at 1 or 2 days prior to expected delivery (i.e., GD 20 for rats, GD 30 for rabbits; GD 60 for dogs, GD 109 for pigs). The dose for rats was 10 mg/kg bw and fetuses were removed 1 h following dosing. In the remaining species, the dose was 5 mg/kg bw and fetuses were removed at 2 h following dosing. All fetuses in 6–9 litters were examined for all species. The study authors reported that that radioactivity was uniformly distributed throughout the litters. Fetal uptake of radioactivity was not affected by fetal sex or fetal position within the uterus. Individual tissues were analyzed in fetal dogs and pigs and it was reported that uptake of radioactivity in individual tissues was also unaffected by uterine position.

Strenths/Weaknesses: The Ikeda et al. 1983 paper *(47*) is well done. The conclusions regarding distribution of the 14C-acrylamide are well justified by the data. A weakness is that it would have been useful to have more quantitative detail on the tissue distribution of the material that was administered. The evaluation of total radioactivity restricts the interpretation to a mixture of unchanged acrylamide and its metabolites.

Utility (Adequacy) for CERHR Evaluation Process: Ikeda et al.'s conclusions add to the overall weight of evidence of the ready uptake of acrylamide into fetuses from the maternal circulation. The observation of placental transfer in four species with different types of placentas supports the conclusion that placental transfer in humans is likely to occur in a similar manner.

2.1.3 Metabolism

It has been reported that major metabolic pathways for acrylamide are qualitatively similar in humans and experimental animals, but that quantitative differences must be considered when conducting risk assessments for humans *(17*). A proposed metabolic pathway is illustrated in Figure 2.

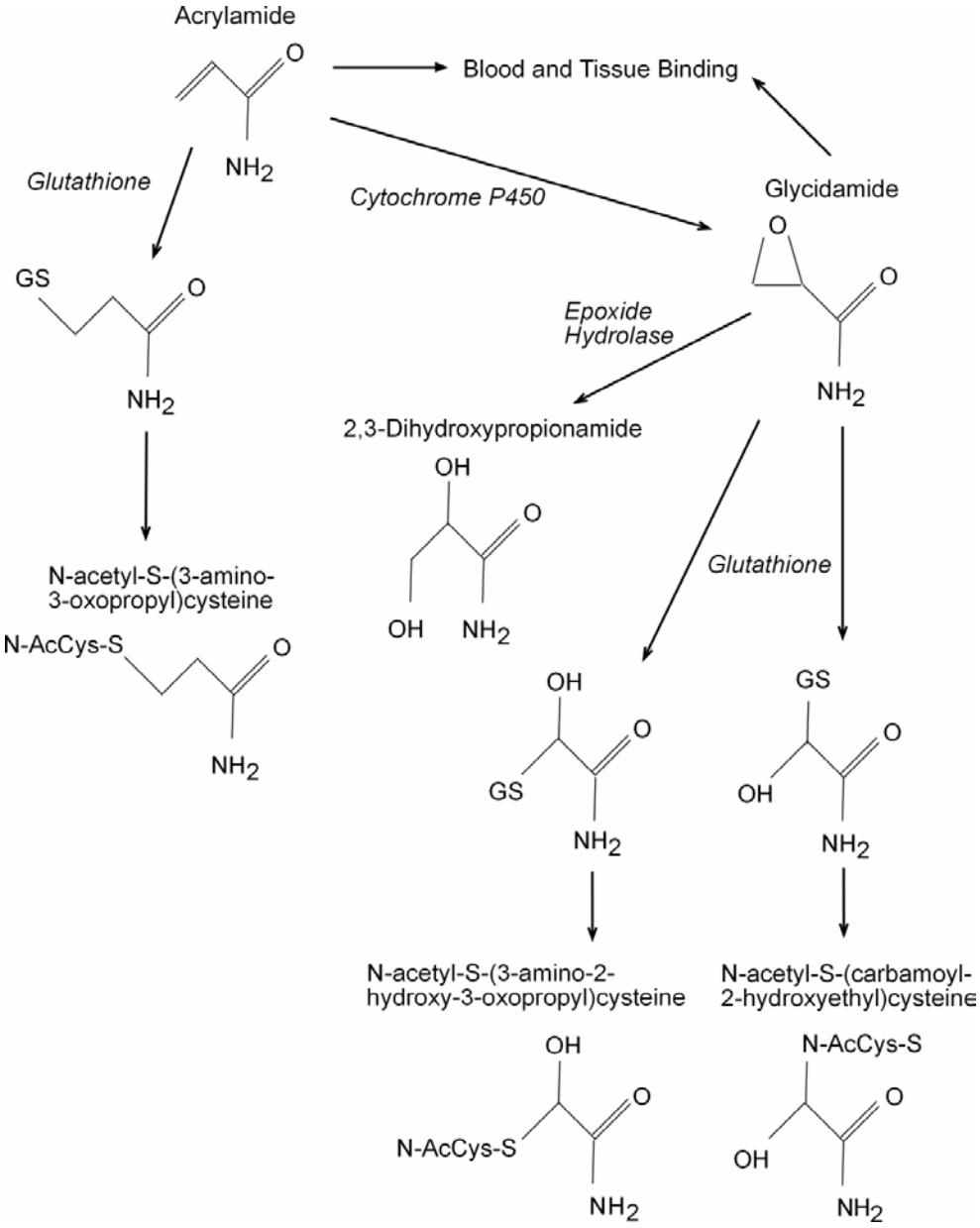


Figure 2. Acrylamide Metabolic Pathway, Adapted from Kirman et al. *(48)*

In experimental animal studies, acrylamide is rapidly eliminated through biotransformation. It has been reported that <2% is excreted unchanged through urine or bile in rats *(6, 38, 39*). In other rat studies, 80% of radiolabel was excreted within 7 days, 90% as metabolites *(7*). One major pathway for acrylamide biotransformation is first order conjugation with glutathione, catalyzed by hepatic glutathione-S-transferase (GST) *(38, 48*). The pathway leads to the formation of the urinary metabolite N-acetyl-S-(3-amino-3-oxypropyl) cysteine in rats and mice. N-acetyl-S-(2-carbamoylethyl) cysteine is the metabolite excreted in human urine *(14*). GST is present in the liver, kidney, brain, and erythrocyte of mice and rats, but the enzyme is 3 times more efficient at conjugating acrylamide in the liver versus the brain of rats *(39*). The second major metabolite of acrylamide, glycidamide, is formed through a saturable reaction with cytochrome P450 *(48*). There is some evidence that acrylamide can induce cytochrome P450 *(27*). Cytochrome P450 2E1 (Cyp2E1) is the specific enzyme involved in this reaction in mice *(49*). Glycidamide is metabolized through conjugation with glutathione to form mercapturic acids or metabolized by epoxide hydrolase or epoxide hydratase *(27, 48*).

It appears that the percentage of acrylamide converted through each major pathway may vary by species and dose. Conversion of acrylamide to glycidamide is greater in mice versus rats as evident by the observation that in rats, N-acetyl-S-(3-amino-3-oxypropyl) cysteine represents 70% of urinary metabolites, while this compound represents 40% of urinary metabolites in mice *(39*). Consistent with that observation, it was reported that the proportion of glycidamide-derived metabolites in urine following a dose of 50 mg/kg bw was 59% in mice and 33% in rats *(11*). The percentage of glycidamide excreted in the urine of mice and rats was 16.8 and 5.5%, respectively, following a dose of 50 mg/kg bw *(27*).

The excretion rate of radiolabel was independent of dose in rats orally administered 1.0–100 mg/kg bw radiolabeled acrylamide *(42*). In rats, high doses of acrylamide can inhibit GST or deplete GSH *(6, 7, 39*). One model suggested that appreciable glutathione depletion is not expected in the rat until doses exceed 10 mg/kg bw/day *(48*). Conversion of acrylamide to glycidamide appears to saturate because it was reported that the proportion of glycidamide produced is inversely related to dose *(17*). Consistent with that observation was a non-linear dose response for glycidamide adduct formation in rats i.p. injected with acrylamide doses ranging from 5 to 100 mg/kg bw; percent conversion to glycidamide was estimated at 51% following a 5 mg/kg bw dose and 13% following a 100 mg/kg bw dose of acrylamide *(39, 50*). The formation of acrylamide adduct was linear in rats at doses up to 100 mg/kg bw. It was noted in an IARC *(11*) review, that although specific urinary metabolites were not studied at doses below 50 mg/kg bw, increases in glycidamide-derived metabolites and decreased in acrylamide-derived metabolites would be expected at low doses. **[The Expert Panel finds the study of Bergmark et al. *(50*) to be ciritical in identifying the relationship between acrylamide dose and glycidamide adduct formation]**

The detection of glycidamide-hemoglobin adducts in workers exposed to acrylamide demonstrates that humans also metabolize acrylamide to glycidamide *(27*). The average AUC ratio of glycidamide to acrylamide in the workers was 0.3, a value that is lower than the ratio estimated for rats (0.58). Calleman stated that the lower ratio in humans indicates either lower conversion of acrylamide to glycidamide or increased glycidamide elimination in humans. Further investigation revealed that levels of urinary mercapturic acids were much lower in humans compared to rats and suggested that the acrylamide elimination rate in humans may be at least 5 times slower than the rate for rats. Based on estimates of free acrylamide in plasma, hemoglobin valine adducts, urinary mercapturic acid levels, and a lower integrated concentration-time ratio for glycidamide to acrylamide in humans, IARC *(11*) estimated that tissue doses of glycidamide may be higher in humans versus rats at equivalent doses of acrylamide. **[The Expert**

Panel believes the IARC authors may have misunderstood the Calleman report. The difference in rates of elimination of both acrylamide and glycidamide in humans and rats will already have been reflected in their respective AUCs].

2.1.4 Elimination

Half-lives for acrylamide urinary excretion were reported at 2.2 h and 7 h in two male subjects who consumed an unspecified amount of acrylamide by eating potato chips or crisp bread *(26)*. Additional details of this study are included in Section 2.1.1. **[The Expert Panel considers the Sorgel et al. *(26)* data, based on only 2 individuals, to be unreliable]**.

Clearance of parent compound in rats is represented by a single compartment model, while clearance of total ^{14}C label is represented by a biphasic curve *(38)*. At doses of 10–20 mg/kg bw acrylamide in rats, the half-life of parent compound in plasma is reported at ~2 h *(17, 38)*. The half-life for the metabolite glycidamide is also reported at ~2 h following oral exposure of rats to 20 mg/kg bw or i.p. exposure to 50 mg/kg bw acrylamide *(39)*. Biphasic distribution and elimination is noted for total ^{14}C-label in rats with an initial half-life of 5 h and a terminal half-life of 8 days in most tissues *(6, 38)*. The initial part of the half-life (5 h) is thought to result from biotransformation of acrylamide and binding of metabolites to macromolecules; the terminal portion of the half-life (8 days) is most likely due to the release of metabolites from tissues and degradation of adducts. The initial elimination half-life for testes (8 h) in rats was slightly longer than the 5-h half-life observed in most other tissues *(42)*.

Excretion half-life of parent compound through rat urine is reported at ~8 h *(6, 7)*. In animal studies where radiolabeled acrylamide was given by the oral, dermal, or inhalation routes, 40–90% of label was eliminated through urine *(27, 39)*. Four to six percent of the dose is eliminated as carbon dioxide in exhaled air (indicating an unidentified metabolic pathway) and 6% of the dose is eliminated in feces within 7 days *(6, 38, 39)*; because the amount of radiolabel entering bile is estimated at 15%, it appears that acrylamide or its metabolites undergo enterohepatic circulation. Calleman et al. *(27)* noted that exhalation of radiolabeled carbon dioxide was not consistently observed among different studies and only occurred when acrylamide was labeled at the carbonyl carbon but not the vinyl carbon.

2.1.5. Physiologically-based pharmacokinetic (PBPK) models

The JIFSAN/NCFST *(39)* review noted an attempt to develop a PBPK model for acrylamide; the report was eventually published as Kirman et al. *(48)*. It was noted that the model was developed using data from six published studies and provides a good description of acrylamide and glycidamide kinetics in the rat. The model focuses on internal dose measures for possible mechanisms of toxicity, including genetic toxicity, reaction with sulfhydryl groups, dopamine agonist activity, and glutathione depletion. Variations in dose-metrics were found to result from model parameters characterizing tissue binding and biotransformation through cytochrome P450 and GST. **[The Panel notes that although an excellent paper, the Kirman et al. report is limited to rats given a single dose of acrylamide. No fetal compartment was included in the model. This paper is useful, however, in reviewing and modeling tissue-level data].**

2.2 General Toxicity

2.2.1 Human data

Information on acrylamide toxicity in humans is based upon case studies and occupational epidemiological studies. Exposure levels in such studies are not well characterized due to exposure usually occurring through multiple routes (e.g. oral, inhalation, and dermal) and lack of a reliable biological index to determine total body burden *(7, 8)*.

In cases of acute or subacute poisoning, CNS effects develop within hours or days of exposure *(7)*. Common CNS effects include confusion, memory problems, sleepiness, slurred speech, inability to concentrate, and hallucinations *(7, 38)*. Peripheral neuropathies develop insidiously following a latency period of days to weeks. Axonopathies are most commonly observed and impairment occurs in sensory fibers prior to motor fibers *(6)*. Common symptoms or clinical signs of peripheral neuropathies include loss of sensation, paraesthesia, numbness, muscle weakness and/or wasting in extremities, and decreased tendon reflexes *(7, 38)*. Tremors and gait disturbances may occur as a result of midbrain and cerebellum disturbances *(6)*. Anorexia, weight loss, and nystagmus have also been observed with acrylamide exposure *(7, 38)*. In some cases, the reporting of symptoms such as sweating, peripheral vasodilation, and difficulty urinating and defecating suggest autonomic nervous system involvement *(7)*. The most consistent electrophysiological finding is reduced nerve action potential amplitude in the distal portion of sensory neurons *(7)*. Generally neurologic symptoms continue to deteriorate for three to four weeks after exposure ends and then a gradual improvement occurs over a period of months to years *(6, 7)*. A full recovery occurs in most poisoning victims.

Symptoms consistent with CNS involvement followed by the development of peripheral neuropathies were observed in a 48-kg woman who ingested 18 g of acrylamide (375 mg/kg bw); additional symptoms included seizures, gastrointestinal bleeding, respiratory distress, and liver toxicity *(6, 8)*. CNS symptoms and subsequent development of peripheral nervous system effects were also observed in adults of a Japanese family following contamination of their well water by an acrylamide grout used in sewer repair *(6, 8, 51)*. The adults recovered within 4 months. Less severe effects were observed in a 10-year-old and 13 year-old child, presumed to be due to lower exposures because of school attendance during the day. An acrylamide level of 400 ppm was measured in the well water at a single time point.

In occupational settings where exposures occurred through inhalation and dermal contact, dermatitis characterized by peeling of skin on palms was the first sign of toxicity; it was followed by the development of peripheral neuropathies, as described above *(38)*. Such effects were noted in workers of a Chinese acrylamide factory who did not have adequate personal protection and were exposed dermally to a 27–30% aqueous acrylamide solution from 1 to 18 months. Air concentrations were not measured for most time periods but were reported at 5.6 to 9.0 mg/m^3 during a 4-month period of heavy activity. Air concentrations were reduced to 0.03 mg/m^3 following renovations at that plant. Some cases of dermatitis and peripheral nervous system effects were noted in additional occupational surveys addressed in a review by the European Union *(8)*. The review noted that prevalence of symptoms related to peripheral neuropathies is increased in workers exposed to >0.3 mg/m^3 acrylamide (8-h TWA), but it is not possible to determine contribution from skin exposure.

Effects of occupational acrylamide exposure in workers were reviewed by NICNAS *(52)*. Clinical examination and nerve conduction studies showed abnormalities most consistently in workers with symptoms, but the authors of one study *(53)* concluded, "Electroneuromyographic changes, including a decrease in the sensory action potential amplitude, neurogenic abnormalities in electromyography, and prolongation of the ankle tendon reflex latency, are of greater importance in the early detection of acrylamide neurotoxicity since they can precede the neuropathic symptoms and signs." According to the review, atmospheric acrylamide concentrations in the workplace ranged from 0.03 to 9 mg/m^3, and skin contact with acrylamide-contaminated water also occurred. Estimates of personal doses of affected and unaffected workers were not provided.

2.2.2 Experimental animal data

Neurologic effects such as ataxia, tremors, convulsions, and muscular weakness were observed in rats, mice, guinea pigs, rabbits, and cats following acute acrylamide poisonings *(7)*. Additional signs observed included circulatory collapse and weight loss. Death can occur following acute exposure to high acrylamide levels and LD_{50}s observed in various species are listed in Table 8.

Table 8. Ranges of LD_{50}s Reported from Acrylamide Exposures

Species (route)	LD_{50} in mg/kg bw	References
Rat (oral)	107–251	IPCS *(7)*, European Union *(8)*, NTP *(54)*
Rat (dermal)	400	IPCS *(7)*, NTP *(54)*
Rat (i.p.)	90–120	IPCS *(7)*, NTP *(54)*
Mouse (oral)	107–170	IPCS *(7)*, NTP *(54)*
Guinea pig (oral)	150–180	IPCS *(7)*, European Union *(8)*
Rabbit (dermal)	1,148	European Union *(8)*
Cat (i.v.)	85	IPCS *(7)*

Neurologic effects are also observed following repeated exposures of experimental animals to acrylamide. Effects are similar to those noted in humans and result from a peripheral neuropathy that starts at distal portions of limbs, then advances to proximal regions *(7)*. Overt signs are consistent among different species and include tremors, incoordination, motor dysfunction, neuromuscular weakness, and reduced motor nerve conduction velocity *(7, 39)*. Histologic evidence of neurotoxicity includes degeneration of distal portions of long sensory and motor peripheral nerve fibers. Degeneration of long axons in the spinal cord, Purkinje fibers in the cerebellum, the optic nerve, and autonomic fibers has also been reported in experimental animal studies. Demyelination of sciatic, tibial, median, and ulnar nerves has been noted. Dose-responses are best characterized in rats, since various studies were conducted to identify NOAELs. Similar effects were noted in other species including mice, cats, dogs, and monkeys *(39)*. However the majority of studies in those species were not designed to identify NOAELs. In a JIFSAN/NCFST review, it was noted that dose rate appears to determine time of onset but not magnitude of neurologic toxicity, thus suggesting a cumulative effect of acrylamide exposure.

In the IRIS review of acrylamide, a subchronic drinking water exposure study in rats by Burek et al. *(55)* was used in the risk assessment of neurologic toxicity *(56)*. The NOEL for neurotoxicity was identified at 0.2 mg/kg bw/day. A LOAEL of 1 mg/kg bw/day was identified based on a slight but significant increase in peripheral axolemmal invaginations in the left sciatic nerve as observed by electron microscopy. The study by Burek et al. *(55)* is reviewed in detail below because it included an examination of reproductive organs.

The European Union *(8)* used a two year drinking water study in rats by Johnson et al. *(57)* to conduct a risk assessment of neurologic toxicity. A NOAEL of 0.5 mg/kg bw/day was identified in that study. A LOAEL of 2 mg/kg bw/day was identified based on degenerative lesions in the tibial nerve. The study by Johnson et al. *(57)* is reviewed in detail in the Carcinogenicity section (Section 2.4.2).

Other organs that were affected with higher acrylamide doses in animal studies included kidney, liver, and the hematopoietic system *(7, 8)*.

Burek et al. *(55)* examined subchronic toxicity of acrylamide administered in drinking water to Fischer 344 rats in an industry sponsored study. At six weeks of age, male and female rats were

administered acrylamide (>99% purity) in drinking water at doses resulting in exposure to 0, 0.05, 0.2, 1, 5, or 20 mg/kg bw/day. Stability and concentrations of dosing solutions were verified. Males were dosed for 92 days and females for 93 days. Numbers of rats treated included 10 females per group and 23–29 males per group. Ten males were used for the subchronic study, 10 were held for a 144-day recovery period, and 3–9 were used for interim sacrifices and ultrastructural analysis of nerves by electron microscopy. Data generated in this study were analyzed by one-way ANOVA followed by Dunnett's test. During the treatment period, rats were observed daily and body weights, food, and water intake were monitored. Body weight gain was significantly reduced in males and females of the 20 mg/kg bw/day group. In the 20 mg/kg bw/day group, water intake was consistently and significantly reduced in female rats beginning on day 21 of the study and was significantly reduced in males during 4 of 13 periods. Hindlimb splay tests were conducted weekly in the control and high dose groups. Splaying was observed in the 20 mg/kg bw/day group on day 22 and became more pronounced on day 29, when the test was stopped. Other clinical signs in the 20 mg/kg bw/day group included curled toes, incoordination, crossing of back legs when held up by tail, and posterior weakness. Clinical signs progressed during the study and eventually led to dragging of back legs. No splaying was noted in the 5 mg/kg bw/day group and none of the dose groups exposed to ≤5 mg/kg bw/day developed clinical signs of neurotoxicity.

At sacrifice, rats were necropsied. Blood was collected for an analysis of hematology and clinical chemistry and urine was collected for urinalysis. All major organs were collected and preserved in phosphate buffered 10% formalin and a select number of males were prepared for electron microscopic examination of nerves. The only statistically and toxicologically significant clinical chemistry observations were decreased serum cholinesterase activity and marginally increased serum alkaline phosphatase activity in females of the 20 mg/kg bw/day group. Packed cell volume, red blood cell count, and hemoglobin concentration were significantly reduced in females of the 5 mg/kg bw/day group and males and females of the 20 mg/kg bw/day group. No treatment-related abnormalities were observed in the urinalysis. Significant organ weight effects in the 20 mg/kg bw/day group included decreased absolute weight of brain, heart, liver, kidneys, thymus, and testis; increased relative weights of brain, heart, liver, and kidneys; and decreased relative weight for thymus (females only) and testis. The authors questioned whether a significant increase in absolute and relative liver weight in the 5 mg/kg bw/day males was related to treatment.

Gross treatment-related observations in the 20 mg/kg bw/day group included perineal soiling, reduced adipose tissue, reduced liver size, dark kidneys, mottled foci on lungs, decreased testicular size or flaccidity, reduced male accessory genitalia size, decreased uterus size, changes in peripheral nerve appearance, skeletal muscle atrophy in posterior body, distended urinary bladder, and diffuse mural thickening of stomach. Histologic evaluations revealed severe degeneration of peripheral nerves and slight degeneration of the spinal cord in the 20 mg/kg bw/day group. Less severe degeneration of the peripheral nerves was observed in the 5 mg/kg bw/day group. In males examined by electron microscopy, very slight nerve degeneration was observed at 1 mg/kg bw/day. Additional histologic findings believed to be treatment-related included skeletal muscle atrophy, slightly increased hematogenous pigment in the spleen (females only), ulcerative gastritis or hyperkeratosis in the stomach (males only), mesenteric fat atrophy (females only), testicular atrophy (n=10/10), mineral in the seminiferous tubules (n=5/10), cellular debris and decreased spermatogenic elements in epididymides (n=9/10), vacuolization of the urinary bladder smooth muscle, and inflammation in the lungs. **[It was not stated but presumed that the histologic findings were observed in the 20 mg/kg bw/day group.]** No treatment-related lesions were found in the brains of rats administered 20 mg/kg bw/day acrylamide.

Male rats treated with 20 mg/kg bw/day and allowed to recover for 144 days demonstrated improvement of clinical neurologic symptoms and partial or complete reversal of neurological lesions. Complete reversal of neurologic lesions occurred in rats of the 1 and 5 mg/kg bw/day groups. Although some testicular lesions in the 20 mg/kg bw/day group were partially reversed, slight testicular effects remained after the recovery period. The effects included focal or multifocal atrophy of individual seminiferous tubules and mineral and cellular debris in tubules with no effect on spermatogenesis. A slight decrease in red blood cell numbers remained in rats of the 20 mg/kg bw/day group up to 92 days in the recovery period. Body weight gain was recovered in the 20 mg/kg bw/day group.

Stengths/Weaknesses: This study was competently performed and reported and is considered reliable. The inclusion of a recovery period is an important strength.

Utility (Adequacy) for CERHR Evaluation Process: The Burek et al. study is important in showing effects on the nervous system and other organ toxicity at 20 mg/kg bw/day but not generally at lower doses. This paper is very useful in showing a dose–response relationship and has important information not only on the nerves but also with regard to other tissues, particularly the testes.

2.3 Genetic Toxicity

The Expert Panel notes the review of Dearfield et al. *(58)*, which includes modeling approaches to assessing heritable genetic risk from exposure to acrylamide. This review concluded that exposure of men to acrylamide in drinking water might result in up to three children with heritable genetic disease among 100 million offspring. The Expert Panel chose to place very little weight on the estimated risks due to the uncertainties associated with the assumptions employed in the model.

2.3.1. Somatic or bacterial cells

Because thorough reviews of the genetic toxicity of acrylamide in somatic or bacterial cells were conducted by numerous authoritative agencies, this section of the report will be based on the most recent and complete review, conducted by the European Union *(8)*.

Results of *in vitro* genetic toxicity tests are listed in Table 9. Those for in vivo genetic toxicity tests are listed in **Table 10**. In terms of *in vitro* genotoxicity, the European Union *(8)* reported:

- Positive results for clastogenicity in Chinese hamster cells, both with (one study) and without (two studies) metabolic activation, and in mouse lymphoma L5178Y cells, without metabolic activation (one study).
- Positive results for mutagenicity in mouse lymphoma L5178Y cells, with (one study) and without metabolic activation (two studies). In the one study that evaluated mutant colony size (without metabolic activation), the increase in mutagenicity was associated with an increase in the frequency of small colonies, an indicator of chromosomal damage rather than the induction of point mutations. This conclusion was supported by the presence of chromosomal damage in these cells.
- Inconsistent results for the induction of point mutations in mammalian cells, with one equivocal (with and without metabolic activation) and two negative studies (with, with and without metabolic activation) at the hypoxanthine-phosphoribosyl transferase (HPRT) locus in Chinese hamster cells. However, due to the absence of toxicity levels appropriate for negative results, these studies are considered inadequate. More recently.

Besaratinia and Pfeifer *(59)* have reported that acrylamide is positive for mutations at the CII transgene in BigBlue® mouse embryonic fibroblasts (without metabolic activation). The increase was associated with an excess of G→C transversions and A→G transitions.

- Consistently negative results for the induction of point mutations in *Salmonella typhimurium, Escherichia coli*, and *Klebsiella pneumoniae*, with and/or without metabolic activation (10 studies). In one *E. coli* study (without metabolic activation), acrylamide was reported to inhibit transfection; however, the significance of this finding in terms of genotoxicity is not clear.
- Inconsistent results in *ex vivo* rodent hepatocyte unscheduled DNA synthesis (UDS) assays (tests to detect DNA damage recognized by excision repair processes), with three positive and two negative studies.
- Inconsistent results for the induction of sister chromatid exchanges (SCEs), an indicator of replication on a damaged DNA template, in Chinese hamster cells, with two positive studies (with, with and without metabolic activation) and one negative study (with and without metabolic activation). The adequacy of the negative study was questioned in the European Review *(8)*.
- Relatively consistent positive responses for cell transformation in BALB/3T3, C3H/10T1/2, or NIH/3T3 cells, with and without metabolic activation (four of five studies).
- Positive results for effects on cell division (e.g., polyploidy, spindle disturbances, malsegragation) in Chinese hamster and human fibrosarcoma cells, in the absence of metabolic activation.
- Negative results for DNA amplification in Chinese hamster cells, without metabolic activation; the significance of this finding is unclear.

In *in vivo* studies in somatic cells, acrylamide is reported to be positive for the induction of:

- Structural chromosomal aberrations in bone marrow cells (3 studies with one study reported by the author as negative but by the European Union *(8)* as likely positive).
- Numerical chromosomal damage (aneuploidy, polyploidy) in bone marrow cells of mice administered acrylamide by i.p. injection (one study) and in feed for up to three weeks (one study) (reported by the author as negative but by the European Union *(8)* as likely positive).
- Micronucleated erythrocytes scored in bone marrow and blood and micronucleated splenocytes (after mitogen-stimulation) collected from mice administered acrylamide acutely by i.p. injection or orally by gavage (7 of 8 studies) (micronuclei arise from either structural or numerical chromosomal damage). No efforts were made by the investigators to identify the mode of action.
- SCE in mitogen-stimulated splenocytes of mice treated by i.p. injection (one study).
- Mutations at the *LacZ* locus in bone marrow cells of MutaMice® treated five times by i.p. injection.

In contrast to these positive *in vivo* studies, acrylamide was reported as negative for DNA damage in a rat liver UDS study, for chromosomal aberrations in mitogen-stimulated splenocytes collected from treated mice, or for the induction of SCE in bone marrow cells.

Based on these results, the European Union *(8)* concluded that acrylamide is genotoxic in cultured mammalian cells and in somatic cells of treated animals, with the pattern of results indicating clastogenicity or interference with chromosomal segregation rather than the induction of point mutations. These conclusions are consistent with those of a JIFSAN *(39)* panel, which

stated that ". . . acrylamide is not a direct acting mutagen in bacterial or mammalian cell assay systems. Acrylamide does, however, have weak clastogenic effects." However, the adequacy of the *in vitro* mammalian point mutation assays (HPRT) is questionable and the study by Besaratinia and Pfeifer *(59*) demonstrates the ability of acrylamide to induce gene mutations in the CII locus. In addition, acrylamide appears capable of inducing cell transformation *in vitro*. In these i*n vitro* studies acrylamide is genotoxic in both the presence and absence of metabolic activation, and the presence of exogenous metabolic activation does not appear to modify its genotoxic activity, suggesting that acrylamide is not metabolized to an inactive metabolite.

The findings reported for the *in vivo* rodent studies support the conclusions of the *in vitro* studies. The most common positive observation is structural chromosomal damage and micronuclei (representing structural and/or numerical chromosomal damage). The observation that mitogen-stimulated cultured splenocytes collected from treated animals exhibit an increase in the frequency of SCE and micronuclei suggests that persistent DNA lesions are induced by acrylamide. The results obtained for the MutaMouse® study are consistent with the ability of acrylamide to be a weak inducer of point mutations.

Table 9. *In Vitro* Genetic Toxicity Studies of Acrylamide

Reference	Acrylamide Concentration	Testing with Metabolic Activation	Species or Cell Type/Strain	End Point	Result
Knaap et. al. (1988) as cited in: European Union *(8)*	≤3,000 µg/mL	Yes	V79 Chinese hamster	Chromosomal aberrations	↑ with and without metabolic activation
Tsuda et al. (1993) as cited in: European Union *(8)*	≤355 µg/mL	No	V79H3 Chinese hamster	Chromosomal aberrations and polyploidy	↑ without metabolic activation
Tsuda et al. (1993) as cited in: European Union *(8)*	71–500 µg/mL	No	V79H3 Chinese hamster	Mutation at the HPRT locus	↔ without metabolic activation
Godek et al. 1982 as cited in: European Union *(8)*	37.5–900 µg/mL	Yes	Chinese Hamster Ovary	Mutagenicity at the HPRT locus	Equivocal with and without metabolic activation, inadequate test due to lack of appropriate toxicity
Godek et al. 1984 as cited in: European Union *(8)*	≤1,500 µg/mL	Yes	Chinese Hamster Ovary	Mutagenicity at the HPRT locus	↔ with and without metabolic activation, inadequate test due to lack of appropriate toxicity
Knaap et al. (1988) as cited in: European Union *(8)*	300–7,500 µg/mL	Yes	Mouse lymphoma L5178Y $TK^{+/-}$	Mutagenicity at the thymidine kinase locus	↑ with and without metabolic activation
Moore et al. (1987) as cited in: European Union *(8)*	≤850 µg/mL	No	Mouse lymphoma L5178Y $TK^{+/-}$	Mutagenicity at the thymidine kinase locus	↑ at ≥500 µg/mL without metabolic activation (dose-related increase in small colonies, mainly small colonies at ≥750 µg/mL)

Reference	Acrylamide Concentration	Testing with Metabolic Activation	Species or Cell Type/Strain	End Point	Result
Besaratinia and Pfeifer *(59)*	32 nM–16 mM **[0.0023–1137 µg/mL]**	No	Big Blue mouse embryonic fibroblasts containing λ phage CII transgene	Mutagenicity in the CII transgene	↑ at 3.2–320 µM; ↔ at higher doses (increase associated with an excess of G →C transversions and A →G transitions)
Sorg et al. (1982) as cited in: European Union *(8)*	≤500 µg/mL	Yes	Chinese hamster ovary	Sister chromatid exchange	↔ with and without metabolic activation
Knaap et. al. (1988) as cited in: European Union *(8)*	≤3,000 µg/mL	Yes	V79 Chinese hamster	Sister chromatid exchange	↑ with and without metabolic activation
Tsuda et al. (1993) as cited in: European Union *(8)*	≤213 µg/mL	No	V79H3 Chinese hamster	Sister chromatid exchange	↑ without metabolic activation
Naismith and Matthews (1982) as cited in: European Union *(8)*	≤100 mg/mL **[100, 000 µg/mL]**	N/A	Rat hepatocyte	Unscheduled DNA synthesis	↑ at 1–33 mg/mL (no clear dose response)
Miller and McQueen (1986) as cited in: European Union *(8)*	≤3.55 mg/mL **[3,550 µg/mL]**	N/A	Rat hepatocyte	Unscheduled DNA synthesis	↔
Butterworth et al. (1992) as cited in: European Union *(8)*	≤710 µg/mL	N/A	Rat hepatocyte	Unscheduled DNA synthesis	↔ without metabolic activation
Barftnecht et al. (1987, 1988) (available only as abstracts) as cited in: European Union *(8)*	≤2 mg/mL **[2,000 µg/mL]**	N/A	Rat hepatocyte	Unscheduled DNA synthesis	↑

Reference	Acrylamide Concentration	Testing with Metabolic Activation	Species or Cell Type/Strain	End Point	Result
Miller and McQueen (1986) as cited in:European Union *(8)*	0.7–710 µg/mL	N/A	Rat hepatocytes	Unscheduled DNA synthesis (tested by autoradiography after exposure of cells to ultraviolet (UV)light);	Slight ↑ in net nuclear grain counts compared to UV alone at 710 µg/mL.
				DNA repair (centrifugation on cesium chloride)	↔for DNA repair (tested at 710 µg/mL only)
Banerjee and Segal (1986), Microbiological Associates (1984, 1982, 1982), Tsuda et al. (1993) as cited in: European Union *(8)*	NS	Yes	BALB/3T3, C3H/10T1/2, or NIH/3T3	Cell transformation	↑ with or without metabolic activation in 4 of the 5 assays
Adler et al. (1993) as cited in: European Union *(8)*	≤1 mg/mL **[1,000 µg/mL]**	No	Chinese hamster V79	Cells with spindle disturbances	↑ without metabolic activation
Sickles et al. 1995 as cited in: European Union *(8)*	≤710 µg/mL	NS	Human fibrosarcoma	Adverse effect on chromosomal segregation and migration	↑
Vanhorick and Moens (1983) as cited in: European Union *(8)*	≤150 µg/mL	NS	CO60 Chinese hamster	DNA amplification	↔ (significance of finding unclear)
Bull et al. (1984), Godek et al. 1982, Hashimoto and Tanii (1985), Jung et al. (1992), Knaap et al. (1988) Lijinsky and Andrews (1980), Muller et al. (1993), Tsuda et al. (1993), Zeiger et al. (1987) as cited in: European Union *(8)*	100–50,000 µg/plate	Yes	*Salmonella. typhimurium* strains TA1535, TA1537, TA98, TA100, TA102, TA1538 *Escherichia.(E.) Coli* strain *WP2 uvrA*	Mutagenicity at the histidine operon in S. typhimurium, and the tryptophan operon in E. coli	↔ with and without metabolic activation

Reference	Acrylamide Concentration	Testing with Metabolic Activation	Species or Cell Type/Strain	End Point	Result
Knaap et al. (1988) as cited in: European Union *(8)*	100–10,000 µg/mL	NS	*Klebsiella . pneumoniae*	Mutagenicity to streptomycin resistance genes	↔
Vasavada and Padayatty (1981) as cited in: European Union *(8)*	≤10 µg [sic; concentration not given]	NS	*E. coli* CR 63	Inhibited transfection (considered a potential indicator of mutagenicity)	↑

↑ Increased in response to treatment; ↔ No effect of treatment; NS Not specified; N/A Non-applicable; HPRT hypoxanthine-phosphoribosyl-transferase (HPRT) locus; $TK^{+/-}$ thymidine kinase

Table 10. *In Vivo* Genetic Toxicity Studies of Acrylamide in Somatic Cells

Reference	Species	Acrylamide Dose (Route)	Cell Type	End Point	Result
Adler et al. (1988) as cited in European Union *(8)*	Mouse	50–125 mg/kg bw (ip) × 1	Bone marrow	Micronucleus	↑
Backer et al. (1989), Cao et al. (1993), Cihak and Vontorkova (1988, 1990), Knaap et al. (1988), Russo et al. (1994) as cited in European Union *(8)*	Mouse	≤150 mg/kg bw (i.p), single or repeated dosing	Bone marrow, spleen, or peripheral blood	Micronucleus	↑
Sorg et al. (1982) as cited in European Union *(8)*	Mouse	75 mg/kg bw (oral gavage) ×1 or ×2	Bone marrow	Micronucleus	↔(EU questioned the sampling times in this study)
Cihak and Vontorkova (1988) as cited in European Union *(8)*	Mouse	100 mg/kg bw (ip) ×1	Bone marrow	Chromosomal aberrations	↑
Adler et al. (1988) as cited in European Union *(8)*	Mouse	50–150 mg/kg bw (ip) ×1	Bone marrow	Chromosomal aberrations	↑
Shiraishi (1978) as cited in European Union *(8)*	Mouse	500 ppm (~60 mg/kg bw/day in diet) for 1, 2, or 3 weeks	Bone marrow	Chromosomal aberrations, aneuploidy, polyploidy, sister chromatid exchange	↑ (Based on European Union conclusion, which is in contrast to author conclusion)
		100 mg/kg bw (ip) ×1	Bone marrow	Chromosomal aberrations aneuploidy, polyploidy	↑ (Based on European Union conclusion, which is in contrast to author conclusion)
Backer et al. (1989) as cited in European Union *(8)*	Mouse	50–125 mg/kg bw (ip) ×1	Spleen lymphocytes	Chromatid aberrations	"↑" at 125 mg/kg bw (not statistically significant)
				Sister chromatid exchange	↑
Butterworth et al. (1992) as cited in European Union *(8)*	Rat	30–100 mg/kg bw, single dose or repeated × 5	Liver	Unscheduled DNA synthesis	↔
Hoorn et al. (1993) and Myhr (1991) as cited in European Union *(8)*	Transgenic mouse	50 mg/kg (ip) ×5	Bone marrow	LacZ (assay unvalidated)	↑

↑ Statistically significant increase in response to treatment; "↑" Increase in response to treatment characterized by the authors of the report but not statistically significant. ↔ No effect of treatment. NS Not specified

2.3.2. Germ cells

2.3.2.1. Chromosome aberrations and related end points in male germ cells

Studies in which males were treated with acrylamide and germ cells evaluated for chromosomal changes (or related end points) are summarized in **Table 11**. Two issues considered by investigators in these studies included whether acrylamide is predominantly clastogenic or also associated with germ cell aneuploidy, and whether exposure of stem cell spermatogonia results in genotoxicity that is transmissible to subsequent generations of germ cells.

The assumptions made by these authors include the likelihood that genetic toxicity in male germ cells would persist with fertilization and that the resultant offspring would manifest abnormal development. Support for this assumption was provided by dominant lethal and other studies in which conceptuses sired by acrylamide-treated males were shown to develop abnormally and in some instances to have identifiable chromosomal or genetic alterations (discussed below). Generally, cytogenetic analysis of male germ cells has proven a reliable means of detecting germ cell mutagens, but certain limitations should be clarified, as follows:

- The majority of (although not all) mutagens are S-phase-dependent inducers of aberrations; i.e., aberrations are only evident in cells undergoing DNA synthesis at or near the time of exposure. In spermatogenesis, DNA synthesis occurs last in preleptotene spermatocytes (about 17 days prior to meiotic division in the rat). The most active DNA replication in the testis is reported to occur during the pre-mitotic S-phase of B-type spermatogonia and the pre-meiotic S-phase of pre-leptotene spermatocytes.
- When mitotic metaphases are analyzed cytogenetically, the majority will be those of B-type spermatogonia because of their short cell cycle length relative to that of stem cell spermatogonia. In the rat, for example, more than 6 cell divisions intervene between exposure and analysis at the first meiotic metaphase (when effects in stem cells can be most reliably evaluated). However, many chemically induced aberrations will be cell-lethal, killing stem cells containing significant chromosomal deletions or asymmetrical exchanges in their first or second division. Hence, this kind of analysis is not particularly useful for assessing effects in stem cells since they don't survive long enough; in fact, it is common to see that agents positive in B-type spermatogonia are not positive in stem spermatogonia, probably because of chromosomal damage-related cell death.
- The observations above suggest that a significant stem cell clastogen may be associated with a transient period of infertility related to sperm production. Acrylamide affects the ratio of spermatid stages in mice and has well described fertility effects. Efforts to distinguish the cause of fertilization failure in such cases are discussed in Section 4.
- Induction of aberrations in post-meiotic germ cells can only be evaluated after pronuclear DNA synthesis during the first cleavage metaphase in the fertilized egg (as discussed in Section 2.3.2.3), and chemically induced aberrations in oocytes are also best measured at this stage.

The Expert Panel had the following observations about selected papers from **Table 11**:

The paper by Shiraishi et al. *(60)*, though providing extensive data, appears to have some problems with the strict time dependency of spermatogenesis. For example, the authors state that spermatocytes were evaluated 11 or 12 days after treatment, "…at which interval these cells were in S-phase and/or early prophase of meiosis." No S-phase occurs during meiosis; the last S-phase occurs in preleptotene spermatocytes and all primary spermatocytes are 4N during differentiation.

It is not clear, then, how to interpret the data presented, but one could surmise that the authors are actually looking at effects in spermatogonia rather than spermatocytes. Overall, the value of this paper is further limited by the lumping of "spermatogonia" and "primary spermatocytes," which prevents specific assignment of sensitive stages, as well as the paper's rather scanty experimental detail.

The study by Xaio and Tates *(61)* in which micronuclei were measured in early spermatids reports increases in micronuclei in spermatids derived from cells exposed as leptotene and zygotene primary spermatocytes. The mechanism whereby acrylamide would be clastogenic in non-S-phase stages like these is unclear; it seems more likely that the authors may have been misled by an overreliance on exact spermatogenic kinetics, i.e., that the day 15 elevations may simply represent the most advanced stage pre-leptotene spermatocytes that were in the final stage of S-phase when exposed 15 days earlier. Estimates of exposed cell stage are relatively imprecise.

The work by Dobrzynska & Gajewski *(62)* is problematic. This study evaluated the induction of abnormal sperm morphology, for which no strict link to genotoxic damage has been demonstrated. It is particularly difficult to accept that abnormal sperm one day after exposure can in any way be related to (a) acrylamide exposure and (b) genetic damage therefrom. Interestingly, the authors reported negative results in bone marrow, at odds with most other published acrylamide results. This paper is not considered reliable and is included in the table only for completeness.

Finally, it is of interest that chromosome aberration data indicating that spermatogonia may be the most "sensitive" to acrylamide do not correlate well with dominant lethal test results (section 2.3.2.2). Embryo death in the dominant lethal test is presumed to be due to induction of major aneuploidies or large chromosomal deletions that cause death of the embryo in early stages. Dominant lethal assessments of acrylamide indicate that epididymal spermatozoa and late-stage spermatids are the most affected stages, compared to the apparently most sensitive spermatogonia and early spermatocytes reported in the studies in **Table 11**. No chromosomal aberration study reported evaluated first cleavage metaphases in fertilized eggs, so direct comparisons are not possible.

Table 11. Male Germ Cell Studies with Chromosome-Related End points (Chronological Order)

Reference/funding source	Species	Acrylamide dose	Treated cell type (per authors)	End point	Result	Comments
Shiraishi *(60)*/Ministry of Education of Japan[a]	ddY mouse	500 ppm (dietary) **[1000 mg/kg/day based on 0.2 kg/kg food factor (EPA Biological Reference Values, 1988)]**	Spermatogonium	Aneuploidy/polyploidy		**[The original paper does not show statistical comparisons clearly. The results were analyzed by CERHR using one-tailed Fisher exact test, which is equivalent to using the analyzed germ cell as the statistical unit. There were 3–5 mice per treatment group].**
				Treatment for 7 days	↑	
				Treatment for 14 days	↑	
				Treatment for 21 days	↑	
				Breaks		
				Treatment for 7 days	↔	
				Treatment for 14 days	↑	
				Treatment for 21 days	↑	
				Chromatid exchanges		
				Treatment for 7 days	↔	
				Treatment for 14 days	↑	
				Treatment for 21 days	↑	
		100 mg/kg i.p.		Aneuploidy/polyploidy		
				12 h after treatment	↔	
				24 h after treatment	↔	
				11 days after treatment	↑	
				12 days after treatment	↑	
				Breaks		
				12 h after treatment	↔	
				24 h after treatment	↔	
				11 days after treatment	↑	
				12 days after treatment	↑	
				Chromatid exchanges		
				12 h after treatment	↔	
				24 h after treatment	↔	
				11 days after treatment	↔	
				12 days after treatment	↔	
		500 ppm (dietary) **[1000 mg/kg/day based on 0.2 kg/kg food factor (EPA Biological Reference Values, 1988)]**	Primary spermatocyte	Sex-chromosome univalents		
				Treatment for 7 days	↔	
				Treatment for 14 days	↑	
				Treatment for 21 days	↑	
				Autosome univalents		
				Treatment for 7 days	↔	

Reference/funding source	Species	Acrylamide dose	Treated cell type (per authors)	End point	Result	Comments
				Treatment for 14 days	↑	
				Treatment for 21 days	↑	
				Fragments		
				Treatment for 7 days	↔	
				Treatment for 14 days	↑	
				Treatment for 21 days	↑	
				Rearrangements		
				Treatment for 7 days	↔	
				Treatment for 14 days	↔	
				Treatment for 21 days	↔	
		50 mg/kg i.p.		Sex-chromosome univalents		
				11 days after treatment	↔	
				12 days after treatment	↔	
				Autosome univalents		
				11 days after treatment	↔	
				12 days after treatment	↔	
				Fragments		
				11 days after treatment	↔	
				12 days after treatment	↔	
				Rearrangements		
				11 days after treatment	↔	
				12 days after treatment	↔	
		100 mg/kg i.p.		Sex-chromosome univalents		
				11 days after treatment	↔	
				12 days after treatment	↔	
				Autosome univalents		
				11 days after treatment	↑	
				12 days after treatment	↑	
				Fragments		
				11 days after treatment	↑	
				12 days after treatment	↑	
				Rearrangements		
				11 days after treatment	↔	
				12 days after treatment	↔	

Reference/funding source	Species	Acrylamide dose	Treated cell type (per authors)	End point	Result	Comments
Backer et al. *(63)*/EPA	Mouse, C57BL/6J	50, 100, and 125 mg/kg i.p.	Spermatogonium	Percent damaged cells	↔	
				Chromatid break/fragment	↔	
				Isochromatid break/fragment	↔	
				Hyperploidy	↔	
		50, 100, and 125 mg/kg i.p.	Spermatocyte	Autosomal univalents	↔	
				XY univalents	↔	
				Chromatid break/fragment	↔	
				Isochromatid break/fragment	↔	
				Hyperploidy	↔	
				Synaptonemal complex aberrations	↑ by trend testing	
Adler *(64)*/funding not stated	Mouse (102/E1 × C3H/E1)F_1	100 mg/kg i.p.	Diplotene	Autosomal & sex univalents	↔	Chromosome aberrations evaluated in diakinesis metaphase I; 5–6 males/group, 100 cells/male. Chi-square statistic used, according to table legend, although mean ± SEM is shown.
				Gaps	↔	
				Fragments	↑5.3-fold	
			Pachytene	Autosomal & sex univalents	↔	
				Gaps	↔	
				Fragments	↑4-fold	
			Zygotene	Autosomal & sex univalents	↔	
				Gaps	↔	
				Fragments	↑7.3-fold	
			Leptotene	Autosomal & sex univalents	↔	
				Gaps	↔	
				Fragments	↔	
			Preleptotene	Autosomal & sex univalents	↔	
				Gaps	↔	
				Fragments	↑4-fold	
				Autosomal & sex univalents	↔	

Reference/funding source	Species	Acrylamide dose	Treated cell type (per authors)	End point	Result	Comments
			Differentiating spermatogonium	Gaps	↔	
				Fragments	↔	
Collins et al. *(65)*/US EPA	Mouse, C57BL/6J	Experiment 1:				
		50 mg/kg i.p.	Leptotene-zygotene	Spermatid micronuclei (MN)	↑	One-tailed trend test used for analysis
				Kinetochore positive spermatid MN	↑	
		100 mg/kg i.p.		Spermatid micronuclei (MN)	↑	
				Kinetochore positive spermatid MN	↑	
		Experiment 2:				
		10 mg/kg i.p.		Spermatid micronuclei (MN)	↔	
				Kinetochore positive spermatid MN	↔	
		50 mg/kg i.p.		Spermatid micronuclei (MN)	↑	
				Kinetochore positive spermatid MN	↑	
		100 mg/kg i.p.		Spermatid micronuclei (MN)	↑	
				Kinetochore positive spermatid MN	↑	
		10 mg/kg i.p.	Diakinesis-metaphase I	Spermatid micronuclei (MN)	↔	
				Kinetochore positive spermatid MN	↔	
		50 mg/kg i.p.		Spermatid micronuclei (MN)	↔	
				Kinetochore positive spermatid MN	↑	
		100 mg/kg i.p.		Spermatid micronuclei (MN)	↑	
				Kinetochore positive spermatid MN	↔	
Russo et al. *(66)*/EEC	Mouse, BALB/c	50 mg/kg, i.p.	Golgi phase spermatids, 2 days after treatment	Spermatid micronuclei	↔	Sampling regimen ensured cells were in meiotic (2 days) or in last pre-meiotic S phase (14 and 16 days). G-test, based on the same general assumptions as of chi-square test, used for statistical analyses
			Golgi phase spermatids, 14 days after treatment		↔	
			Golgi phase spermatids, 16 days after treatment		↔	

Reference/funding source	Species	Acrylamide dose	Treated cell type (per authors)	End point	Result	Comments
		100 mg/kg, i.p.	Golgi phase spermatids, 2 days after treatment	Spermatid micronuclei	↔	
			Golgi phase spermatids, 14 days after treatment		↔	
			Golgi phase spermatids, 16 days after treatment		↔	
		50 mg/kg, i.p. × 4	Golgi phase spermatids, 16 days after first treatment	Spermatid micronuclei	↔	
		50 mg/kg, i.p.	Cap phase spermatids, 2 days after treatment	Spermatid micronuclei	↔	
			Cap phase spermatids, 14 days after treatment		↑	
			Cap phase spermatids, 16 days after treatment		↑	
		100 mg/kg, i.p.	Cap phase spermatids, 2 days after treatment	Spermatid micronuclei	↔	

Reference/funding source	Species	Acrylamide dose	Treated cell type (per authors)	End point	Result	Comments
			Cap phase spermatids, 14 days after treatment		↔	
			Cap phase spermatids, 16 days after treatment		↑	
		50 mg/kg, i.p.× 4	Cap phase spermatids, 16 days after first treatment	Spermatid micronuclei	↑	
		50 mg/kg, i.p.	Spermatogonia	Sister chromatid exchange	↑	
		100 mg/kg i.p.			↑	
Xiao & Tates[a] *(61)*/EEC	rat, Lewis	50 mg/kg i.p.	Pre-leptotene	Spermatid micronuclei	↔	One way ANOVA
		100 mg/kg i.p.	spermatocyte		↑	was used, taking
		50 mg/kg/day i.p. × 4			↑	the treated male as
		50 mg/kg i.p.	Leptotene-	Spermatid micronuclei	↔	the statistical unit
		100 mg/kg i.p.	zygotene		↑	
		50 mg/kg/day i.p. × 4	spermatocyte		↑	
		50 mg/kg i.p.	Diplotene-	Spermatid micronuclei	↔	
		100 mg/kg i.p.	diakinesis/		↔	
		50 mg/kg/day i.p. × 4	pachytene spermatocyte		↔	
Lähdetie et al. *(67)*/Commission of European Communities	Rat, Sprague-Dawley	50 mg/kg i.p.	Pre-leptotene spermatocyte, intermediate & type B spermatogonium	Spermatid micronuclei	↔	The treated male was the statistical unit.
		100 mg/kg i.p.			↔	
		50 mg/kg/day i.p. × 4			↑	
		50 mg/kg i.p.	Leptotene-zygotene spermatocyte	Spermatid micronuclei	↔	
		100 mg/kg i.p.			↔	

Reference/funding source	Species	Acrylamide dose	Treated cell type (per authors)	End point		Result	Comments
		50 mg/kg/day i.p. × 4				↔	
		50 mg/kg i.p.	Diplotene-diakenesis/pachytene spermatocyte	Spermatid micronuclei		↔	
		100 mg/kg i.p.				↔	
		50 mg/kg/day i.p. × 4				↔	
		5 μg/mL *in vitro*	Diplotene-diakenesis/pachytene spermatocyte	Spermatid micronuclei		↔	
		10 μg/ml *in vitro*				↔	
		50 μg/mL *in vitro*				↔	
Gassner & Adler *(68)*/Commission of European Communities	Mouse, (102/E1 × C3H/E1)F_1	120 mg/kg i.p.	Spermatogonia	Meiotic delay		↑	Postulated chromosome loss in micronuclei.
				Hypoploidy		↑	
				Hyperploidy		↔	
Bjørge et al. *(69)*	Rat, Wistar	100 μM *in vitro*	Mixed testicular cells	Single-strand DNA breaks		↔	
		300 μM *in vitro*				↔	
		1000 μM *in vitro*				↔	
	Human	30 μM *in vitro*	Mixed testicular cells	Single-strand DNA breaks		↔	
		100 μM *in vitro*				↔	
		300 μM *in vitro*				↔	
		1000 μM *in vitro*				↑	
Schmid et al. *(70)*/European Union	Mouse, (102/E1 × C3H/E1)F_1	60 mg/kg i.p.	Spermatogonia	3-color FISH (X,Y,8)	Diploidy	↔	
					Disomy	↔	
		120 mg/kg i.p.			Diploidy	↔	
					Disomy	↔	

1

Gassner & Adler, *(71)*	Mouse, (102/E1 × C3H/E1)F_1	80 mg/kg i.p.	24 h later	Immuno-fluores-cent staining	Spindle abnormalities	↔	Different staining methods on cells from the same animals gave different results. Analysis appears to have been per cell (chi square) without regard to treated male of origin.
					Misplaced chromatin	↔	
		120 mg/kg	6 h later		Spindle abnormalities	↑	
					Misplaced chromatin	↔	
			24 h later		Spindle abnormalities	↑	
					Misplaced chromatin	↔	
		80 mg/kg i.p.	24 h later	Differen-tial staining	Spindle abnormalities	↔	
					Misplaced chromatin	↑	
		120 mg/kg	6 h later		Spindle abnormalities	↔	
					Misplaced chromatin	↑	
			24 h later		Spindle abnormalities	↑	
					Misplaced chromatin	↑	
Dobrzynska & Gajewski *(62)* [a]	Mouse, Pzh:Sfis	75 mg/kg i.p.	1 day later	Mouse sperm morphology test (percent abnormal morphology)		↑	The mouse sperm morphology test assumes morphologic abnormalities are associated with genetic damage.
			7 days later			↑	
			14 days later			↔	
			21 days later			↑	
			28 days later			↑	
			35 days later			↔	
			42 days later			↑	

↑Statistically significant increase compared to control. ↔ No significant difference from control. [a]See comments in text.

2.3.2.2. Dominant lethality

Dominant lethal testing in male rodents has been performed with acrylamide exposures in drinking water, by gavage, by i.p. injection, and by dermal application. Studies are summarized in Table 12**.** The traditional dominant lethal end point is the proportional deficit of live fetuses from females mated with treated compared to control males, expressed as a percent of the number of live implants in the control females. The assumption is that the deficit in live fetuses represents pre- and post-implantation embryos that died due to the production of lethal genotoxicity. Some studies use pre- and post-implantation loss as end points that together are comparable to percent dominant lethals. Pre-implantation loss is calculated in rats based on the assumption that all corpora lutea give rise to fertilized oocytes and pre-implantation loss is calculated as the difference between number of identified implants in the uterus and number of corpora lutea in the ovary, expressed as a percentage of the number of corpora lutea. In mice, pre-implantation loss is calculated as the difference in implantation sites between control and treated groups. Post-implantation loss is the difference between the number of live fetuses and the number of implantations, expressed as a percentage of implantations. Fertilization failure is not detectable by any of these methods and will appear as dominant lethality or as preimplantation loss. In the usual presentation of data, percent dominant lethality is expressed for each treatment group without statistical analysis. Values are not presented for the control group when, by definition, the control percent is zero. Pre- and post-implantation loss data are presented for all groups, including the control, and are usually analyzed statistically using the female as the statistical unit. When mating has occurred by cohabiting one male with one female for a given time period, per-female analysis is equivalent to using the male (the treated animal) as the statistical unit. When multiple females are mated to each treated male, the use of the female as the statistical unit may not permit statistical consideration of the treated animal **[In general, the treated animal (male or female) should be considered the statistical unit for maximal precision. In the event that multiple females are mated a single treated male, group means may be employable to derive data appropriate for parametric analysis. In cases where a single female is mated to each male during each time period, the female for practical purposes may be considered the statistical unit without impacting the precision of the analysis. Additional statistical considerations might include the use of clustering analysis to achieve desirable distribution and variance characteristics to enable the use of parametric testing procedures with their attendant greater discrimination. In this process, pregnant females (assuming a single male:female breeding design) are randomly assembled into groups of 4 or 5 to achieve approximate normal distributions and homogeneous variances for each clustered variable. Some Expert Panel members feel that these statistical considerations are less important for dominant lethal studies in which there is uniformity of affected females and a strong treatment effect]**.

Based on the time after treatment that the male is mated, the germ cell type that is sensitive to acrylamide-induced toxicity can be elucidated. The studies involving treatment of male rats and mice with acrylamide show significant increases in pre- and post-implantation loss and in percent dominant lethals when epididymal spermatozoa and late spermatids are exposed. Lowest effective doses (based on cumulative acrylamide by the time of mating) in rats were 30 ppm in drinking water (about 200 mg/kg cumulative dose by the time of mating; *(72)*) and 15 mg/kg/day by gavage (75 mg/kg cumulative dose by the time of mating, *(73)*. In mice, the lowest effective i.p. dose was 75 mg/kg *(74)* **[based on CERHR chi-square using live and dead implants with mating 5-8 days after treatment]**). The lowest effective dermal dose in mice was 25 mg/kg/day (125 mg cumulative dose by the time of mating *(75)*). The lowest effective dose in drinking water in mice was 6.78 mg/kg/day for 20 weeks (cumulative dose 949 mg/kg; *(76)* **[This figure was**

calculated by CERHR from the NTP final report (RACB90022) based on mean week 16 water consumption of 226 g/kg/day with an acrylamide concentration of 30 ppm (Table 2-7 of the report)].

[The dominant lethal data provide firm in vivo, post-metabolic evidence of genotoxicity in mammals. They provide a degree of reassurance as it relates to spermatogonia after acute exposures, but the chronic dose effects demonstrate the potential for genotoxicity to a population. Acrylamide was effective via all routes in all species, and at comparable doses. Stage effect was consistent. The dominant lethal test is a low tech alternative to more costly and resource-intensive tests for mutagenic potential. Properly interpreted the dominant lethal test can be an effective, if gross, predictor of genotoxic effect. For example, the dominant lethal test does not effectively assess damage in spermatogonial stem cells, arguably the cell stage of most interest, since the degree of chemically induced damage is generally so great as to be lethal. Preimplantation loss, confounded as it is by potential effects on fertilization, can still be a valuable component of the test, since the most potent mutagens may induce only preimplantation loss. When assessed in conjunction with assessment of mating rate and methods for the direct quantification of fertilization rate (e.g., oviductal or uterine flushing and embryo culture), the dominant lethal test provides an important component for the overall risk analysis. Studies that include long-term exposure and short-term mating are less useful in determining mechanism of effect, but are useful in predicting genotoxic potential. At the same time, caution is necessary in assigning stage-specific effects based on the kinetics of spermatogenesis, given that some chemical agents (including, perhaps, acrylamide) may alter the kinetics of spermatogenesis. An exception may be the use of flow cytometry-based approaches to assess ploidy. In the case of acrylamide, the dominant lethal studies most likely indicate an effect on the ability of epididymal spermatozoa and spermatids to fertilize an oocye, along with potential pre- and post-implantation genetic effects. The anti-fertilization effect may well be due to non-genetic actions. Since the dose to elicit these effects is relatively high, and unsupported by human data, the utility of these studies to predict human risk is limited].

It has been proposed (discussed more fully in section 2.3.2.6) that the dominant lethal effects of acrylamide are due to metabolism to glycidamide. Adler et al. *(77)* tested this hypothesis by inhibiting metabolism of acrylamide to glycidamide with 1-aminobenzotriazole. Dominant lethals were decreased two weeks after treatment. During the first week after treatment, however, 1-aminobenzotriazole did not decrease the dominant lethal effect of acrylamide, suggesting either that acrylamide itself has dominant lethal effects or that 1-aminobenzotriazole requires more than a week to completely prevent metabolism to glycidamide.

Strengths/Weaknesses: This study demonstrates an attempt to link acrylamide to the demonstrated mutagenicity of glycidamide; however, this study has several weaknesses: (a) The purity of acrylamide was not established; (b) The lack of a good explanation of the delay before effect; (c) As the authors note, there is a decrease in the rate of dominant lethals in their study compared to other studies in mice (they suggest that possible differences in mouse colonies might explain the difference). The modest increase of dominant lethals with acrylamide, therefore, would make any antagonistic effect of 1-aminobenzotriazole less dramatic, weakening the statistical power of the study to show an effect. (d) Interpretation of the effect of 1-aminobenzotriazole on P450 metabolism in this study is made difficult by failure to demonstrate a reduction of the acrylamide-metabolizing P450 isoenzyme in either the liver or the testes; (e) the acrylamide and 1-aminobenzotriazole + acrylamide groups had significantly depressed fertilization rates the first 4-7 days after mating. A spermiogram (without statistical analysis)

indicated that 1-aminobenzotriazole was also spermatotoxic, and did not effectively antagonize the spermatotoxic effect of acrylamide treatment. (f) Given the lack of data about the efficacy of 1-aminobenzotriazole in inhibiting/destroying P450 in this study, the modest decrease in dominant lethality when acrylamide and 1-aminobenzotriazole were given together is insufficient evidence to support a role for P450 or glycidamide in the mechanism of toxicity of acrylamide. However, a non-genotoxic action of acrylamide directly on sperm fertilization ability is indicated. (g) No effort was made to assess possible alterations in libido from fertility or genotoxicity effects. (h) It seems unlikely that the minor differences in "fast" sperm frequency between the acrylamide group and the acrylamide + 1-aminobenzotriazole group could explain the differences in pregnancy rates between these two groups.

Utility (Adequacy) for CERHR Evaluation Process: This paper provides confirmatory data that acrylamide induces dominant lethal mutations in mice. The metabolic inhibitor work, while interesting, is not compelling given the lack of direct confirmatory evidence that 1-aminobenzotriazole is actually affecting acrylamide metabolism and the inconsistency in effect on dominant lethals. The sperm quality studies are a nice addition, but, again, the effect of 1-aminobenzotriazole is somewhat unclear. Overall, the paper does not provide compelling evidence for the effect of 1-aminobenzotriazole treatment.

Table 12. Dominant Lethal Studies, Chronological Order

Reference/ funding	Species	Acrylamide dose	Treatment–fertilization	Germ cell affected	End point	Result	Comments
Shelby et al., *(1)*/ NTP + DOE	Mouse: $(C3H \times 101)F_1$ males; females were T-stock or $(SEC \times C57BL)F_1$ hybrid (figures in table are for T-stock females)	125 mg/kg i.p., single dose	0.5–3.5 days 4.5–7.5 days 8.5–11.5 days 12.5–15.5 days 16.5–19.5 days 20.5–23.5 days 24.5–27.5 days 28.5–31.5 days 32.5–35.5 days 36.5–39.5 days 40.5–43.5 days 44.5–45.5 days	sperm spermatids spermatocytes spermatogonia 	% Dominant lethals[a]	49 52 30 15 1 5 - 12 1 1 - -	Strain of female influenced dominant lethal results.
Smith et al., *(72)*/ EPA	Long-Evans rat	15 ppm water = 112.6 ± 7.4 mg/kg 30 ppm in water = 204.2 ± 22.5 mg/kg 60 ppm in water = 432.2 ± 24.4 mg/kg	Drinking water exposure for 80 days prior to mating	All germ cell types would have been exposed	Preimplantation loss[b] Post-implantation loss[c] Preimplantation loss Post-implantation loss Preimplantation loss Post-implantation loss	↔ ↔ ↔ ↑2.3-fold ↑2.4-fold ↑6.4-fold	Dose = cumulative dose in drink-ing water at time of mating, mean ± SEM. Statistical unit = the male
Zenick et al., *(78)*/ EPA	Long-Evans rat	100 ppm in water = 544 mg/kg cumulative dose	Drinking water exposure for 10 weeks prior to mating	All germ cell types would have been exposed	Post-implantation loss	↑4-fold	Statistical unit = the male
Working et al. *(79)*/ CIIT	Fischer 344 rat	30 mg/kg/day by gavage × 5 days	1 week 2 weeks	sperm	Preimplantation loss Post-implantation loss % Dominant lethal Preimplantation loss Post-implantation loss	↑4.6-fold ↑4-fold 36.0 ↑6.3-fold ↑10-fold	Statistical unit = the female

Reference/ funding	Species	Acrylamide dose	Treatment–fertilization	Germ cell affected	End point	Result	Comments
					% Dominant lethal	60.7	
			3 weeks	spermatids	Preimplantation loss	↑3.8-fold	
					Post-implantation loss	↑4.4-fold	
					% Dominant lethal	49.1	
			4 weeks		Preimplantation loss	↑2.4-fold	
					Post-implantation loss	↔	
					% Dominant lethal	20.6	
			5 weeks	spermatocytes	Preimplantation loss	↔	
					Post-implantation loss	↔	
					% Dominant lethal	-	
			6 weeks		Preimplantation loss	↔	
					Post-implantation loss	↔	
					% Dominant lethal	-	
			7 weeks	spermatogonia	Preimplantation loss	↔	
					Post-implantation loss	↔	
					% Dominant lethal	-	
			8 weeks		Preimplantation loss	↔	
					Post-implantation loss	↔	
					% Dominant lethal	7.8	
			9 weeks		Preimplantation loss	↔	
					Post-implantation loss	↔	
					% Dominant lethal	0.5	
			10 weeks		Preimplantation loss	↔	
					Post-implantation loss	↔	
					% Dominant lethal	-	
Shelby et al. *(80)*/ NIEHS + DOE	Mouse: Males (C3H × 101)F_1, female (SEC × C57BL)F_1	40 mg/kg/day i.p. × 5 days	7-10 days	sperm + spermatids	% Dominant lethals	73	
Sublet et al., *(73)*/ EPA	Long-Evans rat	5 mg/kg/day by gavage × 5 days	1 week	sperm	Preimplantation loss	↔	Statistical unit = the female
					Post-implantation loss	↔	
			2 weeks		Preimplantation loss	↔	

Reference/ funding	Species	Acrylamide dose	Treatment–fertilization	Germ cell affected	End point	Result	Comments
					Post-implantation loss	↔	
			3 weeks	spermatids	Preimplantation loss	↔	
					Post-implantation loss	↔	
			4 weeks		Preimplantation loss	↔	
					Post-implantation loss	↔	
		15 mg/kg/day by gavage × 5 days	1 week	sperm	Preimplantation loss	↑3.8-fold	
					Post-implantation loss	↔	
			2 weeks		Preimplantation loss	↔	
					Post-implantation loss	↑2.7-fold	
			3 weeks	spermatids	Preimplantation loss	↔	
					Post-implantation loss	↑2.3-fold	
			4 weeks		Preimplantation loss	↔	
					Post-implantation loss	↔	
		30 mg/kg/day by gavage × 5 days	1 week	sperm	Preimplantation loss	↑5-fold	
					Post-implantation loss	↔	
			2 weeks		Preimplantation loss	↔	
					Post-implantation loss	↑4.5-fold	
			3 weeks	spermatids	Preimplantation loss	↔	
					Post-implantation loss	↑5.2-fold	
			4 weeks		Preimplantation loss	↔	
					Post-implantation loss	↔	
			7 weeks	spermatogonia	Preimplantation loss	↔	
					Post-implantation loss	↔	
			10 weeks		Preimplantation loss	↔	
					Post-implantation loss	↔	
		45 mg/kg/day by gavage × 5 days	1 week	sperm	Preimplantation loss	↑4.4-fold	
					Post-implantation loss	↔	
			2 weeks		Preimplantation loss	↑2.9-fold	
					Post-implantation loss	↑10.7-fold	
			3 weeks	spermatids	Preimplantation loss	↑5.2-fold	
					Post-implantation loss	↑8.2-fold	
			4 weeks		Preimplantation loss	↔	
					Post-implantation loss	↔	
			7 weeks	spermatogonia	Preimplantation loss	↔	

Reference/ funding	Species	Acrylamide dose	Treatment–fertilization	Germ cell affected	End point	Result	Comments
					Post-implantation loss	↔	
			10 weeks		Preimplantation loss	↔	
					Post-implantation loss	↔	
		60 mg/kg/day by gavage × 5 days	1 week	sperm	Preimplantation loss	↑5-fold	
					Post-implantation loss	↔	
			2 weeks		Preimplantation loss	↑6.4-fold	
					Post-implantation loss	↑13.7-fold	
			3 weeks	spermatids	Preimplantation loss	↑8.6-fold	
					Post-implantation loss	↑7.4-fold	
			4 weeks		Preimplantation loss	↑7.5-fold	
					Post-implantation loss	↑3.4-fold	
			7 weeks	spermatogonia	Preimplantation loss	↔	
					Post-implantation loss	↔	
			10 weeks		Preimplantation loss	↔	
					Post-implantation loss	↔	
Dobrzynska et al. *(81)*/ National Institute of Hygiene (Poland)	Mouse, Pzh:SFISS outbred	75 mg/kg i.p.	1 week	sperm	% Dominant lethals	0.73	
			2 weeks			9.44	
			3 weeks	spermatids		negative	
			4 weeks			0.20	
			5 weeks	spermatocytes		6.68	
			6 weeks			negative	
			7 weeks	spermatogonia		negative	
		125 mg/kg i.p.	1 week	sperm	% Dominant lethals	23.76	
			2 weeks			22.48	
			3 weeks	spermatids		3.59	
			4 weeks			5.44	
			5 weeks	spermatocytes		negative	
			6 weeks			4.22	
			7 weeks	spermatogonia		negative	
		75 mg/kg i.p. + x-ray 0.25 Gy	1 week	sperm	% Dominant lethals	negative	
			2 weeks			13.49	
			3 weeks	spermatids		negative	
			4 weeks			negative	

Reference/ funding	Species	Acrylamide dose	Treatment–fertilization	Germ cell affected	End point	Result	Comments
			5 weeks	spermatocytes		negative	
			6 weeks			negative	
			7 weeks	spermatogonia		negative	
		125 mg/kg i.p. + x ray 1.00 Gy	1 week	sperm	% Dominant lethals	43.78	
			2 weeks			43.45	
			3 weeks	spermatids		29.71	
			4 weeks			17.61	
			5 weeks	spermatocytes		22.24	
			6 weeks			27.70	
			7 weeks	spermatogonia		22.59	
Ehling and Neuhäuser-Klaus *(74)*/ Commission of the European Communities	Mouse: (102/E1 × C3H/E1)F_1 males mated to test stock females	50 mg/kg i.p.	1-4 days	sperm	% Dominant lethals	4.4	Dominant lethal testing performed in conjunction with specific locus mutation testing.
			5-8 days			3.6	
			9-12 days	spermatids		5.2	
			13-16 days			5.2	
			17-20 days			4.5	
			21-24 days	spermatocytes		negative	
		75 mg/kg i.p.	1-4 days	sperm	% Dominant lethals	9.6	
			5-8 days			8.3	
			9-12 days	spermatids		negative	
			13-16 days			4.5	
			17-20 days			0.5	
			21-24 days	spermatocytes		4.6	
		100 mg/kg i.p.	1-4 days	sperm	% Dominant lethals	18.7	
			5-8 days			14.1	
			9-12 days	spermatids		12.3	
			13-16 days			7.6	
			17-20 days			5.9	
			21-24 days	spermatocytes		1.5	
		125 mg/kg i.p.	1-4 days	sperm	% Dominant lethals	40.5	
			5-8 days			28.7	
			9-12 days	spermatids		30.3	
			13-16 days			6.5	
			17-20 days			negative	

Reference/ funding	Species	Acrylamide dose	Treatment–fertilization	Germ cell affected	End point	Result	Comments
			21-24 days	spermatocytes		1.1	
			25-28 days			negative	
			29-32 days			1.0	
			33-36 days			6.0	
			37-40 days	spermatogonia		negative	
			41-44 days			4.9	
			45-48 days			0.2	
Gutierrez-Espeleta et al. *(75)*	Mouse: (C3H/RI × 101/R1)F_1 males and (C3H/RI × C57Bl)F_1 females	25 mg/kg/day dermal × 5 days	7-8 days	sperm	% Dominant lethals	10	
			9-10 days	spermatids		negative	
		50 mg/kg/day dermal × 5 days	7-8 days	sperm		25	
			9-10 days	spermatids		4	
		75 mg/kg/day dermal × 5 days	7-8 days	sperm		70	
			9-10 days	spermatids		38	
		100 mg/kg/day dermal × 5 days	7-8 days	sperm		83	
			9-10 days	spermatids		49	
		125 mg/kg/day dermal × 5 days	7-8 days	sperm		91	
			9-10 days	spermatids		77	
NTP *(76)*	Mouse, CD-1 Swiss	3 ppm in water = 0.72 mg/kg/day	20 weeks	all germ cell stages would have been exposed	Early resorptions	significant trend across doses	Daily dose calculated from table of water intake at week 16 or 20-week dosing period.
					Late resorptions	↔	
					Dead fetuses	↔	
					Total post-implantation death	significant trend across all doses	
		10 ppm in water = 2.2 mg/kg/day			Early resorptions	significant trend across all doses	
					Late resorptions	↔	

Reference/ funding	Species	Acrylamide dose	Treatment–fertilization	Germ cell affected	End point	Result	Comments
					Dead fetuses	↔	
					Total post-implantation death	significant trend across all doses	
					Early resorptions	↑2-fold	
		30 ppm in water = 6.78 mg/kg/day			Late resorptions	↔	
					Dead fetuses	↔	
					Total post-implantation death	↑2-fold	
Nagao *(82)*/funding not indicated	Mouse, ICR	62.5 mg/kg i.p.	1-21 days	Postmeiotic	Implants/female	↔	% early death (fetuses < 6 mm long) reportedly increased when postmeiotic cells treated with 125 or 5 × 50 mg/kg, expressed per pregnant female
		125 mg/kg i.p.				↔	
		50 mg/kg/d i.p. × 5				↓15%	
		62.5 mg/kg i.p.	64-80 days	Spermatogonial stem cells	Implants/female	↔	
		125 mg/kg i.p.				↔	
		50 mg/kg/d i.p. × 5				↔	
Holland et al. *(2)*/ NIEHS	Mouse: C57Bl/6J males and C3H/J females	50 mg/kg i.p. × 5 days	1-2 weeks	sperm and spermatids	Preimplantation loss	72.1%	Statistical comparison to control not given; difference implied at all time points.
					Post-implantation loss	12.7%	
			3-4 weeks	spermatids and spermatocytes	Preimplantation loss	41.3%	
					Post-implantation loss	8.2%	
			5 weeks	spermatocytes	Preimplantation loss	18.5%	
					Post-implantation loss	10.1%	

Reference/ funding	Species	Acrylamide dose	Treatment–fertilization	Germ cell affected	End point	Result	Comments
Adler et al. *(77)*/ funding source not indicated	Mouse, (102/E1 × C3H/E1)F_1 males and females	125 mg/kg i.p. 125 mg/kg + ABT 125 mg/kg 125 mg/kg + ABT 125 mg/kg 125 mg/kg + ABT 125 mg/kg 125 mg/kg + ABT	week 1 week 2 week 3 week 4 	sperm spermatids spermatocytes		Exp 1 / Exp 2 19.5 / 49.5 12.6 / 50.0 33.6 / 21.6 4.7 / 3.0 8.0 / 29.4 -4.1 / 15.8 3.6 / 11.2 2.3 / 1.0	ABT = 3 × 50 mg/kg 1-aminobenzo-triazole to inhibit metabolism to glycidamide
Tyl *(83)*/ Acrylamide Producers Association	Rat, Fisher 344	0.5 mg/kg/day in drinking water 2.0 mg/kg/day in drinking water 5.0 mg/kg/day in drinking water	2 days after a 64-day treatment	all germ cell stages would have been exposed	Implantations/dam Live implants/litter Post-implantation loss Implantations/dam Live implants/litter Post-implantation loss Implantations/dam Live implants/litter Post-implantation loss	↔ ↔ ↓40% ↔ ↔ ↔ ↓14% ↓20% ↑2.3-fold	Part of a multigener-ation study; presented also in Table 32

[a]Dominant lethals = [1–(live fetuses in treated)/(live fetuses in control)] × 100
[b]Preimplantation loss = {[(number of corpora lutea) – (number of implants)]/number of corpora lutea]} × 100
[c]Post-implantation loss = {[(number of implants) – (number of fetuses)]/number of implants]} × 100
↑,↓ Statistically significant increase, decrease compared to vehicle-treated control. ↔ No significant difference from vehicle treated control. For % dominant lethal, actual numbers are given without statistical analysis. For Holland study, % given in place of statistical results due to lack of presentation of statistical analysis.
Dash appears in table when original table contained a dash with no explanation.

2.3.2.3. Chromosome aberrations in conceptuses after treatment of the male

Pacchierotti et al. *(84)*, in a study supported by the Commission of the European Community, administered acrylamide **[purity not given]** in HBSS i.p. to male B6C3F$_1$ mice at single acute doses of 0, 75, or 125 mg/kg or 5 divided daily doses adding to 250 mg/kg (5×50 mg/kg/day). All males were mated 7 days after the last treatment to untreated females and the 125-mg/kg treated males were also mated 28 days after treatment. Females were superovulated with pregnant mare's serum followed by hCG. Plug-positive females were given colchicine 26 h after the hCG and were killed 5 h later. Zygotes were flushed from the oviducts and treated with hyaluronidase to remove cumulus cells and to partially digest the zona pellucida. Fixed cells were air-dried and C-banded. First cleavage metaphases were evaluated if they contained at least 35 chromosomes. At least 100 metaphases were evaluated per dose group **[they were apparently pooled within dose group without regard to sire or dam]** except in the 5 × 50-mg/kg/day dose group for which 55 zygotes were analyzed. Proportions of abnormal metaphases were evaluated by chi-square. The proportion of zygotes with chromosome aberrations increased in a dose-related fashion. With mating seven days after treatment in the 0-, 75-, 125-, and the 5 × 50-mg/kg/day groups, the percent of zygotes with aberrations was, respectively, 0.8, 7.6, 26.3, and 85.4. Most of the aberrations were fragments and dicentrics, with much lower proportions of rings and translocations. Chromatid breaks and exchanges were also unusual (0–0.02 events/zygote), regardless of treatment group. When 125 mg/kg group males were mated 28 days after treatment, only 5.1% of zygotes had aberrations, which was not significantly different from the control. A subset of male mice underwent flow cytometric evaluation of testicular germ cell populations (discussed in section 4.2.2). There was a decrease in percent mated females seven days after the last acrylamide treatment that was not dose-related. The percent mated (plug-positive) was 86.7, 57.1, 54.1, and 61.0 in the 0-, 75-, 125-, and 5 × 50-mg/kg/day groups, respectively. When males in the 125-mg/kg group were cohabited with females 28 days after treatment, 86.7% of females showed evidence of mating, a result identical to that in the control group. The results of the mating studies are discussed more fully in Section 4.2.2.

Strengths/Weaknesses: This study was well-designed and carefully conducted and includes an extensive evaluation of the induction of chromosomal aberrations in males germ cells as assessed in first cleavage metaphase zygotes, coupled with specific analysis of mating rates, fertilization rates, and toxic effects on spermatogenic cells, permitting a definitive analysis of the effect of acrylamide. A strength of this study is its multi-dimensional comparison of treatment effects. The low (and possibly variable) recovery of zygotes per female may have required that zygotes be pooled per group. The statistical issue of pooling zygotes and not treating the male as the statistical unit is minor in the context of the findings; however, the number of males per group was not specified in the paper. Flow cytometry experiments referred to subsets within each time and dose group of 5 and 6 male mice taken for analysis (16 solvent controls) but the total group size is not stated. The results of the admittedly tedious task of evaluating chromosomal and chromatid abnormalities indicated that the dose response was curvilinear. However, the curve of the authors' Figure 3, percent zygotes with aberrations plotted against dose, is forced through the origin. A straight-line fit to the three positive responses could just as well have indicated a threshold dose of about 50 mg/kg.

Utility (Adequacy) for CERHR Evaluation Process: This study is very useful in suggesting mechanisms of acrylamide reproductive toxicity. Although the conclusions the authors draw from the data are consistent with work by others in the field, ignorance of the group size and therefore unknown recovery of zygotes per female make it impossible to assess the variability of the data. Although i.p. dosing is common in studies of acrylamide genetic effects in mice, and

may be appropriate for mechanistic studies, the route is not relevant to humans and limits the utility of this study in the evaluation of human risk.

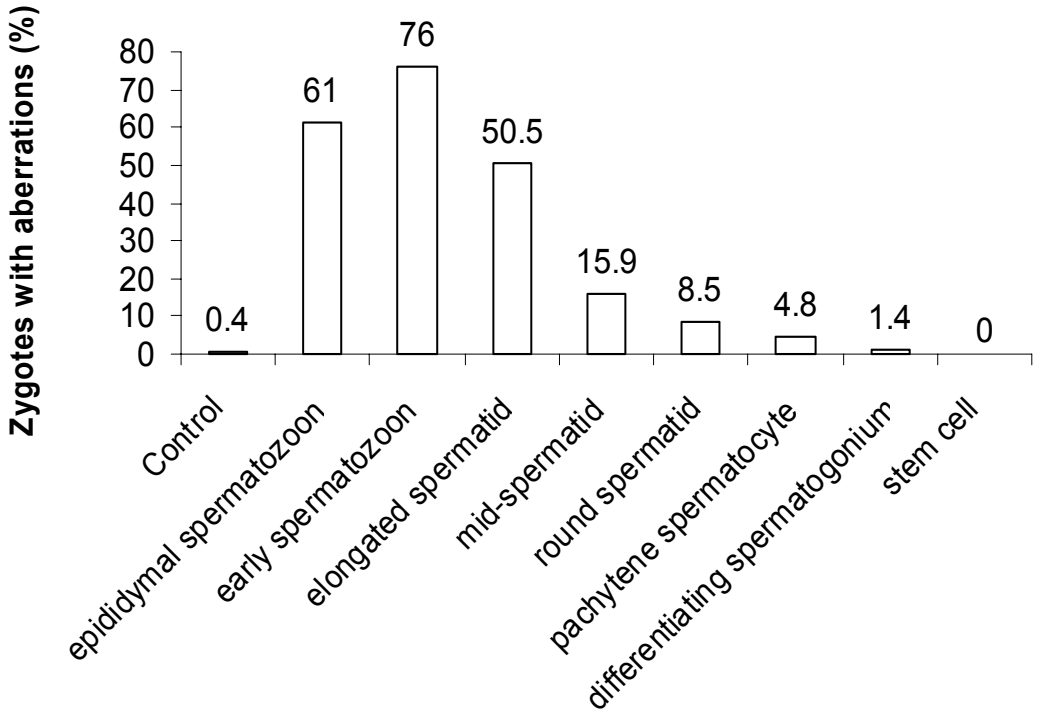
Marchetti et al. *(3)*, supported by DOE and NIEHS, gave acrylamide **[purity not specified]** to male $B6C3F_1$ mice at 50 mg/kg/day by i.p. injection for 5 consecutive days. Mice were mated with untreated females of the same strain at 2.5, 6.5, 9.5, 12.5, 20.5, 27.5, 41.5, and 48.5 days after the last acrylamide injection, to produce fertilization by sperm that had been exposed to acrylamide at the epididymal spermatozoon, early spermatozoon, elongated spermatid, mid-spermatid, round spermatid, pachytene spermatocyte, differentiating spermatogonium, and stem cell stages, respectively. Females were superovulated with pregnant mare's serum followed by hCG, then mated. Plugged females were given colchicine 24 h after hCG to arrest zygote development at metaphase of the first cleavage. Females were killed 6 h after colchicine and zygotes harvested. Chromosomes were evaluated using fluorescence *in situ* hybridization with five probes (chromosomes 1, 2, 3, X, Y). Chromosomes with color junctions were identified as translocations or dicentrics, with the distinction made by identification of the centromere using 4',6-diamidino-2-phenylindole (DAPI) fluorescence. The authors report, based on previous work, that this method permits detection of 36.5–38.1% of chromosome exchanges in the male pronucleus. **[The frequencies of translocations and dicentrics were analyzed by the Fisher test, which takes the harvested zygote as the statistical unit. The sire of origin was not indicated; in fact, the number of males is not stated except that matings were 1:1 and "[z]ygotes harvested from 10–15 females were pooled…"]**. The proportion of zygotes with chromosome aberrations was increased with mating from 2.5 to 27.5 days after acrylamide treatment, indicating an effect on pachytene spermatocytes and later stages. The incidence of aberrations is shown in **Figure 3**, suggesting particular susceptibility of late spermatids and spermatozoa, paralleling the stages sensitive to dominant lethality. The authors included a figure comparing their results with the dominant lethal results of Shelby et al. *(1)* with the proportion of zygotes with unbalanced rearrangements in the current study, showing good agreement and supporting the belief that zygotes with unbalanced rearrangements would be most likely to die *in utero*. **[These authors conclude that meiotic cells are also affected. To support this conclusion, they cite their data (4.8% zygotes with aberrant chromosomes after exposure of spermatocytes) plus the data of Pacchierotti et al. *(84)* (showing 5.1% of zygotes with aberrations when mating occurred 28 days after treatment) and Adler *(64)*, which did not involve assessment of conceptuses. The Expert Panel notes that the Pacchierotti finding was not statistically significant].**

Strengths/Weaknesses: Statistical mistreatment of the data has led to an over-interpretation of results in this study, limiting the value of some of its more far-ranging conclusions. While the data are supportive of similar data collected by Pacchierotti et al. *(84)* as relates to late stage spermatids and epididymal spermatozoa, the conclusions about pachytene spermatocyte effects are not supportable. A mere 6 of 125 zygotes examined at this time point had increased chromosomal aberrations, and the use of the rather forgiving and non-discriminating Fisher's exact test contributed to the problem. Dominant lethal testing, as summarized in Section 2.3.2.2, does not confirm the clastogenic activity of acrylamide in this stage spermatogenic cell, further casting doubt on the claim. The decreased fertility rate reported late (41.5 days after treatment) could be accounted for by clastogenic effects in early spermatogonia since they will tend not to survive the 4 to 6 cell divisions required to get to the spermatid stage. The finding, however, is likely skewed by the statistical treatment. The spermatocyte data may be problematic because qualitatively they involved only DAPI acrocentrics with no PAINT exchanges. However, the DAPI was more sensitive. These results need to be evaluated compared to negative and positive findings in other studies evaluating chromosomal aberrations. The paper attempts to demonstrate that a strong correlation exists between the ability of acrylamide to induce chromosomal

abnormalities, and dominant lethal and heritable translocation events. A dose regimen of acrylamide was used that, based on previous studies, would be predicted to induce a maximum number of dominant lethal events; therefore, no dose–response evaluation was attempted. This study involves technically demanding and patient scoring of pronuclei with aberrant chromosomes. However, the reader is referred to previous publications for many of the experimental details, and left in the dark as to how the zygotes were counted, and how the number on a slide was determined. Between 100 and 284 zygotes were examined by in situ hybridization for chromosomal structural abnormalities at selected time points, but this number obviously represents a selected population, and how the population was selected in not specified in the article. The authors' Figure 2 implies that multiple experiments were performed, but does not indicate the number, except for data from single experiments at 20.5 and 41.5 days. Given the design of the experiments, only statistical analysis based on the unit of a zygote is possible. This analysis is appropriate only if the selection of the zygotes was truly random and consistent, and the slides were read blind. The observation of a low level of abnormality inflicted on spermatocytes that is the same value (about 5%) as reported by Paccheirotti et al. must be considered intriguing but coincidental at this time. However, subsequent work by Holland et al. *(2*), discussed below, supports the authors' conclusion. The authors' data support the random nature of the chromosomal damage, and is consistent with the suggestion that chromosomal protein, rather than DNA, is the target of acrylamide.

Utility (Adequacy) for CERHR Evaluation Process: Despite the structural weaknesses of this paper in describing methodology and weak statistical analysis, for the purpose of risk assessment, the paper does offer insight into the mechanism of acrylamide toxicity, consistent with an ability of acrylamide to induce chromosomal abnormalities in late stages of spermatogenesis. An effect on spermatocytes may occur. This paper is supportive of the Pacchierotti et al. study but misuse of statistics limits the value of additional conclusions.

Figure 3. Incidence of Chromosome Aberrations Among Zygotes Harvested from Females Mated with Mice After Acrylamide Treatment (5 × 50 mg/kg/day), from Data Presented by Marchetti et al. *(3)*. The timing of mating was used to identify the germ cell stage that was affected.



Titenko-Holland et al. *(85)*, in a study sponsored by NIEHS, treated male C57BL/6J mice with acrylamide **[purity not given]** 50 mg/kg/day (n=92) or PBS (n=80) i.p. for five days. About 10% of acrylamide-treated males were lost within 24 h of the last injection. Males were mated with untreated C3H/J females by placing 3 or 4 females with each male overnight. Mating was assumed to have occurred at midnight. Females were killed at 86-88 h after mating and 1 h after treatment with colcemid to increase the proportion of metaphase embryonic cells. Corpora lutea were counted and were taken to represent the number of ovulated oocytes. The uterine horns were flushed with tissue culture medium and embryos or oocytes recovered. Embryos were evaluated under a dissecting microscope for morphology. All abnormal embryos and an approximately equal number of normal-appearing embryos were taken from each treatment group for cytologic analysis **[how distributed by sire of origin is not indicated]**. Embryos were fixed in methanol:acetic acid:ice-cold water (4:1:5) followed by drop-wise methanol:acetic acid (1:1) until the embryos broke and spread. Spread embryos were evaluated by phase contrast microscopy following which they were stained with DAPI for epifluorescence microscopy. Cells were assessed for nuclear fragmentation and the presence of micronuclei. A modified FISH procedure was applied using a pancentromeric probe for evaluation of a less expensive method of micronucleus identification. Statistical analysis was by Fisher exact test using proportions of embryos or proportions of cells without regard to sire of origin**.** There was an increase in the frequency of abnormal embryos (per dose group) in pregnancies sired by acrylamide-treated males compared to controls (88.7% vs. 14.8%). Single-cell "embryos," which were either unfertilized oocytes or uncleaved zygotes, accounted for 38.2% and 8.1% of embryos in the acrylamide and control groups, respectively. Acrylamide-group embryos had a smaller cell number and a lower incidence of metaphases than embryos from the control group **[single-cell "embryos." which may include unfertilized oocytes, were included in the analysis; an**

accompanying figure lumps single-cell "embryos" with embryos having up to 10 cells, suggesting that unfertilized oocytes may have been included in the determination of embryo cell number and frequency of metaphase cells]. Using the embryonic cell as the unit of analysis, micronucleus frequency increased from 4/1000 cells in morphologically normal embryos of the control group to 41/1000 cells in morphologically normal embryos of the treated group. Micronucleus frequency was 93/1000 cells in morphologically abnormal embryos of the treated group. On a per embryo basis, the frequency of micronuclei was 13.6% in the control group and 21.9% in the treated group ($P \leq 0.05$). Within the treated group, 47.0% ($P \leq 0.001$ compared to control) and 8.3% of the normal and abnormal embryos, respectively, had micronuclei. There was no significant difference between the frequency of micronuclei in abnormal treated embryos and normal control embryos. The number of micronuclei per embryo was not different among normal control, normal treated, abnormal treated, or total treated embryos, according to the table, although the text states that the number of micronuclei per embryo was significantly greater in the treated group than in the controls **[CERHR used the Kruskas-Wallis statistic and found no significant difference among groups. Pair-wise testing by CERHR of the control embryos with normal treated embryos, abnormal treated embryos, and total treated embryos showed significant differences by unpaired *t*-test with Welch's correction for unequal variances, using the number of embryos to determine degrees of freedom because number of sires was not given]**. Other findings in this study included an effect of paternal acrylamide on decreasing nuclear area and increasing fragmented nuclei in morphologically normal embryos.

Strengths/Weaknesses: Overall, this paper represents a poorly-presented and analyzed study, which is unfortunate since it represents a tremendous effort on the part of the investigators and, in fact, is supportive of the published literature. Specific weaknesses are as follows: (a) Incomplete data presentation. The authors' Table I is also said to show that male mating behavior was not compromised by acrylamide treatment. It does not. The number of females mated to each male is not specified, but averages about 2.5. (b) It is not entirely clear, but all data collected between days 5 and 17 seem to be pooled, perhaps to raise the *n*. According to the Methods section, embryos were evaluated at specific breeding intervals post-treatment, but the data are not presented separately. The rationale for this reporting method is not clear, particularly given the well-known time relationship of acrylamide affects. This is a questionable approach at best. (c) Inclusion of uncleaved/unfertilized embryos in the "abnormal" category. While it is true that one cannot distinguish unfertilized embryos from those that are fertilized but fail to divide because of treatment effects, the practice of including them potentially overestimates the imputed genotoxic effects of acrylamide. This problem is particularly important here since acrylamide is *known* to have effects on fertilization (Sublet et al. *(73)* and many others). If one eliminates embryos in this category, the fraction of abnormal embryos falls to 50.5% in the treated group and 6.6% in the control group, which still demonstrates genotoxic effect. (d) Use of the embryo as the statistical unit, which inflates statistical significance. For example, 21.9% of preimplantation embryos in the treated group have micronuclei, compared to 13.8% in the control group, a difference reported as statistically significant ($P<0.05$; Author's Table III). If the analysis instead used the treated male as the statistical unit, which would be correct, would this difference still be statistically significant? (e) There is a high background of aberrations and the lumping of single-cell forms with embryos having up to 10 cells. (f) Clearly the "normal" embryos from treated animals had fewer cells. This disparity distorts the calculation of micronuclei per embryo (the authors' Table III), and creates a large difference in micronuclei per 1000 cells. If the "abnormal" embryos from the treated animals are included in this statistic (which apparently includes unfertilized/single cell zygotes), the distortion becomes even larger. Why, for example, does the number of nuclei per embryos with micronuclei in the treated group become so large (567 vs. 150 for the control) while the number of nuclei per embryo without micronuclei in the

treated group becomes less than control (290 vs. 390) (the authors' Table IV)? The authors' conclusion that acrylamide treatment resulted in smaller embryos, delayed proliferation, and more cell death is supported by their data, but a large gap lies between chromosomal events and the apparent induction of micronuclei in treated animals.

Utility (Adequacy) for CERHR Evaluation Process: The lack of distinction between unfertilized eggs and early embryos detracts from the utility of this study from in the evaluative process. The paper tries to bridge the gap between the chromosomal abnormalities induced in late stage sperm and mechanisms of embryotoxicity. It partially succeeds, but ignorance of the mechanism of micronucleus formation precludes a mechanistic explanation for the delay of proliferation other then the general one of chromosome abnormalities leading to cell death.

Holland et al. *(2)* performed a mouse dominant lethal study and measured chromatin adducts as part of a larger study on effects of acrylamide on preimplantation embryo development (discussed in section 3.2.3). The study was performed with Department of Energy and NIEHS funding. In the dominant lethal study, acrylamide **[purity unstated]** was given to C57Bl/6J male mice by daily i.p. injection, 50 mg/kg/day, for 5 days. An unspecified number of males was mated with approximately six females per group **[the term "group" probably represents a one-week mating period]** each week for five weeks from the end of treatment. Females were killed at 15–16 days **[plug day unspecified]** and evaluated for pre- and post-implantation loss. A control level of 12.3 and 7.3% for pre- and post-implantation loss was subtracted to give an acrylamide-induced loss rate. The protocol was not specified except by reference to Shelby et al. *(1)*. The data were expressed and analyzed as total percent loss **[without regard to sire]** Preimplantation loss in the groups mated at 1–2 weeks, 3–4 weeks, and 5 weeks after treatment of the male **[representing exposure of, respectively, spermatozoa, spermatids, and spermatocytes]** was, respectively, 72.1, 41.3, and 18.5%. Post-implantation loss at these intervals was 12.7, 8.2, and 10.1%. A comparison of the preimplantation loss rates at these times with the incidence of abnormal embryos reported in this paper showed similar rates (72.9, 40.1, and 10.1%), at least in the first two time intervals, suggesting to the authors that the preimplantation loss in the dominant lethal study was attributable to abnormal preimplantation embryos. In the evaluation of abnormal embryos, however, unfertilized eggs and zygotes were not distinguished. During weeks 1 and 2 after treatment of the male, 57% of day-4 "embryos" were represented by these single-cell forms, and during weeks 3 and 4, 22% were represented by these single-cell forms. It appears possible, then, that a substantial portion of what was called preimplantation loss could have been failure of fertilization rather than toxicity to the early embryo.

Holland et al. *(2)* also measured chromatin adducts in mouse sperm using accelerator mass spectrometry. Ten males were treated with 50 mg/kg ^{14}C acrylamide (3 mCi/mol **[= 10.7 mCi/dose]**) **[route not specified, but all other treatments in this paper were i.p.]**. Two males were killed every three days and sperm isolated from caudae epididymides. Sperm nuclei were separated by sonication and centrifugation. The dried pellet was converted to graphite by combustion to carbon dioxide followed by reduction of the carbon dioxide to graphite on cobalt. ^{14}C content relative to ^{13}C content was expressed as acrylamide equivalents in µg/g sperm **[when or how the sperm were weighed is not indicated]**. The results indicated to the authors that chromatin adducts peaked 9 days after treatment **[although day 6 data were not provided]**, consistent with involvement of late-step spermatids, testicular sperm, and epididymal sperm. The authors acknowledge that they could not distinguish protamine adducts from DNA adducts using their technique.

Strengths/Weaknesses: This paper is essentially a preliminary study of the one described in the paragraph above *(85*), and, like that one, is an interesting approach to looking at preimplantation developmental effects. This study is also flawed by significant inconsistencies in data presentation, methods description, the use of often inadequate methods and insufficient rigor, and a questionable statistical approach. Specific weaknesses include: (a) The number of embryos used for each stage is too small at many time points, with a low of 20 in the authors' Table 2 and 50 in the authors' Table 3. These numbers are entirely too small to draw such sweeping conclusions. Misuse of statistical analysis, using the embryo as the statistical unit, amplifies the problem since it gives the impression that something has actually been demonstrated. (b) The grouping of unfertilized eggs with abnormally developed embryos all as "abnormal embryos" is incorrect. Such a practice classifies a fertility effect as a genotoxic effect (the abnormal development). (c) Methods are unclear. For example, it is never clear to how many females each treated male was mated, what the division of mated females between chromosomal aberration studies and dominant lethal testing was, how decisions were made regarding grouping of data, and why only 2 males per time point were used for adduct quantification. The text indicates that corpora lutea were counted before embryos, thus accounting for inconsistencies in recovery, but the authors' Table 1 footnotes state that embryos were counted before corpora lutea "to avoid bias." (d) Fertilization rate is said to be unaffected by acrylamide treatment, but the fertilization rate in the control group is abnormally low (~20%), with no comment made. (e) The authors' Table 1 appears to contain several calculation errors. (f) Successful mating performance (which would correctly be defined as the number of plugged females per male) is said not to vary, but is a mean of 11.2 in treated animals at 50 mg/kg × 5 vs. 14.3 in controls and was as low as 4 in lower dose groups. (g) The authors' Tables 3 and 4 do not include data from control animals and it is not possible to tell how many males were treated or from which females the embryos were retrieved, further confounding data interpretation. The authors' Table 2 includes some control data, but data from selected time points are missing. (h) Data are often summarized inappropriately in the tables, grouping data from embryos collected at different time periods after treatment and then conducting a statistical analysis on them as if combining these data were appropriate. (i) The discussion of the effect, or lack of it, of female body weight and corpora lutea number, is puzzling, in part because body weights did not differ among groups (so there was no effect) and in part because it is difficult to envision an effect of this type on females when the males were treated; the authors do concede that body weight had little impact, however. (j) There is uncertainty that the tracer experiment measured adducts vs. drug and metabolites reaching the tissue compartment; i.e., there is no evidence the drug is bound to target of interest from this experiment alone. On the positive side, the number of abnormal embryos (as defined by the authors' criteria) match well with reported dominant lethality data. Significant numbers of high-dose males yielded abnormal embryos out to 5 weeks post-treatment, supporting the conclusions of Marchetti et al. *(3*) of chromosomal events in spermatocytes. These effects occurred even though acrylamide binding to mouse sperm was essentially at background by 18 days post-treatment.

Utility (Adequacy) for CERHR Evaluation Process: This study is generally supportive of other studies, but by itself is not of use in the evaluation process. The correlation of acrylamide binding, abnormal embryo numbers, and dominant lethal events are consistent and mutually supportive.

Nagao *(82*) (support not indicated) gave ICR mice 62.5 or 125 mg/kg acrylamide or 5 daily doses of 50 mg/kg/day. Acrylamide **[purity not stated]** was administered i.p. In addition to the evaluation of number implants, and early/late implant death (presented in Table 12), the author reported that there was no increase in external malformations among live fetuses sired by acrylamide-treated males. **[Data were analyzed by combining all fetuses within a dose group,**

without regard to sire of origin]. There were 20 males per dose group, with at least 2 pregnant females per male. Reduction of implantation was observed only in high dose, post-meiotic exposed sperm and increased post-implantation loss occurred at the 5 × 50 mg/kg and 125 mg/kg doses. The concurrent control rate of external abnormalities was higher than historical control.

Strengths/Weaknesses: It was not clear whether the lower number of pregnant females in the second period reflected reduced opportunity or reduced mating frequency. When so-called early and late deaths are combined, dominant lethal effects were observed at the mid and high doses during the first mating period (the postmeiotic germ cell stage), but not at the low dose. No effects were seen in animals mated during the spermatogonial germ cell time period. The separation of embryonic deaths into early and late deaths is probably unnecessary; the conclusion that early deaths were affected is perhaps misleading given the subjective nature of the classification.

Utility (Adequacy) for CERHR Evaluation Process: This study is supportive of other studies and is not of utility on its own.

2.3.2.4. Heritable translocations

Shelby et al. *(80)* (supported by NTP and DOE) administered acrylamide (> 99% purity) i.p. to male mice at 50 or 40 mg/kg/day for 5 days in two experiments. The 40 mg/kg/day dose was used in the second experiment in an attempt to increase numbers of progeny by decreasing the rate of dominant lethality. Acrylamide solutions were prepared in HBSS. Male (C3H × 101)F_1 mice were mated with female (SEC × C57BL)F_1 mice. The female offspring were killed and the male offspring retained and weaned. Male progeny were mated and if the first litter contained 10 or more offspring, the male was considered fertile. If the first litter contained fewer than 10 offspring, a second litter was evaluated. If the second litter contained 10 or more offspring, the male was considered fertile. If the second litter contained fewer than 10 offspring, the male was considered potentially partially sterile and was mated with three females. The females were killed on about GD 14 (plug day not specified) and uterine contents evaluated. The procedure of mating with three females was repeated to confirm reduced fertility. A random sample of ten males with reduced fertility were analyzed cytogenetically for the presence of reciprocal translocations in diakinesis metaphase I spermatocytes **[the method of analysis is not given except by reference to Evans et al. 1964]**. The proportions of male progeny that were sterile or semisterile after paternal treatment with 50 and 40 mg/kg/day × 5 days were 49/125 and 39/162 compared with 17/8095 in the historical control **[statistical analysis not perfomed; sire of origin not considered]**. All ten of the semisterile males sampled for cytogenetic analysis of spermatocytes showed translocations.

Strengths/Weaknesses: The authors estimate the frequency of heterozygous translocation male offspring derived from parental males treated with acrylamide. The timing of fertilizations was such that the offspring screened for translocations resulted from acrylamide exposures of spermatozoa or spermatids. The authors concentrated on these spermatogenic stages based on a strong dominant lethal response in these stages. Ten randomly chosen semi-sterile males from the 5 × 50 treatment group were cytogenetically analyzed and all confirmed as reciprocol translocation heterozygotes. The individual chromosomes involved in the translocations were not determined. Data were presented as the frequency of semi-sterile or sterile males for each treatment group and allow independent statistical analysis. The authors compared the frequency of combined semi-sterile + sterile males in treatment vs. control groups. Thus, the individual screened male offspring was the experimental unit for comparisons. The implied assumption in this study is that translocations are induced by treatment of the late spermatogenic germ cell

stages and that the resultant offspring, even littermates, represent the independent treatment of different male germ cells. If these assumptions are true, a statistical analysis without consideration of the breeding structure of the population is justifiable. The authors do not mention the presence or absence of clustering (more than one semi-sterile or sterile male offspring recovered from a particular treated male). Since all semi-sterile and sterile males were not cytogenetically analyzed and the cytogenetic analyses did not identify the chromosomes involved in the translocation, the results as presented cannot definitively exclude the occurrence of clustering. However, based on the number of parental males treated and the number of male offspring screened per dose group, there were approximately 1–1.3 male offspring screened for litter-size reduction/sterility per parental male. This observation strongly suggests that clustering was absent and supports the statistical analysis as reported.

Utility (Adequacy) for CERHR Evaluation Process: This is study is one of the three published studies that estimates the effect of acrylamide treatment on transmitted germ-line cytogenetic end points and is critical for the evaluation of acrylamide. This study clearly demonstrates that acrylamide is an effective inducer of translocations in postmeiotic germ cells of rodents but lacks adequate dose-response information.

Adler *(64)* (European Community) administered acrylamide **[purity not specified]** intraperitoneally to C3H/E1 mice at 50 mg/kg/day for five consecutive days. Each male was mated to two female 102/E1 mice 7–11 and again 36–42 days after the end of treatment. No control group was reported. The author states that the litter size was reduced in the first mating interval due to the dominant lethal effect of acrylamide **[statistical analysis was not performed by the author. CERHR performed an ANOVA with post-hoc Neuman-Keuls multiple comparison test on the two replicates at each mating interval. The overall ANOVA gave a *P* value of < 0.0001 with the post-hoc test showing a difference between both 7–11 day matings and both 36–42 matings in litter size, with a reduction from about 6 pups/litter to about 2 pups/litter at birth].** The male and female offspring from the 7–11 day-matings were mated to unexposed (102/E1 × C3H/E1)F_1 mice and the male and female offspring from the 36–42-day matings were mated to one another (avoiding sibling matings). Male offspring were suspected of being translocation carriers based on reduced litter size. These animals were unilaterally orchidectomized and meiotic chromosome preparations were used to confirm translocation status. There were 23 translocation heterozygotes among 105 progeny from the offspring of the 7–11-day mating interval **[the number of treated males giving rise to these offspring is not stated]**. Among the offspring of the treated males there were 17 male translocation carriers among 58 male offspring and 6 female translocation carriers among 48 female offspring (male vs. female, $P < 0.05$). In the second mating interval (36–42 days after treatment), 1004 offspring were produced of which two 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.

Strengths/Weaknesses: In this study, repeated acrylamide exposure was employed. In the first group, treated spermatozoa and spermatids were assayed for the occurrence of heterozygous reciprocal translocation carriers in the resultant offspring. Both male and female offspring were screened for semi-sterility or sterility. All suspect reciprocal translocation carriers were cytogenetically analyzed and the individual chromosomes involved, with breakpoints, identified. The data are reported for the numbers of semi-sterile, sterile, and confirmed translocation carrier offspring among the animals screened, which allows an independent analysis of the results. The screened offspring was the experimental unit for comparison (breeding structure from which the population was derived was ignored), which is justifiable if the assumptions that the translocations are induced by treatment of the late spermatogenic germ cell stages and that the

resultant offspring, even littermates, represent the independent treatment of different male germ cells are correct. The authors do not mention the presence or absence of clustering of semi-sterile or sterile offspring among the descendents of a particular treated parental male. However, results of the cytogenetic analyses confirm the assumption that clustering did not occur (all reciprocal translocations recovered were unique). The observed difference in the frequencies of translocation carriers identified in male vs. female offspring should be considered when comparing these results to Shelby et al. (*(80)*; only male offspring screened). The Expert Panel suspects that the testing procedure for semi-sterility may be dependent on the genetic background of the females mated. The second experimental group is potentially very interesting. Offspring resulting from treated early spermatogenic stages (36-42 days post-treatment fertilizations) were screened for reduced fertility by mating male offspring from the treated parental males to female offspring from treated parental males. The observed increase of translocation carriers in male offspring needs to be repeated or utilized cautiously in the acrylamide evaluation.

Utility (Adequacy) for CERHR Evaluation Process: This study is one of three published reports on the frequency of heritable reciprocol translocations in germ cells of the mouse and is essential for an evaluation of acrylamide. First, it provides an independent assessment of the effects of acrylamide in treated late stage spermatogenic stages of the mouse on the frequency of translocations. Second, it provides definite cytogenetic data on the recovered semi-sterile and sterile males. These results are important in addressing the question of clustering in both studies and the occurrence of multiple translocation events in some offspring. This study provides clear evidence that acrylamide induces structural chromosomal damage in postmeiotic cells of rodents, but lacks dose-response information.

Adler et al. *(86)* (European Community) treated male C3H/El mice with 50 or 100 mg/kg bw acrylamide. Each male was mated to 2 102/El mice 7–16 days post-treatment. Male and female offspring were tested for semi-sterility/sterility as described above in Adler 1990. All suspect translocation carriers were cytogenetically analyzed to confirm the presence of chromosomal rearrangements and to identify the chromosomes involved and the positions of the breakpoints. 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, 3/8700. As in the previous studies (above) the experimental unit of comparison was the individual offspring screened for the presence of a translocation. Clustering was not apparent as indicated by the fact that all translocations were unique. Even two independent T(5;6) mutations could be distinguished based on the positions of the breakpoints. There was a possible prevalence of male translocation carriers (9/12); however, the frequency data were not reported nor analyzed separately by sex of offspring screened. These results and the 5 × 50 mg/kg treatment results from Adler's lab were submitted to a dose-response analysis. Results suggested that the 5 × 50 treatment is more effective than a linear extrapolation from the single acute dose points to the accumulated total dose as the determinant of response.

Strengths/Weaknesses: The use of cytogenetic analysis to determine the involved chromosomes and the positions of the breakpoints is an important strength in this study.

Utility (Adequacy) for CERHR Evaluation Process: This study is one of three published reports on the frequency of heritable reciprocol translocation in germ cells of the mouse and is essential for an evaluation of acrylamide. This study provides clear evidence that acrylamide induces structural chromosomal damage in postmeiotic cells of rodents, and is the only study for transmitted genetic effects in mammals in which a dose response analysis was attempted.

2.3.2.5. Specific locus

Russell et al. *(87)* used (101/R1 × C3H/R1)F_1 male mice treated with acrylamide (>99% pure) 50 mg/kg i.p. every day for 5 days. This dose regimen was used to decrease the lethality associated with single i.p. doses greater than 125 mg/kg. Males were mated at specific intervals after mating to T-stock females homozygous for *a* (non-agouti), *b* (brown), c^{ch} (chinchilla), *p* (pink-eyed dilution), *d* (dilute), *se* (short ear), and *s* (piebald). Offspring of matings were evaluated at about three weeks of age for mutant phenotypes associated with these loci and for other recognizable external variations. Presumed mutants were bred to determine whether a heritable mutation was present. Findings included a marked decrease in the production of offspring when mating occurred 1 or 2 weeks after acrylamide treatment (attributed by the authors to dominant lethality). Specific locus mutations occurred in 5 of 28,971 offspring with exposures 1 to 7 weeks after treatment, which was significantly higher than the historical control rate of 43 in 801,406 (P=0.026 in a Fisher one-tailed exact test **[which assumes the offspring as the statistical unit]**. The 2 mutants arising from matings 1 and 2 weeks after treatment represented a significantly higher mutation rate than the 3 mutants arising from matings in weeks 3–7; the rate in this latter period was not significantly greater than the control rate. No mutations were recovered in 17,112 offspring derived from treated stem cell spermatogonia (fertilizations occurring >49 days post-treatment). The authors indicate that this observation excludes at the 5% level an induced mutation rate greater than 2.3 times the historical control rate, following the procedure of Selby and Olson. The historical control rate used the combined historical control data of Oak Ridge, Harwell and Neuherberg, 43/801,406.

Strengths/Weaknesses: The authors have sampled all spermatogenic stages in treated males within the limits of laboratory capacity. Original mutation frequency results were presented and allow statistical evaluation. Where possible, all recovered mutations were genetically confirmed, characterized for homozygous viability, and cytogenetically analyzed. The major conclusions are that acrylamide is mutagenically active in late spermatids–spermatozoa, the recovered mutations are associated with chromosomal aberration-type events (deletions and/or translocations), and acrylamide is not mutagenically active in stem-cell spermatogonia. The statistical analysis is based on the screened F1 offspring as the unit of analysis (not the treated male), which is acceptable in treating the development of each germ cell as an independent event. There was no clustering of repeat mutations at a particular locus within the offspring of a single treated male as evidenced by the allelism test results of the mutations recovered. If such clustering had occurred, the recovered mutations could have been pre-existing rather than induced. Further, ignoring the family structure of the offspring provides an unbiased estimate of the mutation frequency when clustering is absent.

Utility (Adequacy) for CERHR Evaluation Process: These results represent one of two studies on transmitted germ cell specific-locus mutations and are essential for a consideration of the genotoxicity of acrylamide.

Ehling and Neuhäuser-Klaus *(74)* performed a mouse specific locus test sponsored by the Commission of the European Communities. Male mice were (102/E1 × C3H/E1)F_1 hybrids, treated with acrylamide **[purity not stated]** in distilled water at 0, 100, or 125 mg/kg i.p. Immediately after treatment, males were housed with untreated test-stock females homozygous for *a* (non-agouti), *b* (brown), c^{ch} (chinchilla), *p* (pink-eyed dilution), *d* (dilute), *se* (short ear), and *s* (piebald). Females were replaced every four days for 20 days. On day 21, 100-mg/kg males were housed with females for an unspecified period of time **[but at least 43 days]**. Offspring were evaluated for mutant phenotypes. Their conception was dated as their day of birth minus a presumed 20-day gestation length. Evaluation for mutant phenotypes began at the day of birth

and continued until 21 days of age. Statistical evaluation was by Fisher exact test **[thus taking the offspring as the statistical unit].** At 5–8 and 9–12 days after treatment, specific locus mutation and dominant lethals (see Table 12) were increased. There were three mutant offspring among 4647 total offspring at these time points in the 100-mg/kg group and three mutant offspring among 3872 total offspring in the 125-mg/kg group. The control rate was 22 mutants among 248,413 offspring. The acrylamide group rates were significantly different from the control rate. With mating ≥ 43 days after treatment (indicating fertilization with sperm exposed as spermatogonia), there were 6 mutants among 23,489 offspring, which was significantly increased compared to the control rate ($P = 0.03$).

Strengths/Weaknesses: Two, single acute doses of acrylamide were employed in this specific locus mutation test. Original mutation frequency results were presented and allow statistical evaluation. As in the Oak Ridge study, all recovered mutations were genetically confirmed and characterized for homozygous viability, where possible. The major conclusions from the sampled post-spermatogonial stages are that acrylamide is mutagenically active in spermatozoa and late spermatids and that the recovered mutations are deletion-type events. The authors sampled treated post-spermatogonia up to day 21 post-treatment in shorter mating intervals than the Oak Ridge study. Offspring arising from fertilizations occuring 21 through 42 days post-treatment were combined into a single treatment group. Because both the Oak Ridge and the Neuherberg results indicated no increased mutation frequencies in post-spermatogonial stages outside of spermatozoa–late spermatids this combination is acceptable. Statistical analyses were based on F_1 offspring being the experimental unit, not the treated parental male. As in the Oak Ridge experiment, no clustering of specific locus mutations was reported and ignoring the family structure of the offsping for statistical analysis was justified. In general, there are no discrepancies in the results from Oak Ridge and Neuherberg for the post-spermatogonial stages.
For treated spermatogonia, the observations from Oak Ridge and Neuherberg differ and are statisticaly significant (Fisher's test, $P = 0.0430$). There is a difference in experimental protocol, which Ehling and Neuhäuser-Klaus discuss: Oak Ridge employed a 5×50 mg/kg bw dosing, whereas the Neuherberg experiment used single, acute exposure doses.

Utility (Adequacy) for CERHR Evaluation Process: The Neuherberg results are one of only two experiments providing transmitted germ line specific locus mutation estimates in the mouse and essential for an evaluation of the genotoxicity of acrylamide. The study provides evidence for specific-locus mutations in spermatid and spermatozoal stage germ cells of male mice, but lacks dose-response information.

2.3.2.6. Mammalian spot test

Neuhäuser-Klaus and Schmahl *(88)* performed a mouse spot test, supported in part by the Umweltbundesamt, Berlin. T-stock females were mated with HT males (2 females to 1 male) and the day of a vaginal plug was counted as GD 1. Females were treated with acrylamide (analytical grade) in distilled water at 50 or 75 mg/kg i.p. once on GD 12 or daily on GD 10, 11, and 12. Control females were injected with distilled water. Five replicate experiments were conducted. The mouse spot test involved non-agouti black mice heterozygous at several coat color loci. Mutations of the dominant wildtype allele at any of the heterozygous coat color loci were detected as "spots of genetic relevance" as distinguished from coat color changes due to pigment cell inactivation or misdifferentiation, which were characterized as not of genetic origin. Offspring with spots of genetic relevance were counted and group comparisons made with the control by Fisher's exact test **[thus taking the offspring as the statistical unit]**. After a single injection on GD 12, offspring with spots of genetic relevance were increased in both acrylamide groups. The proportions were compared based on weaned offspring rather than offspring at birth. These proportions in the 0, 50, and 75 mg/kg groups were 5/212 (2.4%), 14/213 (6.6%), and

13/211 (6.1%), respectively. The proportions of weaned offspring with spots of genetic relevance were also increased after the three-dose regimen in both acrylamide groups: 0, 50 mg/kg × 3, and 75 mg/kg × 3 proportions were 6/225 (2.7%), 26/196 (13.3%), and 21/215 (9.8%), respectively.

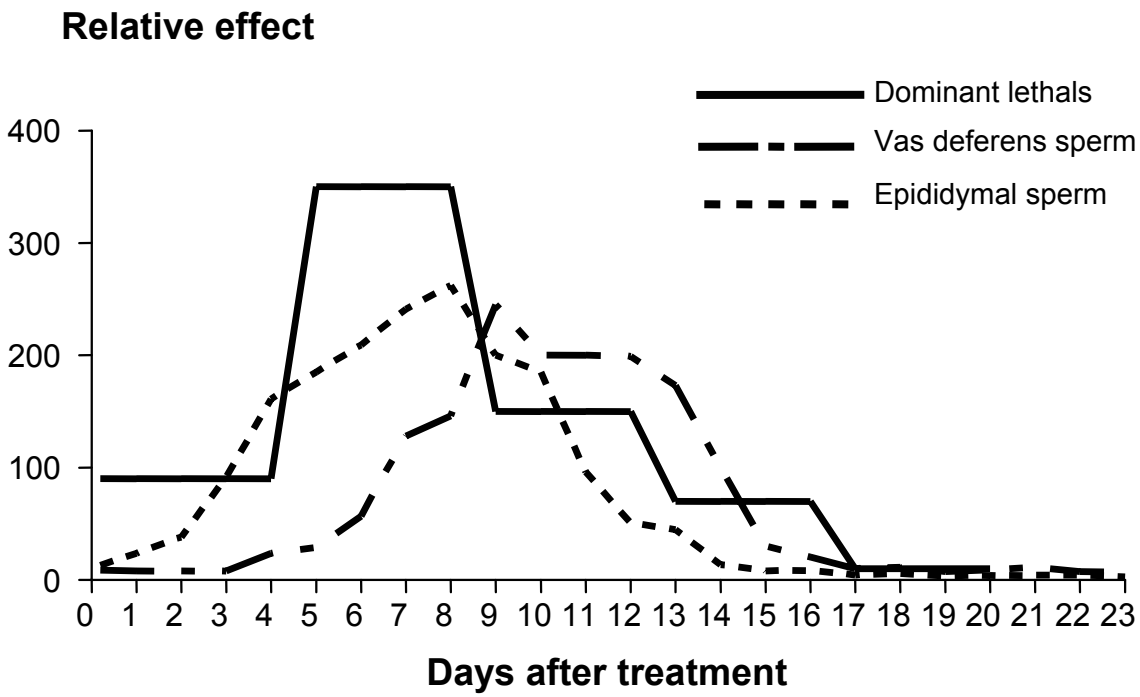
Strengths/Weaknesses: The authors assessed the mutagenic activity of acrylamide in the mammalian somatic spot test, which involves in vivo treatment at the embryonic stage. Two single acute dose exposures as well as two multiple dose exposures were included as well as concurrent controls. Spots were phenotypically distinguished as "spots of genetic relevance," i.e. spots expressing a homozygous recessive color consistent with mutation of the wildtype allele at one of the 7 heterozygous coat color loci screened, or spots due to cell killing or mis-differentiation. All treatment group frequencies of genetically relevant spots were statistically higher than the respective concurrent control value. The original frequency results are presented and allow an independent statistical evaluation. The authors do not clearly state if there was clustering or lack of clustering of spots in the offspring examined. The paradigm of the mammalian somatic spot test is that spots of genetic relevance represent a somatic mutational event in the pigment-producing melanocytes of the treated embryo. Each offspring examined represents the treatment of a population of melanocytes and the susceptibility status between individual pregnant females and the embryos within each pregnant female is assumed similar. Thus, results were reported for the individual offspring as the experimental unit. In the present study the frequency of spots is extremely low and probably not adequate to test this assumption.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for an evaluation of the mutagenic effect of acrylamide in mammals. The mammalian spot test represents specific locus mutations in somatic cells and is regarded as a short-term test with good predictive value for the mouse germ cell specific locus test.

2.3.2.7. Effects on DNA and protamine in male germ cells

Sega et al. *(4)* injected male (C3H × 101) F_1 mice with ^{14}C-labeled acrylamide (1.00 mCi/mmole) at an intraperitoneal dose of 125 mg/kg. Six males per time point were killed 4 h later and daily for 23 days after the injection. Vasa deferentia and caudae epididymides were dissected. Sperm heads were isolated by filtration and sonication followed by centrifugation through sucrose. Radioactivity in sperm heads was measured using scintillation counting after the addition of scintillation fluid. Separate aliquots of sperm heads were used for isolation of DNA and of protamines, and radioactivity in these fractions was measured using scintillation counting. The time-course of sperm head radioactivity in this study was compared to the time-course of dominant lethality in the same strain of mice in Shelby et al. *(1)*. Statistical comparisons were not performed, but inspection of the data suggested parallel effects, with late spermatids and early spermatozoa the most affected stages (Figure 4). Very little radioactivity was found in the DNA fraction, whereas protamine radioactivity and total sperm head radioactivity following superimposable time-course curves. Amino acid analysis of hydrolyzed sperm protamine showed a radioactivity peak that co-chromatographed with *S*-carboxyethylcysteine. The authors postulated that acrylamide alkylation of cysteine sulfhydryl groups in protamine occurred, resulting in *S*-formamidoethyl cysteine. The acid hydrolysis step could have resulted in *S*-carboxyethylcysteine through loss of the amino group. The same data and conclusions were presented in a subsequent publication *(89)*.

Figure 4. Comparison of Timing of Dominant Lethality and Sperm Head Alkylation with Dominant Lethal Data from Shelby et al. *(1)* and Alkylation Data From Sega et al. *(4)*. Y-axis is dominant lethality (% above controls) multiplied by 10 for clarity or ^{14}C dpm per 10^6



Sega and Generoso *(90)* (supported by DOE/NICHD) measured DNA breakage in the germ cells of male (C3H × C57BL/10)F_1 mice using an alkaline elution procedure. Radiolabeled DNA from treated and control animals was applied to a 4-μm pore size polycarbonate filter and exposed to a buffer at pH 12.0. The amount of DNA passing through the filter was taken to represent fragmented DNA. Males were treated with acrylamide (99.9% purity) at a dose of 100 mg/kg intraperitoneally the day after intratesticular injection of labeled thymidine. Sperm were obtained from the vas deferens 1-21 days after acrylamide injections. Additional experiments were conducted using doses of acrylamide from 25 to 125 mg/kg i.p. with harvesting of sperm from the vas deferens 10 days later. Pachytene spermatocytes and spermatids were isolated from testes by centrifugal elutriation for evaluation 90 minutes, and 1, 2, 3, and 4 days after acrylamide treatment (100 mg/kg i.p.). Statistical analyses were not presented and the number of males contributing germ cells was not given; however, the data tables indicate the number of "independent experiments" **[not further explained]**. The percent of sperm DNA eluted was increased compared to an untreated control when sperm were obtained 1–16 days after treatment with 100 mg/kg acrylamide **[identified as increased based on ANOVA with post-hoc Dunnett test performed by CERHR]**. Seventeen to 21 days after treatment, the percent eluted sperm DNA was similar to the untreated control value. The percent of sperm DNA eluted 10 days after acylamide was related to acrylamide doses between 25 and 75 mg/kg; higher doses up to 125 mg/kg had no additional effect **[the data are shown only in graphic form with no indication of the number of independent observations; the identification of statistical difference from control was made by CERHR using unpaired *t*-tests and assuming 21 observations in the control (which appeared to be the same control used for the previous comparison) and at**

least two observations per acrylamide-treated group]. Pachytene spermatocytes showed a significant increase in DNA eluted 90 minutes, 1 day, 2 days, and 3 days after acrylamide treatment. By 4 days after treatment, the DNA elution percentage was similar to that of the control **[based on ANOVA with post-hoc Dunnett test performed by CERHR from data presented in a table].** The authors stated that DNA elution was increased in early spermatids with the greatest effect occurring 1 day after acrylamide treatment [ANOVA with post-hoc Dunnett test by CERHR shows a difference from the control only for the day 1 value. This analysis may not be appropriate given the large standard errors for some of these data]. The authors state that DNA breakage appeared to be greatest in mid/late spermatids [ANOVA with post-hoc Dunnett test by CERHR shows significant increases compared to control and 1, 2, 3, and 4 days post-treatment]. The authors interpreted their results as consistent with DNA breakage due to DNA alkylation in spermatocytes and early spermatids. By day 17 after treatment, treated germ cells would have entered a phase during which DNA repair occurs, resulting in elimination of the vulnerable DNA. The increase in DNA breakage of mid/late spermatids and spermatozoa was attributed by the authors to alkylation of protamines with DNA breakage secondary to stresses imposed on chromatin by the damaged protamines. The authors concluded that the demonstration of sensitivity of mid/late spermatids and spermatozoa was consistent with the sensitivity of dominant lethality to acrylamide treatment during these stages.

Sega et al. *(91)* (supported by DOE and NIEHS) evaluated unscheduled DNA synthesis (UDS) in the germ cells of male (C3H × 101)F_1 mice after treatment with acrylamide (99.9% purity). Radiolabeled thymidine was injected into the testes of mice. Acrylamide was given at doses of 0, 7.8, 15.6, 31.2, 62.5, and 125 mg/kg i.p. from 0 to 48 h prior to injection of labeled thymidine. Cauda epididymal sperm were obtained 16 days after treatment, representing sperm that were in the early spermatid stage at the time of acrylamide treatment. The maximal incorporation of radiolabeled thymidine occurred 6 h after acrylamide treatment, suggesting that time was required for direct acrylamide-mediated DNA damage or for metabolism of acrylamide to the proximate DNA-damaging agent. Evaluation of the slope of the dose response curves for radiolabeled thymidine incorporation showed a five-fold steeper curve when the labeled thymidine was given 6 h after acrylamide compared with simulataneous administration of labeled thymidine and acrylamide. The authors state that intratesticular radiolabeled thymidine is available for only about 4 h; thus, the lack of incorporation of radiolabeled thymidine when injected simultaneously with the acrylamide treatment supported the conclusion that acrylamide-associated DNA-repair was delayed at least 4 h.

In a second experiment reported in the same paper, male (C3H × BL10)F_1 mice were treated i.p. with 0 or 125 mg/kg acrylamide with evaluation of epididymal sperm every 2 or 3 days between 1 and 30 days after treatment. Radiolabeled thymidine was given 6 h after acrylamide. There were 4 mice per dose group at each sampling time point. An increase in radiolabeled thymidine incorporation into DNA was evident 12–27 days after treatment [statistical analyses were not presented and the graph cannot be analyzed statistically due to the pooling of sperm from the four males per dose group at each time point]. According to the authors, the sensitive time period corresponds to treatment of germ cells at the early spermatocyte to mid-spermatid stage.

In a final experiment, radiolabeled acrylamide was given to male (C3H × BL10)F_1 mice by i.p. injection at a dose of 46 mg/kg bw. Testes and livers were recovered from each of 4 animals per time point from 1, 2, 4, 6, 8, and 24 h after acrylamide treatment for evaluation of radioactivity incorporated into the DNA. The peak number of adducts (expressed per million deoxynucleotides) was reached in the testis at 6 h, whereas in liver, the peak occurred at 1 or 2 h [no statistical analysis was presented and it is not possible to analyze the graphed data statistically due to pooling of samples from the four animals used at each time point; in the liver, the 1 and 2 h values could be statistically similar]. In addition, the number of adducts in the liver was an order of magnitude higher than in the testis.

The authors pointed out that no other chemical in their experience showed such a long delay between administration and UDS as did acrylamide. They considered unlikely the possibility that direct damage of DNA by acrylamide was a slow process, due in part to the observation *in vitro* that acrylamide binding to DNA is rapid. They concluded that their experiments supported the hypothesis that acrylamide effects on DNA are mediated through metabolism to an active intermediate such as glycidamide.

Strengths/Weaknesses: By using the same protocol as was used for dominant lethal studies this series of mechanistic studies in mice was able to correlate alkylation of protamine (and lack of alkylation of DNA) with dominant lethal effects (percent dead implants) attributed to exposures of late spermatids and early spermatozoa. Aliquots of the same samples were analyzed for both protamine and DNA adducts *(89, 90*), allowing direct comparisons. Protamine alkylation was shown convincingly to involve reaction with free sulfhydryls in protamine and to occur at times when protamine is present in mature spermatids (late stages of spermatogenesis) and before inter- and intra-molecular disulfide bonds cross-link the protamine. Thus the vulnerable window of development for this aspect of acrylamide toxicity is defined based on a molecular mechanism**.** The alkaline elution studies *(91*) confirmed the presence of DNA breakage in late spermatids and early spermatozoa (thought to be secondary to protamine alkylation) and also in pachytene spermatocytes (thought to be direct, but reparable, hence not resulting in dominant lethality). The time course for the latter is consistent with a delay and implicates a requirement for metabolism to an active molecule. The results of the UDS experiments *(92*) are consistent with DNA damage to spermatocytes and early spermatids being repaired before the cells become sperm, and hence the lack of dominant lethality. Thus, the studies in this series logically complement one another and provide a compelling model to explain the dominant lethality (percent dead implants) of acrylamide. Weaknesses of this series of papers are as follows: Sega et al. *(89*), attributed all labeling by radioactive acrylamide in sperm heads to covalent binding to protamine. According to the authors, all of the radioactivity in the sperm head was bound to protamine, presumeably to Cys, as carboxyethyl-Cys was demonstrated. Other proteins in sperm have Cys-why weren't these proteins labeled? Since sperm from the vas and epididymis were taken, prior labeling of histones would have been lost, since the histones are replaced by the protamines at this stage of development. Sega et al. *(91*) demonstate that DNA strand breakage occurred in pachytene

spermatocytes (purified fractions were described as 80% pure, but the method of determining purity was not described, although scanning electron micrographs of pachytene spermatocytes and round spermatids were presented). The damage demonstrated 1 day after exposure in the pachytene spermatocytes had disappeared by the time the pachytene spermatocytes had advanced to the post-meiotic stages, presumeably by DNA repair. DNA repair was the subject of the 4th paper of this series, whereby UDS was measured after acrylamide exposure. UDS peaked 6 h after acrylamide exposure; a time when free acrylamide would have been cleared from the tissue. UDS measured 6 h post acrylamide showed a strong acrylamide dose dependency. Error bars representing SEM are given on the graphs in the figures, but no indication of the number of experiments is provided, so the basis for the SEM is a mystery.

Utility (Adequacy) for CERHR Evaluation Process: These studies provide a model by which acrylamide (or a metabolite) may produce dominant lethality (increased percent dead implants) when exposures occur at a particular stage of spermiogenesis by binding to protamine in spermatids and early spermatozoa before protamine is cross-linked as the sperm move from the caput to cauda epididymis. This model also accounts for only minimal if any DNA alkylation at this time when protamine appears to protect DNA from alkylation. This model is biologically plausible and would be expected to be pertinent for both rodents and humans. Thus, it provides at least one basis for extrapolation from rodent test species to humans when characterizing reproductive risks of acrylamide.

Support for glycidamide as the mediator of acrylamide genotoxicity was provided by Generoso et al. *(92)*, supported by DOE and NIEHS. Glycidamide (100% stated purity) was administered i.p. to male (C3H/RL × 101/RL)F_1 mice. Tests were performed for dominant lethality, heritable translocations, and UDS. The glycidamide doses were selected based on preliminary studies to provide the highest dose that did not impair survival or mating ability. **[No attempt was made to use doses comparable to acrylamide doses in previous studies. Although acrylamide and glycidamide are nearly identical in molecular mass (differing only by a single hydrogen atom), the authors did not know the extent to which i.p. acrylamide in male mice would be metabolized to glycidamide].** Dominant lethal testing was performed with a glycidamide dose of 125 mg/kg. Results of the dominant lethal study were analyzed on a per female basis and showed a depression in live implants and an increase in resorptions with mating 0.5–11.5 days after treatment of the male **[Analysis per sire was not possible with the information given. Males were mated with two females/day according to the methods, but there appears to have been a decrease in mating ability. During the first three-day period, there were reported to have been 36 treated males. There should have been 216 mated females (36 males × 2 females/male/day × 3 days). The reported number is 54 mated females. A similar deficit appears in the control group for which 24 males were reported, giving the expected of 144 mated females, with 48 actually reported. Other time periods show more than 48 mated females per 3-day time period. Therefore, it remains unclear how many females were used per male per time period. If the protocol was similar to Shelby et al. *(1)*, two females were housed with each male, but only replaced when one showed evidence of breeding; i.e., not 2 new females per day unless both bred the night before. Since the authors state there is no difference in the number of females bred per time interval, data on implants per female seem valid (despite lack of male as experimental unit). The only "suspicious" period is 0.5 – 3.5 days when the number of mated females that became pregnant is lower than at other times (44% vs. ~60-70% in this study; and only 33% vs. 70-100% in Shelby et al. *(1)*)) implying that some males were not fertile at this time and were not therefore part of the analysis of percent dead implants. This possible discrepancy does not appear to affect the overall interpretation of this study].**

The heritable translocation test was performed using 100 mg/kg i.p. glycidamide. There were 669 male progeny of glycidamide treated sires. Among the progeny, 52 were sterile and 94 were semi-sterile (not defined in this study, but defined in a previous study by these authors as producing fewer than 10 offspring per litter in two consecutive litters). Semi-sterility was taken as evidence of chromosome rearrangements based on the authors' prior experience. Sterile males underwent cytogenetic analysis. The percent translocations **[not defined]** was 20.18, compared to 0.06 from historical control data.

The UDS experiment used glycidamide 150 mg/kg i.p. with vehicle-injected males as controls. Radiolabeled thymidine was given 0, 2, 4, or 6 h after the injection of glycidamide. UDS was increased in the glycidamide treated animals at all time points, with the maximum effect seen when radiolabeled thymidine was given 2 h after glycidamide **[statistical testing was not indicated in the paper, but *t*-tests by CERHR show significant differences at all time points]**. These results were considered by the authors to be consistent with direct DNA damage by glycidamide. According to the authors, comparison of the UDS response to glycidamde in this paper with the UDS response to acrylamide from Sega et al. *(91)* showed reasonable agreement with the theory that acrylamide-associated UDS is due to metabolism to glycidamide, accounting for the differences in injected radioactivity, moles of chemical, and experimental uncertainty.

2.4 Carcinogenicity

2.4.1 Human data

The European Union *(8)* and IARC *(11)* concluded that human data were inadequate to assess the carcinogenicity status of acrylamide. The conclusions were based on two occupational mortality cohort studies *(93, 94)*. These studies reported negative results. IARC concluded that power to detect cancer at any specific site was extremely limited in the Sobel et al. *(94)* study due to small sample, short exposure duration, and short latency. In their review of the Collins et al. *(93)* study, the European Union stated that no firm conclusions could be drawn from that study. The study by Collins et al. *(93)* was later updated and published as Marsh et al. *(95)* and reviewed in JIFSAN/NCFST *(39)*. A JIFSAN/NCFST *(39)* panel reviewed the Marsh et al. *(95)* study, which examined cohorts of workers in 3 plants hired between 1925 and 1973 and followed through 1994. The cohorts originally consisted of 8854 males but was later reduced to 8508 workers due to incomplete records for some workers. Workers were considered exposed to acrylamide if cumulative exposure was >0.001 mg/m^3·years. No statistically significant excess of mortality from cancer at any specific site was noted when compared to expected rates. An increase in respiratory cancer in one plant occurred primarily in workers exposed to muriatic acid. An excess of pancreatic cancer was noted in workers exposed to >0.30 mg acrylamide·year, but Marsh et al. *(95)* noted that information on smoking, a major risk factor for pancreatic cancer, was not available for each member of the cohort. Marsh et al. *(95)* concluded that study results provide little evidence of a relationship between acrylamide exposure and cancer mortality.

CERHR identified two additional epidemiologic studies examining the relationship between diet and cancer. Because those studies have not been addressed in major reviews to date, they are being reviewed and summarized in detail.

Pelucchi et al. *(96)* examined the association between fried or baked potato consumption and cancer by reviewing a series of hospital based case-control studies conducted in Italy and Switzerland between 1991 and 2000. Potatoes were used as an indicator of acrylamide intake, although the authors acknowledged that numerous other starchy foods may contain acrylamide. The types of cancer (n=cases, controls) considered were oral cavity/pharynx (n=749, 1772), esophagus (n=395, 1066), larynx (n=527, 1297), colon (n=1225, 4154), rectum (n=728, 4154),

breast (n=2569, 2588), and ovary (n=1031, 2411). Median ages of subjects ranged from 55 to 62 years. Subjects were questioned about dietary intake during the past two years. Odds ratios were calculated and adjusted for age, gender, location, education, body mass index, energy intake, alcohol intake, smoking, physical activity, and/or parity. No association between potato intake and cancer was noted as indicated by odds ratios between 0.8 and 1.1 for each type of cancer.

Mucci et al. *(97)* examined the association between dietary acrylamide intake and cancer of the large bowel, kidney, and bladder by reanalyzing data from a Swedish population-based case control study that was originally designed to determine the relationship between heterocyclic amines in fried foods and cancer. Subjects of the study were born in Sweden between 1918 and 1942 and resided in Stockholm for at least 1 month between November 1992 and December 1994. Subjects included 538 healthy controls (50.6% male), 591 with large bowel cancer (58.5% male), 263 with bladder cancer (75.7% male), and 133 with kidney cancer (53.4% male). At the time of the study, subjects ranged in age from 51 to 77 years. Subjects were questioned about their intake of 188 food types during the 5 years prior to this study. Acrylamide levels were measured in more than 100 kinds of foods. Acrylamide doses were estimated based on intake frequency of different food types. Odds ratios were estimated using unconditional logistic regression with adjustment for age and gender, as well as confounding factors (e.g., smoking, body mass index, alcohol intake, fruit and vegetable intake, saturated fat density, red meat density, and total energy). There was no evidence of excess risk or trends for large bowel, bladder, or kidney cancer in subjects who ingested large quantities of 14 food types with high (300–1200 μg/kg) or moderate (30–299 μg/kg) acrylamide levels. No association with bladder or kidney cancer was found in an analysis of acrylamide dietary intake quartiles. A 40% reduced risk of large bowel cancer was observed in the highest compared to lowest quartile of acrylamide intake (P=0.01 in trend analysis) **[The Expert Panel notes that the negative results of Pelucchi et al. *(96)* and of Mucci et al. *(97)* may have been due to inadequate power to detect the magnitude of an increase suggested by experimental animal studies. The Panel does not consider these papers to discount the experimental animal results]**.

The FAO/WHO *(17)* noted that an absence of positive cancer findings in epidemiologic studies does not prove that the substance does not cause cancer because such studies have limited power to detect small incidences of increased tumors. It was also noted by the FAO/WHO that the epidemiologic studies did not consider dermal exposure. **[The Expert Panel agrees that the human data are inadequate for a determination of acrylamide carcinogenicity].**

2.4.2 Experimental animal data

Two animal carcinogenicity studies were identified and they were both reviewed in detail since acrylamide exposure was found to result in cancer of reproductive organs.

In an industry sponsored study, Johnson et al. *(57)* examined carcinogenicity in Fischer 344 rats following chronic exposure to acrylamide (enzyme grade; 96–99% purity). At 5 to 6 weeks of age, 90 Fischer 344 rats/sex/group were administered acrylamide through drinking water for 2 years at concentrations that resulted in doses of 0, 0.01, 0.1, 0.5, or 2.0 mg/kg bw/day. Dose selection was based upon the subchronic study by Burek et al. *(55)*. Dosing solutions and drinking water samples were regularly analyzed for acrylamide content by HPLC. Rats were observed daily and weighed monthly. Blood and urine samples were collected from 10 rats/sex/group at 3, 6, 12, and 18 months. Ten rats/sex/group were randomly examined at 6, 12, and 18 months. Non-histologic data were evaluated by Gehan-Wilcoxon test, ANOVA, and Dunnett's t-test. Cumulative mortality of male and female rats in the 2.0 mg/kg bw/day was significantly increased beginning on the 21st month of the study. A slight but significant decrease in body weight gain occurred in male rats of the 2.0 mg/kg bw/day group, but there were no

changes in food or water intake **[data not shown]**. Small and infrequent changes in body weight gain were noted for females in the 2.0 mg/kg bw/day group and males in the 0.5 mg/kg bw/day group. There was moderate degeneration of the tibial nerve in males. The study authors reported no adverse treatment-related effects on hematologic, clinical chemistry, or urinalysis parameters **[data not shown]**.

Johnson et al. *(57)* conducted histopathologic analyses in 60 rats/group/sex; a variety of organs were fixed in 10% formalin and examined including cervix, epididymides, mammary glands, ovaries, oviducts, prostate, seminal vesicles, testes, uterus, vagina, and central and peripheral nervous tissues. Histopathologic data were evaluated by Fisher's exact probability test with Bonferonni correction in cases of ≥6% control incidence. Cochran-Armitage test for linear trend was conducted in the absence of a positive Fisher's test and adjusted for mortality if appropriate and informative. Table 13 lists the incidence of histopathologic findings for which statistical significance or dose-related trends were reported at one or more doses. An increased incidence and severity of tibial nerve degeneration was observed in rats of the 2.0 mg/kg bw/day group and the effect was more pronounced in male rats. No clinical signs of neuropathy were observed. Incidence of testicular mesothelioma was significantly increased at 0.5 and 2.0 mg/kg bw/day. The incidence of testicular mesothelioma in the 0.1 mg/kg bw/day group was not significantly increased, but was said to be greater than concurrent and historical control values by study authors. All other increases in tumor incidences were noted in the 2.0 mg/kg bw/day group and included tumors of the mammary gland (benign and malignant), CNS (malignant), thyroid follicular epithelium (combined benign and malignant), oral tissues (benign), uterus (malignant), and clitoral gland (benign) in females and thyroid follicular epithelium (benign) and CNS in males. An increase in CNS tumors of glial origin in control male rats exceeded historical control values and the authors concluded that the increase in the 2.0 mg/kg bw/day males was most likely related to treatment. An increase in benign pituitary adenomas in female rats and benign pheochromocytomas in male rats were considered to be of questionable biologic significance by study authors due to high incidence in aging rats and low concurrent control value compared to historical controls, respectively. **[It was not stated if non-neoplastic lesions were observed in reproductive organs]**.

Table 13. Selected Histopathologic Effects in Rats Exposed to Acrylamide in Drinking Water for Two Years, Johnson et al. *(57)*.

Observation	Sex	Number of Rats Affected/Number Examined at Each Dose (mg/kg bw/day)				
		0	0.01	0.1	0.5	2.0
Severe degeneration of tibial nerve	m	1/60	1/60	0/60	0/60	4/60[a]T
Malignant mesothelioma of testes, with or without metastasis	m	3/60	0/60	7/60	11/60*	10/60*
Benign primary adenoma of thyroid follicles	m	1/60	0/58	2/59	1/59	7/59*
Focal hyperplasia of hard palate epithelium	m	0/60	1/60	1/60	4/60*	5/60*
Benign primary pheochromocytoma of adrenal gland	m	3/60	7/59	7/60	5/60	10/60*
Total with glial CNS tumor or glial proliferation	m	5/60	2/60	0/60	3/60	8/60
Moderate degeneration of tibial nerve	f	0/60	0/60	0/60	0/60	3/61
Malignant primary adenocarcinoma of mammary gland	f	2/60	1/60	1/60	2/58	6/61[a]T
Total with one or more benign mammary tumors	f	10/60	11/60[b]	9/60	19/58[b]	23/61*
Total with metastatic or nonmetastatic adenocarcinoma of uterus	f	1/60	2/60	1/60	0/59	5/60*
Benign primary adenoma of clitoral gland[e]	f	0/2	1/3	3/4	2/4	5/5*
Total with either adenocarcinoma or adenoma of thyroid follicles	f	1/58	0/59	1/59	1/58	5/60*[d]
Benign primary squamous papilloma of hard palate, lip, or tongue	f	0/60	3/60	2/60	1/60	7/61*
Total with CNS tumor or glial proliferation	f	1/60	2/59	1/60	1/60	9/61*[c]
Benign primary adenoma of pituitary	f	25/59	30/60	32/60	27/60	32/60*

m=male, f=female; [a]T=Linear trend, [b]One rat had two different types of mammary tumors, [c]One rats had both a tumor and glial proliferation, [d]One rat had both an adenocarcinoma and adenoma, [e]Only tissues with gross lesions were examined

**P*=0.05

A second carcinogenicity study in Fischer 344 rats was sponsored by industry *(98)* in order to clarify some of the results observed in the Johnson et al. *(57)* study. The study was designed with sufficient power to detect a 5% increase in scrotal mesothelioma incidence compared to an expected 1.3% incidence in control rats. The other purpose of the study was to characterize dose-response for tumors in female rats. At 44–45 days of age, rats were administered acrylamide (electrophoresis grade with 99.9% purity) in drinking water for 106–108 weeks. Doses administered to males (n=number in each dose group) were 0 (n=102), 0 (n=102), 0.1 (n=204), 0.5 (n=102), or 2.0 (n=75) mg/kg bw/day. Doses given to female rats were 0 (n=50), 0 (n=50), 1.0 (n=100), or 3.0 (n=100). Two control groups were used to better characterize low-incidence tumors. Twenty-five rats of each sex served as sentinels to monitor for infectious diseases. Stability and levels of acrylamide in dosing solutions were verified. During treatment, the animals were monitored for weight gain, clinical signs, and food and water intake. Included among the organs collected and fixed in 10% formalin for histopathologic examination at necropsy were epididymides, ovaries, prostate, seminal vesicles, mammary gland, testes, uterus, and vagina.

Also examined were tissues observed to have lesions or neoplasms in the Johnson et al. *(57)* study such as adrenal glands, central and peripheral nerves, thyroid, oral structures, and pituitary. Examinations were initially conducted in high dose and control groups and intermediate dose levels were examined as necessary. Statistical analyses included ANOVA, Dunnett's *t*-test, and/or pairwise *t*-tests for non-histopathologic data. For the analysis of tumor data, survival estimates were obtained by the Kaplan-Meier method, log rank test, and dose-trend tests. Statistical analyses for lifetime tumor rates that were not time adjusted included the Cochran-Armitage trend test, Tarone's method, interval based methods, and/or logistic score test.

Mortality was increased in the 2.0 mg/kg bw/day male group starting at month 17 and the 3.0 mg/kg bw/day females beginning at month 24. Body weight gain was reduced in males of the 2.0 mg/kg bw/day group and females of the 3.0 mg/kg bw/day group. Table 14and Table 15 list the incidence of histopathologic findings of the Friedman et al. *(98)* study, primarily for effects that identified statistical significance at one or more doses and tissues found to have lesions or neoplasms in the Johnson et al. *(57)* study. An increased incidence of minimal to mild sciatic nerve degeneration was noted in males dosed with 2.0 mg/kg bw/day and females dosed with 3.0 mg/kg bw/day. None of the rats had visible signs of neurotoxicity. Statistically significant increases in tumor incidences included testicular mesotheliomas (2 mg/kg bw/day), mammary gland fibroadenomas and also adenocarcinomas and fibroadenomas combined (≥1 mg/kg bw/day), and combined thyroid follicular adenomas and carcinomas (females: ≥1 mg/kg bw/day; males: 2 mg/kg bw/day). **[The statement about thyroid tumors was made in the results section, which is in contrast with results shown in tables. The study tables indicated a significant increase in follicular cell adenomas in males of the 2 mg/kg bw/day group and combined follicular cell adenomas and carcinomas in females of the 3 mg/kg bw/day group].** Friedman et al. *(98)* noted that tumors observed in testes, mammary gland, and thyroid were consistent with results of the previous study *(57)*, and that the present study demonstrated no significant increase in testicular mesotheliomas at 0.5 mg/kg bw/day. **[The level of significance in the Johnson et al. *(57)* was 0.05 while the level of significance in the Friedman study was <0.001]**. In contrast to results from the previous study, the tumor incidence was not increased in CNS glial cells, the oral cavity, clitoral gland, and uterus **[with the exception of CNS glial cells, data were not shown for these tissues]**.

Table 14. Selected Histopathologic Effects in Male Rats Exposed to Acrylamide in Drinking Water for Two Years, Friedman et al. *(98)*.

Observation	Number of Rats Affected/Number Examined [%] at Each Dose (mg/kg bw/day)				
	0	0	0.1	0.5	2.0
Sciatic nerve degeneration	30/83 [36.1]	29/88 [33.0]	21/65 [32.3]	13/38 [34.2]	26/49 [53.1]
Tunic mesothelioma of testes	4/102 [3.9]	4/102 [3.9]	9/204 [4.4]	8/102 [7.8]	13/75* [17.3]
Follicular cell adenoma of thyroid	2/100 [2.0]	1/102 [0.98]	9/203 [4.4]	5/101 [5.0]	12/75* [16.0]
Total with follicular cell neoplasms (adenomas and carcinomas) of thyroid	3/100 [3.0]	3/102 [2.9]	12/203 [5.9]	5/101 [5.0]	17/75 [22.6]
Total with CNS tumor of glial origin[a]	1/(102+82)	1/(102+90)	2/(98+68)	1/(50+37)	3/(75+51)

*p<0.001. [a]Denominator represents number of (brain+spinal cord) samples analyzed

Table 15. Selected Histopathologic Effects in Female Rats Exposed to Acrylamide in Drinking Water for Two Years, Friedman et al. *(98)*.

Observation	Number of rats affected/number examined [%] at each dose			
	0	0	1.0	3.0
Sciatic nerve degeneration	7/37 [18.9]	12/43 [27.9]	2/20 [10.0]	38/86 [44.2]
Fibroadenoma in mammary gland	5/46 [10.9]	4/50 [8.0]	20/94* [21.3]	26/95* [27.4]
Total with mammary gland neoplasms (fibroadenoma and adenocarcinoma)	7/46 [15.2]	4/50 [8.0]	21/94* [22.3]	30/95* [31.6]
Total with follicular cell neoplasms (adenomas and carcinomas) of thyroid	1/50 [2.0]	1/50 [2.0]	10/100 [10.0]	23/100* [23.0]
Total with CNS tumor of glial origin[a]	0/(50+45)	0/(50+44)	2/(100+21)	3/(100+90)

*p<0.001. [a]Denominator represents number of (brain+spinal cord) samples analyzed

In a later publication *(99)*, the testicular tumors from the Friedman et al. *(98)* study were examined by light and electron microscopy. It was found that tumors developing in the tunica vaginalis of the testes did not differ morphologically in acrylamide-treated versus control animals.

2.4.3 Carcinogenicity classifications

IARC *(11)* classifies acrylamide as a Group 2A compound, probably carcinogenic to humans. The classification was based upon inadequate evidence in humans but sufficient evidence in experimental animals. Also considered in the evaluation were positive genetic toxicity results in *in vivo* and *in vitro* assays, formation of acrylamide and glycidamide DNA adducts in mice and rats, and formation of acrylamide and glycidamide hemoglobin adducts in humans and rats.

NTP classifies acrylamide as a compound that is reasonably anticipated to be a human carcinogen *(12)*

The USEPA *(56)* classifies acrylamide as a B2 compound, probable human carcinogen. The classification is based upon inadequate human data and sufficient evidence of carcinogenicity in animals.

ACGIH *(29)* classifies acrylamide as an A3 compound, Confirmed Animal Carcinogen with Unknown Relevance to Humans.

2.5 Potentially Sensitive Subpopulations

2.5.1 Age-related susceptibility to neurotoxicity in humans and animals

In a case study of a Japanese family poisoned by acrylamide in their well water, more severe neurologic symptoms were reported in adults (aged 40–65 years) versus children (aged 10–13 years) *(51)*. The study author noted that it is unknown if the children were less susceptible than adults or drank less water from the well due to the time they spent at school.

A limited number of animal studies examined susceptibly of young versus mature animals to acrylamide-induced neurotoxicity, but findings were inconsistent.

As part of a series of experiments examining electrophysiologic and histologic evidence of acrylamide-induced neurotoxicity, Fullerton and Barnes *(100)* administered 4 doses of 100 mg/kg bw acrylamide by gavage at weekly intervals to 5, 8, 26, and 52-week-old male and female Porton rats (n=6/group). The 52-week-old animals were severely affected after 3 doses, while the

26-week-old animals were severely affected and youngest animals were only mildly affected after 4 doses. **[Parameters examined in these animals were not specified and data were not shown.]**

Kaplan and Murphy *(101)* examined age-related effects of acrylamide exposure on rotarod performance in male Holtzman rats in a university study funded by a NIOSH grant. At the ages of 5, 7, 11, and 14 weeks, 12 rats/age group were tested daily on a rotarod and then given 50 mg/kg bw/day acrylamide in water by i.p. injection. Older rats failed sooner on the rotarod test with mean failure days (±SEM) of 5.3 ± 0.19, 5.5 ± 0.20, 6.4 ± 0.22, 7.3 ± 0.22 in rats aged 14, 11, 7, and 5 weeks, respectively. Differences between the 5-week-old and 11- and 14-week old rats were statistically significant. However the recovery period occurred later in young rats (19.2 ± 0.4, 15.6 ± 0.5, 13.8 ± 0.66, and 14.8 ± 0.48 days in 5, 7, 11, and 14-week-old rats, respectively). Results in the youngest group of rats were statistically significant compared to all other age groups. The study authors concluded that adult rats were more susceptible to acrylamide toxicity than young rats.

In a study funded by an NIH grant, Suzuki and Pfaff *(102)*, conducted light or electron microscopy examinations of nervous system structures from 30 sucklng Osborne-Mendel rats ("day 1" 5.0–8.0 g) and 28 adult rats (150–300 g) that were i.p. injected with 50 mg/kg bw acrylamide, 3 times weekly, up to 18 times. A control group was injected with saline. There was a shorter latency for appearance of clinical symptoms in the immature animals (5 or 6 injections) versus adult rats (7 or 8 injections), but symptoms were more severe in the adult animals **[The Expert Panel considers the difference between 5–6 and 7–8 injections to be of questionable significance]**. In the immature animals, slight weakness in hind limbs became more pronounced and progressed to an inability to stand on hind legs as dosing continued, but some animals showed signs of recovery during the time period while they were still receiving acrylamide injections. Mature animals experienced hind limb weakness that eventually led to complete paralysis and wasting of hind limb muscles; weakness persisted for one month following the injection period. Axonal and myelin alterations were seen in the younger rats at earlier stages of neuropathy, but the changes became more pronounced and complicated in adult rats. Evidence of neurologic regeneration (e.g., growth cones and axonal sprouts) occurred in immature rats during the injection period, but were not seen in adult rats until 20 days following the last injection. The study authors concluded that young rats are more susceptible to acrylamide but have a greater ability for regeneration than adult rats.

Ko et al. *(103)* examined the effects of age on acrylamide-induced neurotoxicity in male ICR mice in a study conducted at a Taiwanese university. Groups of 3-week-old and 8-week-old mice were given drinking water containing 400 ppm acrylamide. Acrylamide intake was estimated at (mean ± SD) 91.8 ± 20.6 mg/kg bw/day in the 3-week-old mice and 90.8 ± 10.9 mg/kg bw/day in the 8-week-old mice. Control mice for each age group were given drinking water without acrylamide. Time to reach 3 different stages of neurologic symptoms was recorded in the treated mice. An initial stage was characterized by normal appearance but decreased performance on rotarod, and swelling of motor nerve terminals. Signs observed in the early stage included paraparesis and terminal nerve swelling. Symptoms of the late stage included quadriparesis, denervation, and decreased amplitude of muscle action potential. Total numbers of mice treated were not specified but 3–24 mice/group were examined pathologically during three different periods. Each of the stages occurred significantly earlier in the 3-week-old mice versus 8 week-old mice (mean days ± SD): 7.1 ± 1.1 vs. 15.6 ± 4.0 days, respectively for the early stage and 15.3 ± 2.1 vs. 31.7 ± 6.0 days, respectively for the late stage. The study authors concluded that

younger mice are more susceptible to acrylamide-induced toxicity and that pathologic symptoms occur prior to neurologic symptoms.

Husain et al. *(104)* studied the effects of acrylamide exposure on brain neurotransmitters in developing and mature male Wistar rats in a study conducted at an Indian research center. In the first study, dams were orally administered saline or 25 mg/kg bw/day acrylamide during the entire lactation period. The male offspring of the dams were sacrificed at 2, 4, 8, 15, 30, 60, or 90 days of age for an examination of brain neurotransmitter levels. During each time period, brains from 1 to 3 rats were pooled and six observations were made. Compared to rats in the control group, the rats exposed to acrylamide through milk had significantly lower levels of brain noradrenaline and dopamine at 2 to 15 days of age and 5-hydroxytryptamine at 2 to 30 days of age. Monoamine oxidase activity was significantly increased and acetylcholinesterase activity was significantly decreased at 2 to 30 days of age. In a second study, male rats were orally administered saline or 25 mg/kg bw/day acrylamide for 5 days at 12, 15, 21, or 60 days of age. Neurotransmitter levels were measured in various brain regions. It appears that at least 6 animals per group were examined and it was stated that reported values represented the mean of five observations. Significant reductions in neurotransmitter levels in treated compared to control rats that were observed only in immature rats (≤21-days-old) included noradrenaline in the pons medulla and basal ganglia and dopamine in the pons medulla. Neurotransmitter levels reduced in in all ages of treated compared to control rats included noradrenaline in midbrain; dopamine in cerebellum and midbrain; and 5-hydroxytryptamine in pons medulla, hypothalamus, and cerebral cortex. The study authors concluded that immature rats are more vulnerable to neurotoxicity induced by acrylamide exposure and that effects are localized within certain brain regions.

Due to unpublished observations that younger rats are more susceptible to acrylamide-induced paralysis, Dixit et al. *(105)* compared hepatic GST activity and glutathione content in young and mature albino rats. The study was conducted at an Indian research center. At the ages of 9, 15, or 26 days and 4 months, rats were given 50 mg/kg bw/day acrylamide by i.p. injection for 5 days [number of rats in each group not specified]. Control rats were injected with the sodium chloride vehicle. One day following the last injection, livers were homogenized for a determination of hepatic GST activity and glutathione content. Generally, GST activity increased with age and acrylamide treatment reduced GST activity in all age groups. The magnitude of GST reduction was greatest in the 15-day-old rats in which early development of paralysis was reported **[neurotoxicity data not shown]**. Acrylamide treatment resulted in significantly decreases in reduced and oxidized glutathione content only in the 26-day-old rats. **[Methods of statistical analysis were not discussed for any of the data reported in this study]**.

[The Expert Panel finds the literature on age-related susceptibility to be difficult to interpret, with some studies showing greater susceptibility in young animals and others not showing such increased susceptibility].

2.5.2 Ontogeny, polymorphism, and other factors affecting metabolism

2.5.2.1 GST and glutathione

As noted in Section 2.1.3, a major pathway of acrylamide biotransformation is conjugation with glutathione, catalyzed by GST. Cytosolic GST is a family of soluble dimeric enzymes consisting of 13 different subunits from 5 different subclasses *(106)*:

1. alpha (GSTA1, GSTA2, GSTA3, GSTA4)
2. mu (GSTM1, GSTM2, GSTM3, GSTM4, GSTM5)

3. pi (GSTP1)
4. theta (GSTT1, GSTT2)
5. sigma (GSTZ1)

Subunits can dimerize only with members of the same class to form homodimers or heterodimers *(106*). The alpha, mu, and pi enzymes are the most abundant GST classes in mammalian species *(107*).

McCarver and Hines *(106*) reviewed the ontogeny of GST and other phase II metabolizing enzymes. A limited amount of information is available on the ontogeny of human hepatic GST. The ontogeny of human hepatic GST is summarized in Table 16. GSTA1 and GSTA2 are expressed in the fetus and expression reaches adult levels at 1–2 years of age, following a 1.5–4-fold increase. Low levels of GSTM are also detected in the fetus and expression increases 5-fold at birth to reach adult levels. GSTP1 expression is highest in 10–22-week-old fetuses and decreases during the second and third trimesters; GSTP1 is expressed in neonates but not adults. McCarver and Hines *(106*) concluded that though limited, the information on GST ontogeny demonstrates that substantial changes occur during development. Such changes can affect chemical disposition and clinical outcomes, which also depend upon the balance of other phase I and phase II enzymes. Currently, the information is inadequate to predict adverse reactions and determine appropriate therapies for fetuses, neonates, infants, and children.

Table 16. Ontogeny of Human Hepatic GST, McCarver and Hines *(106)*

Gene	Prenatal Trimester			Neonate	1 Month to 1 Year	1 to 10 years	Adult
	1	2	3				
GSTA1/A2	+	+	+	+	+	+	+
GSTM	+	+	+	+	+	+	+
GSTP1	+	+	+	+	-	-	-

GST polymorphisms are recognized, most notably null phenotypes for the GSTM1 (GSTM1*0) and GSTT1 (GSTT1*0) alleles *(107)*. No gene products are expressed in individuals who are homozygous for the null phenotype. Frequency of GSTM1*0 homozygosity is 58% in Chinese, 52% in English, 48% in Japanese, 43% in French, and 22% in Nigerian individuals *(107)*. Frequency of GSTT1*0 homozygosity is 16% in English, 12% in German, 38% in Nigerian, and 32% in West Indian individuals.

Friedman *(14)* noted that reductions in glutathione levels can increase susceptibility to acrylamide-induced toxicity by leaving cell membranes less protected against oxidative stress. Factors that can reduce glutathione levels include diets low in the amino acids cystine and methionine, oxidative stress, and liver damage.

2.5.2.2 Cytochrome P450

As noted in Section 2.1.3, a second major pathway of acrylamide biotranformation is oxidation to glycidamide through cytochrome P450. CYP2E1 was identified as the enzyme responsible for biotransformation of acrylamide in mice and rats, but no information on the specific enzyme in humans could be located **[The Expert Panel finds it reasonable to believe that CPY2E1 is the isoform responsible for acrylamide biotransformation in humans]**. Hines and McCarver *(108)* noted that fetal liver contains 30–60% of total cytochrome P450 content compared to adult liver; levels continue to increase at birth and reach adult levels at ten years of age. However, significant expression differences are noted among individual P450 enzymes. Anzenbacher and Anzenbacherova *(109)*, noted that polymorphisms among cytochrome P450 enzymes could result in defective, qualitatively different, reduced, or enhanced activities of the enzymes. Enzyme activities could also be affected by factors that induce or inhibit enzymes, including diet, age, and health status. Calleman et al. *(27)* noted that mixed results were found in animal studies examining the effects of inducers or inhibitors of oxidative metabolism on acrylamide-induced neurotoxicity. Calleman noted that some inducers or inhibitors can impact other metabolic enzymes, such as GST.

2.5.3 Gender-related differences

There is no indication that males or females are more susceptible to general toxicity induced by acrylamide exposure. Acrylamide has adverse effects on the reproductive systems of males, but not females, as discussed in Section 4.

2.6. Summary of General Toxicology and Biologic Effects

2.6.1. Toxicokinetics and Metabolism

Qualitative absorption of acrylamide through food was demonstrated in humans *(26)* and other studies provide indirect evidence of acrylamide absorption through oral, inhalation and dermal exposure in humans, rats, mice, dogs, and/or pigs *(6-8, 11, 27, 38-40)*. Acrylamide absorbed through any exposure route is rapidly distributed throughout the bodies of rats, pigs, and dogs *(38)*. Higher concentrations were found in livers and kidneys of rats *(6)* but there was no evidence of bioaccumulation in neural tissues *(38)*. In red blood cells acrylamide and/or its metabolite glycidamide react with sulfhydryl groups on hemoglobin to form persistent (long-lived or stable) adducts *(6, 27, 38, 39)*. Glycidamide but not acrylamide was observed to form DNA adducts in rats *(19, 27)*.

Studies in rats indicate that acrylamide is rapidly metabolized with more than 90% of the dose excreted in urine as metabolites within one week *(6, 7, 38, 39)*. In one major pathway of biotransformation, hepatic glutathione-S-transferase (GST) catalyzes the first order conjugation of acrylamide with glutathione, ultimately leading to the formation of the urinary metabolites N-acetyl-S-(3-amino-3-oxypropyl) cysteine in rats and mice and N-acetyl-S-(2-carbamoylethyl) cysteine in humans *(14, 38, 48)*. In the second major pathway of biotransformation in humans and animals, glycidamide is formed through a saturable reaction with cytochrome P450 *(48)*. Cytochrome P450 2E1 (Cyp2E1) is the specific enzyme involved in this reaction in mice *(49)*. Glycidamide is further metabolized through conjugation with glutathione to form mercapturic acids or metabolized by epoxide hydrolase or epoxide hydratase *(27, 48)*. Percent acrylamide conversion through each major pathway varies by species and dose. A greater percentage of acrylamide is converted to glycidamide in mice compared to rats *(11, 27, 39)*. High doses of acrylamide can inhibit GST or deplete GSH *(6, 7, 39)*, but a model suggests that appreciable GSH depletion is not expected to occur in rats until doses exceed 10 mg/kg bw/day *(48)*. A critical study in rats demonstrated saturation of glycidamide formation at i.p. doses between 5 to 100 mg/kg bw/day, as evident by non-dose related formation of glycidamide adducts; glycidamide formation was estimated at 51 and 13% at the low and high dose, respectively *(50)*. There is no information on the departure point for metabolic saturation in either humans or animals.

Human data are unreliable for quantitatively estimating acrylamide excretion in humans. In experimental animal studies, plasma half-lives of 2 h were reported for parent compound and for glycidamide following administration of acrylamide at doses up to or exceeding 20 mg/kg bw *(17, 38, 39)*. Dosing of rats with ^{14}C labeled acrylamide results in biphasic tissue distribution and elimination, with an initial half-life of 5 h and terminal half-life of 8 days *(6, 38)*. The urinary excretion half-life was reported at 8 h in rats *(6, 7)*. The majority of ^{14}C label (40–90%) is eliminated through urine in rats *(27, 39)*, while a smaller percentage (15%) is thought to undergo enterohepatic circulation with elimination of 6% radiolabel through feces *(6, 38, 39)*. Elimination through exhaled air is questionable since it has not been consistently observed in different studies *(27)*.

Human placental transfer of acrylamide was demonstrated in a study that found the acrylamide adduct, N-2-carbamoylethylvaline, in maternal and umbilical cord blood (n=11), with levels in fetuses estimated to be equivalent to mothers when adjusted on a weight basis. *(43)*. The results of the human study are consistent with studies in rats, mice, dogs, rabbits, and pigs, which also demonstrated placental transfer of acrylamide *(44-47)*. In one study conducted with a single i.v. dose of 5 mg/kg bw radiolabeled acrylamide, 82.3% of radiolabel passed through the placenta of dogs on GD 60 and 69% passed through the placenta of pigs on GD 109 *(46)*. Brains of fetal dogs and pigs contained high levels of acrylamide and indicated no effective blood-brain barrier

Transfer of acrylamide to breast milk was demonstrated in a study conducted with two women. Ingestion of 800–1000 µg acrylamide through potato chips resulted in milk levels of 3.17–18.8 ng/mL acrylamide within the time period of 3–8 h following ingestion *(26)*. Exposure estimates based on milk levels are reported in Section 1.2.4.1.

In male mice gavage dosed with 116–121 mg/kg bw radioactive acrylamide, movement of radiolabel paralleled that of spermatids, with detection of radiolabel in testis parenchyma at 1 h, in seminiferous tubules and head of epididymis at 9 h, and in the tail of epididymis and crypts of glans penis epithelium at 9 days *(45)*. In experimental animal studies there is a delay in reaching peak radiolabel levels in testis due to an initial absorption phase *(6, 38)*. The first phase of the elimination half-life (8 h) in testis is slightly longer than the elimination half-life observed in other tissues (5 h) *(42)*.

2.6.2. General Toxicity

2.6.2.1 Human

Effects of acute or subacute acrylamide poisoning are described in numerous reviews. CNS symptoms consisting of confusion, memory problems, sleepiness, slurred speech, inability to concentrate, and hallucinations develop within hours or days of poisoning *(7, 38)*. A latency period of days to weeks is followed by insidious development of peripheral neuropathies characterized by loss of sensation, paraesthesia, numbness, muscle weakness, and/or wasting in extremities, and decreased tendon reflexes *(7, 38)* Axonopathies are most commonly observed and impairment occurs in sensory fibers prior to motor fibers *(6)*. Toxicity to midbrain or cerebellum may result in tremors and gait disturbances *(6)*. Possible autonomic nervous system involvement was suggested by reports of sweating, peripheral vasodilation, and difficulty urinating and defecating in some cases *(7)*. Anorexia, weight loss, and nystagmus have also been observed with acrylamide exposure *(7, 38)*. Reduced action potential in distal sensory neurons is the most consistent electrophysiologic finding *(7)* Most individuals who survive acrylamide poisoning recovery fully over a period of months to years. *(6, 7)*. Symptoms consistent with CNS involvement followed by peripheral neuropathies were reported in a woman who ingested 375 mg/kg bw acrylamide *(6, 8)*. Similar symptoms were also reported in three adults from a Japanese family who were poisoned by acrylamide in their well water (400 ppm at a single time point) but recovered fully within four months *(6, 8, 51)*. Two children in the family (10- and 13-years old) were less severely affected than adults, possibly as a result of lower exposures due to school attendance during the day.

In cases of occupational exposure to acrylamide through inhalation and dermal contact, common symptoms included peeling of skin on palms, followed by development of peripheral neuropathies *(8, 38)* It is difficult to determine exposure levels in workers experiencing toxicity since air levels are infrequently measured and it is difficult to determine extent of dermal exposure. A review by the European Union *(8)* noted that prevalence of symptoms related to peripheral neuropathies is increased in workers exposed to >0.3 mg/m^3 acrylamide (8-h TWA), but contribution from skin exposure is unknown. NICNAS *(52)* concluded that electroneuromyographic changes such as decreased sensory action potential amplitude and prolonged ankle tendon reflex can precede neuropathic symptoms and are therefore important in early detection of neurotoxicity in workers.

2.6.2.2 Experimental Animal

Symptoms of acute acrylamide exposure in laboratory animals such as rats, mice guinea pigs, rabbits, and cats include ataxia, tremors, convulsions, muscular weakness, circulatory collapse weight loss, and/or death *(7*). LD_{50}s observed in various species are listed in Table 8.

Neurologic effects observed with repeated acrylamide dosing of animals such as rats, mice, cats, dogs, and monkeys are consistent with those observed in humans *(7, 39*). Animals develop peripheral neuropathy and overt signs that include tremors, incoordination, motor dysfunction, neuromuscular weakness, and reduced motor nerve conduction velocity *(7, 39*). Histologic findings include degeneration of distal long sensory and motor peripheral nerve fibers, long axons in the spinal cord, Purkinje fibers in the cerebellum and optic nerve, and autonomic fibers. Demyelination of sciatic, tibial, median, and ulnar nerves has also been observed.

Studies designed to identify NOAELs were limited to rats. In risk assessments conducted by IRIS *(56*) and the European Union –*(8*), subchronic and chronic drinking water studies in rats *(55, 57*) were used to select neurotoxity NOAELs ranging from. 0.2–0.5 mg/kg bw/day and LOAELs of 1–2 mg/kg bw/day based on neurological lesions. Severity of neurological lesions increased with dose. Clinical signs of neurotoxicity were only observed in doses exceeding LOAELs and partial to full reversal of lesions was observed during a recovery period. Organs affected at higher acrylamide doses in animal studies included kidney, liver, and the hematopoietic system *(7, 8*).

Reproductive organs in rats were targets of toxicity following 92–93 days of dosing with 20 mg/kg bw/day acrylamide in drinking water *(55*). Effects included decreased size of uterus, testis, and male accessory genitalia, testicular atrophy, mineral in seminiferous tubules, and cellular debris and decreased spermatogenic elements in epididymides. Following a 144-day recovery period, there was partial reversal of testicular effects. Neoplastic lesions observed in reproductive organs are discussed in Section 2.4.2.

2.6.3 Genetic Toxicity

Genetic toxicity in somatic or bacterial cells was reviewed by the European Union *(8*). *In vitro* studies in mammalian cells consistently demonstrated clastogenicity, cell transformation, and cell division effects (e.g., polyploidy, spindle disturbances, malsegregation). With the exception of studies examining cell division effects, metabolic activation was included in at least one study examining each end point. Inconsistent results were obtained for point mutations, unscheduled DNA synthesis, and sister chromatid exchange in mammalian cells. A lack of point mutations was consistently observed in bacterial cells, with and without metabolic activation. *In vivo* tests conducted mostly in mice treated by i.p. dosing and in a few cases orally demonstrated chromosomal aberrations, aneuploidy, polyploidy in bone marrow, and micronuclei in erythrocytes. Negative results were obtained in *in vivo* tests of unscheduled DNA synthesis, sister chromatid exchange, and chromosomal aberrations in mitogen-stimulated splenocytes from mice. The European Union *(8*) and JIFSAN *(39*) concluded that acrylamide is likely clastogenic or interferes with chromosomal segregation but does not cause point mutations. However, the Expert Panel noted that adequacy of point mutation assays in mammalian cells is questionable. In addition, an *in* vitro study using embryonic fibroblasts from BigBlue® mice demonstrated that acrylamide induced mutations at the CII transgene, while an *in* vivo MutaMouse® study reported the induction of mutations at the LacZ locus in bone marrow cells *(59*). Therefore, the Panel noted that there are some indications that acrylamide could be a weak inducer of point mutations. Lastly, the Panel noted that acrylamide was positive in both the presence and absence of exogenous metabolic activation and that metabolic activation did not appear to modify genetic toxicity. This observation suggests either that acrylamide is not metabolized in these *in vitro* systems or, if it is metabolized, the primary metabolite(s) must be as reactive as acrylamide itself.

Germ cell genetic toxicity of acrylamide has been evaluated primarily in male mice and to a lesser extent in male rats. A number of reports were reviewed in which acrylamide treatment was associated with gem cell aneuploidy, chromosome breaks, fragments, exchanges, and univalents *(60, 63, 64, 68, 69*) in germ cells or with micronuclei in spermatids, which represent the consequence of structural or numerical chromosome damage *(61, 65-67*). Based on the timing of exposure prior to collection of the germ cells, sensitive germ cell stages have been estimated. These estimates, however, are inexact and may not be reliable. Some authors *(60, 66, 68*) have described chromosome damage after treatment of spermatogonia, but most studies have reported chromosome abnormalities after exposure of spermatocytes.

Dominant lethal studies have been performed with acrylamide given in drinking water to rats or mice *(72, 76, 78*), by gavage in rats*(73, 79*), by i.p. injection in mice*(1, 2, 74, 80-82*), and by dermal application in mice *(75*). Late spermatids and early spermatozoa appear to be the germ cell stages most sensitive to acrylamide dominant lethal effects. Lowest effective doses of acrylamide (expressed as cumulative amount prior to mating) are about 200 mg/kg in drinking water in rats (derived from an acrylamide concentration of 30 ppm *(72*)), 949 mg/kg in drinking water in mice (from a daily intake of 6.78 mg/kg for 20 weeks *(76*)), 75 mg/kg by gavage in rats (from 5 daily treatments with 15 mg/kg/day *(73*), 75 mg/kg i.p. in mice (single dose) *(74*), and 125 mg/kg dermally in mice (25 mg/kg/day for 5 days *(75*). The possible role of glycidamide in mediating acrylamide dominant lethal effects was investigated by Adler et al. *(77*), who gave 1-aminobenzotriazole to prevent cytochrome P450 metabolism. The dominant lethal effect of acrylamide was not attenuated during the first week after treatment with 1-aminobenzotriazole, raising the possibility that acrylamide itself has dominant lethal effects (or anti-fertility effects that were interpreted as dominant lethal effects); however, the Expert Panel did not believe that the methods used in this study adequately addressed the role of acrylamide metabolism.

The administration of acrylamide i.p. to male mice was associated with chromosome abnormalities in first metaphase zygotes sired by treated males *(3, 84*). Consistent with results of dominant lethal testing, the late spermatid and spermatozoa stages were the most sensitive in the production of this effect. Studies by Holland et al. *(2, 85*) were generally supportive of the production of abnormal pre-implantation conceptuses through induction of genetic damage by acrylamide, although interpretation of these reports was limited by problems in the presentation and analysis of data. Three studies *(64, 80, 86*) identified heritable reciprocal translocations in the offspring of male mice treated with i.p. acrylamide and two studies *(74, 87*) identified specific locus mutations in the offspring of male mice treated with i.p. acrylamide; along with the dominant lethal studies, these reports demonstrate the germ line genetic effects of acrylamide in male mice. In addition, a mouse spot test in GD 12 mice demonstrated the susceptibility of fetal somatic cells to mutation after acrylamide treatment of the dam *(88*). The mechanism of toxicity of acrylamide to the genetic material of male germ cells appears to be mediated by protamine-binding, possibly by glycidamide rather than by acrylamide itself, with DNA breakage secondary to stresses imposed on the chromatin by protamine binding *(89, 91, 92*).

2.6.4 Carcinogenicity

2.6.4.1 Human

Epidemiological studies examining the relationship between cancer and occupational or dietary exposure to acrylamide reported negative findings *(93-97*). However The European Union *(8*), IARC *(11*) and the CERHR Panel judged the studies to be inadequate for assessing cancer risk due to inadequate power to detect the magnitude of effect suggested by experimental animal studies.

2.6.4.2 Experimental animal

Two cancer studies were conducted in rats exposed to acrylamide through drinking water for two or more years *(57, 98*). The more recent study was conducted to clarify some of the effects observed in the first study and was designed with sufficient power to detect a 5% increase in scrotal mesothelioma compared to a 1.3% incidence in control rats. Neoplastic effects (and doses) that were consistently increased in the two studies were testicular mesotheliomas (0.5 and 2 mg/kg bw/day) benign and malignant mammary gland tumors ((1 and 2 mg/kg bw/day), and benign and malignant thyroid tumors ((1 and 2 mg/kg bw/day). Increased incidence of tumors in CNS glial cells, clitoral gland, and oral tissues in rats exposed to 2 mg/kg bw/day acrylamide in first study was not found in the second study. A later publication *(99*) reported no morphologic differences in testicular tumors from acrylamide and control rats from the latter study *(98*).

2.6.4.3 Carcinogenicity Classifications

Table 17 Lists acrylamide cancer classifications by national and international agencies.

Table 17. Acrylamide Cancer Classifications

Agency	Classification	Explanation
IARC	2A	Probably carcinogenic to humans
USEPA	B2	Probably human carcinogen
ACGIH	A3	Confirmed animal carcinogen

2.6.5 Potentially Sensitive Subpopulations

In a Japanese family poisoned by acrylamide in their well water, children (aged 10 and 13 years) experienced less severe neurotoxic effects than adults, but it could not be determined if the children were less susceptible or received a lower exposure due to time spent at school *(51*). A number of studies compared neurotoxicity in young versus mature rats or mice, but the Expert Panel was unable to draw conclusions about age-related susceptibility due to inconsistent findings *(100-104, 105.*)

As noted in Section 2.5, GST-catalyzed conjugation of acrylamide with glutathione represents a major pathway of acrylamide biotransformation. Biotransformation of acrylamide to glycidamide by cytochrome P450, is the second major metabolic pathway. CYP2E1 was identified as the enzyme responsible for acrylamide biotransformation in mice *(49*), and the Panel finds it reasonable to assume that CYP2E1 is the isoform that biotransforms acrylamide in humans. Cytochrome P450 levels or activities can be affected by ontology, polymorphisms, diet, and health status *(14, 106, 107*). The current information is insufficient for predicting the effects of variable metabolic enzyme activity on acrylamide-induced toxicity.

3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human Data

No human data on acrylamide developmental effects were located.

3.2 Experimental Animal Data

3.2.1. Non-neurologic developmental end points

Edwards *(44)* treated Porton strain rats with acrylamide **[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 six weeks of age with weekly weights and observations 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 *(44)*, six pregnant females were given 400 ppm in the diet from the day of mating until 20 days thereafter when they underwent cesarean section. Six control dams were fed the powdered diet without acrylamide. Uteri were examined for resorptions **[presumably uteri: the text says 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 acrylamide 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 acrylamide represents a daily dose of 1.4 mg/kg/day]**. Fetal weights were reduced by acrylamide treatment (acrylamide 2.4 ± 0.05 g, control 3.2 ± 0.05 g, mean ± SEM) **[the *P* value reported by the authors using Student's *t*-test is > 0.2; however, the *t*-test performed by CERHR gave *P* < 0.0001].** Four fetuses were found dead in one uterine horn in the acrylamide-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 *(44)* administered acrylamide 100 mg/kg in water intravenously 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 six weeks during which the day of eye opening was noted and animals were evaluated for gait and weight (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 per 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 between groups in birth weight, pup weight 24 h after birth, pup weight 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.

Strengths/Weaknesses: Weaknesses include use of a limited number of doses, a very limited number of pregnant rats per group, and a limited number of outcomes measured. Because data

necessary for full evaluation are missing from this report, and only a few litters were used in each experiment, the conclusions of the report are questionable.

Adequacy (Utility) for CERHR Evaluation Process: This study is suggestive only that the developing rat may be less sensitive than the dam to the standard neuronal toxicity of acrylamide. The protocol is inadequate to detect subtle changes or draw conclusions regarding dose-response.

An industry-sponsored study conducted at Bio/dynamics Inc. *(110)* examined developmental toxicity in Sprague-Dawley CD rats. Twenty female rats/group were given acrylamide (100% purity) in the diet at 0, 25, or 50 ppm for two weeks prior to mating. The rats were mated with untreated males and following the first sign of mating (GD 0), resumed dietary acrylamide intake for the first 19 days of gestation. The study authors estimated acrylamide intake at 1.75–1.90 and 3.45–3.82 mg/kg bw/day in the 25 and 50 ppm dose groups, respectively. Rats were allowed to give birth and litters were culled to 3 male and 3 female pups on PND 4. Pups were examined for postnatal growth and mortality through the lactation period (PND 21). Statistical analyses included *F*-test and Student's *t*-test, Cochran's approximation, and chi-square. The only effect reported for maternal body weight was a slight but significant reduction in body weight gain in the 50 ppm dams during the pre-mating period. There were no differences in food intake among treatment groups. Alopecia was noted in dams from both acrylamide-treatment groups. Mating and pregnancy indices were comparable among all treatment groups. A total of 13, 15, and 16 litters were available for evaluation in the control, 25 ppm, and 50 ppm dose groups, respectively. There were no differences in mean gestation length, fetal viability at birth, number of live pups at birth, litter size on PND 4, pup weights, or pup survival on PND 4, 14, and 21.

At weaning (PND 21), 4 pups (2/sex) from the control group and 8 pups (4/sex) from the 50 ppm dose group were sacrificed for histopathologic evaluation of brain, spinal cord, and sciatic, tibial, and plantar nerves *(111)*. The samples were fixed in Dalton's chrome osmium solution, dehydrated in ethanol, cleared with propylene oxide, and embedded for examination by light microscopy. Acrylamide exposure did not produce major teratogenic effects in the brain. Some fine structural differences were noted between control and acrylamide-treated animals including scattered nerve fiber degeneration in the sciatic and optic nerves. The study authors noted that nerve fibers from treated animals were more prone to preparation artifacts than control animals. **[Details concerning the incidence and severity of neurologic lesions were not provided in the report.]**

Strengths/Weaknesses: The data presented are suggestive of specific neuronal susceptibility, and the observation that fibers apparently are more fragile with acrylamide treatment raises concern: the observation could mean the findings were over-interpreted or nerve fibers from exposed animals actually may have been affected in additional ways not directly measurable within the evaluation. The group size used was large, but the sample size used for neuropathology evaluation was small. The standard end points of reproductive performance and litter measurements do not suggest toxicity in this study; only the suggestive neuronal effects at weaning suggest developmental toxicity. This study would be stronger if it included either (a) functional offspring measurements, or (b) more extensive neuropathology evaluations, either at weaning or at later ages to evaluate persistence or recovery of the reported findings. Another weakness is the limited dose range. Strengths include estimation of intake and the inclusion of treatment prior to breeding, which can be viewed as a strength for detecting reproductive as well as developmental effects, but a weakness if trying to define sensitive period of exposure (because the results were negative, this potential issue is not critical).

Utility (Adequacy) for CERHR Evaluation Process: From the non-neurologic perspective, there appears to be no indication of standard reproductive and developmental toxicity; the sample size and procedures are adequate for use in this regard. The evidence for lack of developmental toxicity covers only a limited dose range

Walden et al. *(112)* in a study from NIEHS evaluated the activity of five intestinal enzymes in the offspring of acrylamide-treated Sprague-Dawley rats. Animals were treated from GD 6–15 (insemination = GD 0) with acrylamide **[purity not given]** 20 mg/kg/day or water by gavage. 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, treated dams with treated pups, control dams with treated pups, and treated dams with control pups. Four pups were removed from each litter (without regard to sex) for intestinal enzyme analysis on each of PNDs 14, 21, and 60. **[This design suggests that pups were reassigned to dams so that litter size initially was 12/litter. If not, it is unclear how there were 4 pups/litter/age].** 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 spectophotometric assays were performed for alkaline phosphatase, citrate synthase, and lactate dehydrogenase. End point spectrophotometric assays were performed for acid phosphatase and β-glucuronidase. Dams were killed on PND 24, after weaning, and underwent measurement of intestinal enzymes by the same methods. Statistical analysis was performed by Mann-Whitney *U* test using a P value < 0.05 as the criterion for statistical significance. **[The question of multiple comparisons is not addressed. Five enzymes measured at three time points in four groups of animals yields 60 possible comparisons. The Expert Panel notes that if the comparisons were completely independent (which is not likely), 60 comparisons would give a 95% likelihood of identifying a significant difference at a nominal *P* value of 0.05]**.

There were no differences in maternal body weight, or litter averages for pup number, weight, or sex ratio. Dam intestinal enzyme levels did not differ by treatment status. Results for offspring are summarized in Table 18. The authors concluded, "…prenatal and lactational exposure to acrylamide does significantly change intestinal enzyme levels…during the early stages of development…"

Strengths/Weaknesses: While the enzyme data are likely over-interpreted statistically, the pattern of findings presented in the table are suggestive of changes in alkaline phosphatase and perhaps a "developmental" effect (delay or acceleration in normal pattern of enzyme changes?) on PND 21 in β-glucoronidase. The meaning of such changes to animal status and wellbeing was not made clear by the authors and is not obvious.

Utility (Adequacy) for CERHR Evaluation Process: The lack of significant findings in a developmental study with adequate sample size is useful in the evaluation. The utility of this study is limited by use of a single dose and therefore lack of dose–response information.

Table 18. Enzyme Levels in Proximal Intestine of Rat Pups After Prenatal Exposure to Acrylamide. From Walden et al. *(112)*

	Alkaline phosphatase	Citrate synthase	Lactate dehydrogenase	Acid phosphatase	β-glucuronidase
Prenatal effect (C-T vs. C-C)					
PND 14	↑	↔	↔	↑	↔
PND 21	↑	↔	↔	↔	↑
PND 60	↓	↔	↔	↓	↔
Lactational effect (T-C vs. C-C)					
PND 14	↔	↔	↔	↑	↔
PND 21	↑	↔	↑	↔	↑
PND 60	↓	↔	↔	↔	↔
Combined effects (T-T vs. C-C)					
PND 14	↑	↔	↔	↔	↓
PND 21	↑	↔	↔	↓	↑
PND 60	↑	↔	↔	↑	↔

C-C Control dam raising control pups; C-T Control dam raising treated pups; T-C Treated dam raising control pups; T-T Treated dam raising treated pup.
↑,↓ Significantly increased, decreased for indicated comparison at $P < 0.05$, taken from bars graphs shown in the paper.
↔ Indicated comparison did not show a statistical difference at $P < 0.05$.

In a study sponsored by the US EPA, Zenick et al. *(78)* gave acrylamide **[purity not specified]** to Long-Evans hooded female rats at 0, 25, 50, or 100 ppm in drinking water. There were 15 females with regular estrous cycles per group. **[CERHR estimated doses from graphs of body weight and fluid intake over the course of the experiment (see** Table 19**). The weight-adjusted acrylamide dose increased during lactation in all groups and increased during pregnancy in the 100 ppm group due largely to a failure of weight to increase in spite of increasing fluid intake.]** After two weeks of exposure to treated drinking water, untreated males were placed with the females overnight, without a water bottle **[presumably water bottles were replaced when males were removed and presumably acrylamide treatment was continued through weaning; however, the study report is not specific on this question]**. Females were given seven days to mate. The presence of sperm in the vagina or a copulatory plug was taken as GD 1. After seven days of cohabitation without evidence of mating, acrylamide exposure was continued and females were monitored as though pregnant and were killed 23 days later if they had not produced a litter. **[In the male reproduction study reported in the same paper, a reverse 10:14 light:dark photoperiod is described with lights on at 10 pm. The photoperiod for the female reproduction study is not described]**. Dams were allowed to deliver spontaneously and rear their pups until weaning. On PND 4, litters were culled to 4 males and 4 females. It is presumed that exposure of the dam ceased at weaning. At weaning, litters were culled to 2 males and 2 females. Vaginal patency of the pups was evaluated beginning PND 28. Offspring were killed on PND 42. Data were analyzed with repeated measures ANOVA or one-way ANOVA with post-hoc Duncan's test. Linear regression was applied to evaluate the contribution of dam body weight, cumulative acrylamide intake, cumulative fluid consumption, and their interactions on per litter birth and weaning weights.

Body weight was significantly decreased at several times points in the 100-ppm group compared to the control group from 2 weeks of exposure through the end of the study. Body weight in the 50-ppm group was decreased compared to the control group at three time points from day 7 of the lactation period **[mean body weights in all groups appeared to follow uniform trends; the statistical significance of the body weight comparisons may have varied due to changes in**

the variance, which was not shown]. Fluid intake was lower in the 100- and 50-ppm groups than in the control group during the lactation period, and in the 100-ppm group during late gestation. Litter results are summarized in Table 20. There were two litters in the 100-ppm group with 80 and 100% mortality by PND 4, but survival in the remaining litters was above 90%. Litter size and percent survival were not affected by acrylamide exposure. Hind-limb splaying occurred in the first two weeks in the females exposed to 100 ppm acrylamide, and feed and water intake required facilitation, showing a biologic effect of this dose of acrylamide. No other dose groups showed hind-limb splay. The main effect of acrylamide treatment of the dam was on pup weight, which was consistently depressed in the two highest dose groups in a dose-related manner and transiently depressed during the first week after birth in female pups born to dams exposed to 25 ppm. Because of the incapacitation of the dams given 100 ppm acrylamide, their data were excluded from the regression analysis, which showed that birth weight was not influenced by body weight, fluid intake, or cumulative acrylamide intake, or their interactions. Litter weaning weights were, however, influenced by cumulative acrylamide intake ($P \leq 0.01$). The authors concluded that acrylamide exposure of the dam at 50 or 100 ppm in drinking water results in a decrease in offspring weight due either to gestational or lactational exposure or both. **[Possible exposure of pups to drinking water prior to weaning was not discussed. A benchmark dose[1] calculated for litter size at birth appears in** Table 20**; insufficient data were given for benchmark dose calculations on other end points]**.

Strengths/Weaknesses: This study was adequately performed and interpreted. Although the study was designed as a female reproduction study, useful developmental end points were included. The use of a top dose that produced hind limb splay in the dams provides an evaluation of the dose range up to the dose producing the non-reproductive end point (neurotoxicity) of greatest interest in humans. The graphic presentation of the data does not readily permit modeling of the dose-response relationship or use of the benchmark dose approach.

Adequacy (Utility) for CERHR Evaluation Process: This study offers an indication of general postnatal toxicity using standard end points in rats; however, the developmental effects may have been due to maternal toxicity. Since the extended treatment period includes gestation, this study provides useful information on developmental toxicity over the dose range of 25-200 ppm. A developmental lowest observed adverse effect level (LOAEL) of 25 ppm can be selected based on a transient decrease in pup weight **[the authors selected 50 ppm as a developmental LOAEL]**. The maternal LOAEL is 50 ppm.

[1] The benchmark dose method uses a mathematical model of the dose–response curve to estimate the dose at which a given response will occur. Benchmark doses were calculated using the software available from the U.S. EPA web site at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid= 20167. The BMD_{10} represents the dose at which a 10% change from the control group would occur. The BMDL represents the dose corresponding to the lower bound of the 95% confidence interval around a 10% response above/below the control response.

Table 19. CERHR Estimates of Mean Acrylamide Doses (mg/kg/day) in the Female Reproduction Study of Zenick et al., *(78)*

	Baseline	GD-1	GD-21	PND-3	PND-21
25 ppm	4	4	4	4	9
50 ppm	6	5	9	8	14
100 ppm	11	10	14	12	24

Estimates rounded to the nearest whole number were made from graphic representations of mean weight and mean fluid intake over the course of the study.

Table 20. Litter Parameter Summary from Female Reproductive Study by Zenick et al., *(78)*

	25 ppm	50 ppm	100 ppm
Maternal weight gain	↔	↓	↓
Mating performance	↔	↔	↔
Pregnancy rates	↔	↔	↔
Litter size at birth	↔	↔	"↓" 18% ($P \leq 0.1$)
BMD_{10} = 96 ppm BMDL = 40 ppm			
Survival to PND 4	↔	↔	↔
Survival to PND 21	↔	↔	↔
Male pup weight from PND 7	↔	↓[a]	↓
Female pup weight from PND 7	↓[b]	↓	↓
Vaginal patency	↔	↔	delayed 2.8 days

↑,↓ Statistically significant increase, decrease compared to 0-ppm control value. "↓" Decrease indicated by the authors, although criterion for statistical significance not satisfied. ↔ No significant difference from control value.
[a]Mean offspring weight not statistically different from control group on PND 42.
[b]Mean pup weight significantly reduced at PND 1 and 7 in 25 ppm group.
BMD_{10} Exposure level associated with a 10% response, estimated from a mathematical dose–response model. BMDL Dose associated with the lower bound of the 95% confidence interval around the BMD_{10}.

Neuhäuser-Klaus and Schmahl *(88)* performed what they called a teratogenicity study in conjunction with a mouse spot test (discussed in section 2.3.2.6), supported in part by the Umweltbundesamt, Berlin. T-stock females were mated with HT males (2 females to 1 male) and the day of a vaginal plug was counted as GD 1. Females were treated with acrylamide (analytical grade) in distilled water at 75 mg/kg i.p. once on GD 12 or 50 or 75 mg/kg daily on GD 10, 11, and 12. Control females were injected with distilled water. Females were killed on GD 18 and implantation sites, resorptions, and living fetuses counted. Litters with fewer than four embryos were not included in the evaluation, because the authors stated that these embryos are better-nourished and can more easily recover from fetotoxic effects. There were 14 litters examined about a single injection of distilled water and 15 litters examined after three daily injections of distilled water. There were 13 litters examined after a single injection of 75 mg/kg acrylamide. There were 10 and 14 litters examined after three daily injections of 50 and 75 mg/kg acrylamide, respectively. Fetuses were fixed in 8% formalin. Malformed fetuses and five normal fetuses were randomly selected for sagittal sectioning and histologic evaluation (5-6 µm sections stained with hematoxylin and eosin). **[The method of determining that a fetus was malformed is not indicated, but was presumably based on external evaluation inasmuch as the designation of malformed was made prior to sectioning]**. The frequency of fetuses with malformations was analyzed by chi-square test **[thus taking the fetus as the experimental unit]**. The difference

between mean fetal weights was evaluated by *t*-test. A decrease in litter size was described in the group receiving acrylamide 75 mg/kg/day for 3 days (4.7 fetuses vs. 7.3 fetuses; **[SEM or SD not provided]**). The proportion of growth-retarded fetuses (defined as weighing less than 0.6 g) was also said to be increased in this group (18.8% vs. 4.9%, **[SEM or SD not provided: it is likely that these are proportions calculated on pooled fetuses in the dose group rather than per litter figures]**) leading to a decrease in mean fetal weight in this group compared to the control (0.63 ± 0.091 vs. 0.76 ± 0.073 **[it is not stated whether these errors are SEM or SD Even assuming these are SD, *t*-test performed by CERHR does not show a significant difference. The lack of statistical significance may be because of the higher than normal variability seen in this end point, in part already noted by the incidence of "growth retarded fetuses" even in the control group. Regardless of statistical findings or whether litter or fetus was used as the unit for analysis, a 17% decrease in fetal weight is considered by the Expert Panel to be a meaningful change even with small litters excluded].** The malformation noted by the authors was kinked tail, occurring in 9.8% of weaned offspring in the 75 mg/kg × 3 group compared to none of the controls in the accompanying mouse spot test. In the teratology evaluation on GD 18, malformed tails were seen in all groups, including the control. The data from the published table for single treatments are 1.0 and 5.9 for 0 and 75 mg/kg, respectively. For the three daily injections, the data from the published table are 1.6, 1.4, and 4.7 for 0, 50 mg/kg × 3, and 75 mg/kg× 3, respectively **[it is not stated whether these are percent of all fetuses, litter percents, or mean number of fetuses per litter].** The results of the histologic evaluation read, "…examination of 8 fetuses showed hypoplasia of the lymphatic organs as well as of the centers for hematopoiesis in liver and bone marrow. In the placenta hemorrhages were observed frequently." The authors concluded that the mesenchyme "may be the main target of [acrylamide] fetotoxicity."

Strengths/Weaknesses: Extending fetal evaluation to the histologic level is a strength. Methods for summarizing fetal findings are poorly described, and likely were carried out on a per fetus basis. For evaluation of these types of effects, it is preferable to present the data on both a per litter and a per fetus basis, and statistical analyses should use the litter as the unit. The decision not to evaluate surviving fetuses from litters with high resorption rates is a poor one; a litter already determined to be "affected" may provide a few surviving fetuses that could be the best opportunity for characterizing morphologic changes that occur in the absence of death of the embryo/fetus.

Utility (Adequacy) for CERHR Evaluation Process: On its own, the study is only suggestive because there are too many design/analysis issues. The tail findings on an observational level correlate well with the skeletal variations report in mice in the study by Field et al. (discussed below). The histologic findings extend the suggestion of developmental sensitivity in this apparently more sensitive species to soft tissues.

Field et al. *(113)*, under a contract with the National Toxicology Program, evaluated the developmental toxicity of acrylamide in Sprague-Dawley rats (29-30/group) and Swiss CD-1 mice (30/group) in a GLP-compliant study. After cohabitation, the day vaginal sperm (rats) or a copulation plug (mice) was found was designated GD 0. Treatments were by oral gavage on GD 6–20 in rats and on GD 6–17 in mice. Acrylamide was 98% pure, and HPLC prior to and after use showed dosing solutions to be 92-108% of the intended concentrations. The dose volume of each treatment was 5 mL/kg in rats and 10 mL/kg in mice, adjusted daily based on actual animal weights. Concentrations were made up in deionized distilled water. Doses were selected based on preliminary studies and were designed to produce significant maternal or fetal toxicity at the high dose, and no toxicity at the low dose. Rats received acrylamide 0, 2.5, 7.5, or 15 mg/kg/day. Mice received acrylamide 0, 3, 15, or 45 mg/kg/day. Dams were killed on the final day of

treatment. Fetuses all underwent soft tissue dissection and half the fetuses underwent Wilson sectioning of decapitated heads. All carcasses were double-stained for bone and cartilage **[clearing is not mentioned but is assumed]**.

In rats, maternal weight was decreased in a dose-dependent manner (Table 21). Absolute and relative liver weight was unchanged (data not shown). There were no dose-related clinical signs in the dams. The only significant fetal parameter associated with treatment was a dose-related increase in the percent fetuses per litter with variations and the percent litters with variations on trend testing (but not on pairwise comparison). The most common variation was extra lumbar rib but statistical analysis of percent fetuses per litter with extra ribs or of litters with extra ribs did not show a significant association with acrylamide treatment.

In mice, there were decreases in maternal weight gain over the gestation period and the treatment period by linear trend testing as well as by pairwise comparison in the highest dose group (Table 22). When corrected for uterine weight, the trend test remained significant although pairwise comparisons were no longer significant. Gravid uterine weights were decreased 12% and 14% in the 15 and 45 mg/kg/day groups, respectively, and fetal weight per litter was decreased 15% in each sex in the highest dose group. Maternal absolute but not relative liver weight showed a decreasing linear trend with dose. In the high dose group, hind-limb splaying was observed in nearly half the dams. The percent litters with resorptions showed a significant linear trend and a significant increase in the 15 mg/kg/day group; however, at the 45 mg/kg/day dose, there was no increase in resorptions; 24% of litters at the high dose had resorptions compared to 32.1% of litters in the control group. There was a significant linear trend for percent fetuses/litter with extra ribs and percent litters with extra ribs, although none of the pairwise comparisons showed a significant increase compared to control.

In their evaluation of the data, the authors of this report proposed that an increase in extra ribs "may be an indirect reflection of the adverse maternal effects and stress of treatment rather than chemical-specific fetotoxicity." They concluded that, "[acrylamide] exposure during the post-implantation phase of gestation resulted in fetal growth retardation in mice, but only in the presence of well-defined maternal toxicity. The no-observed-adverse-effect level (NOAEL) for maternal and fetal toxicity in mice was 15 mg/kg/day. In rats, [acrylamide] treatment did not alter measured end points of embryo/fetal viability, growth, or development at doses [that] depressed maternal weight gain. The NOAEL for [acrylamide]-induced maternal toxicity in rats was 2.5 mg/kg/day, and 15 mg/kg/day represented a NOAEL for developmental toxicity in rats under the conditions of this study. Neither embryo/fetal viability nor morphological development of rat and mouse offspring were affected by [acrylamide] exposure." **[Fetal weight reduction in the mouse study was appropriate for benchmark dose calculation. A power model appeared to fit the data better than a linear model. The BMD_{10} for male offspring weight reduction was 42 mg/kg/day with a BMDL of 32 mg/kg/day. The BMD_{10} for female offspring weight reduction was 44 mg/kg/day with a BMDL of 36 mg/kg/day].**

Strengths/Weaknesses: This study is very strong and was designed specifically to meet/exceed testing guidelines in dosing period, number of animals/group, multiple means of expressing outcome (for example, number/litter, number of litters affected). The study includes all standard end points evaluated in a developmental toxicology study. Although the authors conclude some end points were not meaningfully affected, and that they may be related to maternal toxicity, a decrease in maternal weight gain of 18% is not severe stress/toxicity, but simply a maternal effect level . A decrease in maternal *weight* of that degree would likely be associated with the types of changes seen here in fetuses strictly on a maternal basis

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluative process and the quantitative evaluation can be considered reliable.

Table 21. Summary of Rat Developmental Toxicity Study, Field et al. *(113)*

	Doses (mg/kg bw/day)			
	0	2.5	7.5	15
No. dams placed on test	30	29	30	29
No. dams withdrawn (dosing errors)	3	2	1	2
No. dams pregnant at termination	23	26	26	24
Maternal weight gain (corrected for uterine weight)[a]	Reference	↔	↓12%	↓18%
Implantation sites/litter		↔	↔	↔
Resorptions/litter		↔	↔	↔
Live fetuses/litter		↔	↔	↔
Male fetal weight/litter		↔	↔	↔
Female fetal weight/litter		↔	↔	↔
% fetuses or litters with malformations		↔	↔	↔
% fetuses or litters with variations[b]		↔	↔	↔

[a]There was a statistically significant inverse linear trend for dose and maternal weight over gestation, over the treatment period, and corrected for uterine weight. [b]There was a significant linear trend for % fetuses per litter with variations and % litters with variations in rats.
↑,↓ Statistically significant increase, decrease on pairwise comparison with control value; ↔ statistically similar on pairwise comparison with control value.

Table 22. Summary of Mouse Developmental Toxicity Study, Field et al. *(113)*

	Doses (mg/kg bw/day)			
	0	3	15	45
No. dams placed on test	30	30	30	30
No. dams withdrawn	0	0	1[c]	2[d]
No. dams pregnant at termination	28	26	29	25
Maternal weight gain (corrected for uterine weight)[a]	Reference	↔	↔	↔
Implantation sites/litter		↔	↔	↔
Resorptions/litter		↔	↔[e]	↔
Live fetuses/litter		↔	↔	↔
Male fetal weight/litter		↔	↔	↓15%
Female fetal weight/litter		↔	↔	↓15%
% fetuses or litters with malformations		↔	↔	↔
% fetuses or litters with variations[b]		↔	↔	↔

[a]There was a statistically significant inverse linear trend for dose and maternal weight over gestation, over the treatment period, and corrected for uterine weight. [b]There was a significant linear trend for % fetuses/litter and % litters with extra ribs in mice. [c]Removed due to liver mass. [d] One removed due to dose .error and one removed due to morbidity not due to test article. [e]% litters with resorptions was increased from 32.1% to 58.6% in this dose group only.
Statistically significant decrease on pairwise comparison with control value; ↔ statistically similar on pairwise comparison with control value.

Rutledge et al. *(114)* presented data from studies in which female mice **[strain not stated, but SEC × C57Bl F1 females were used in a different experiment described in the same paper]** were mated for a 30-minute period, then treated with acrylamide **[purity and source not stated]** at 125 mg/kg **[i.p.]** 1, 6, 9, or 25 h later. The intervals represented fertilization, the early pronuclear stage, pronuclear DNA synthesis, and the 2-cell stage, respectively. Control animals were given HBSS at the respective times **[there is only one control listed for the 9- and 25-h groups; it is not known when that control was treated]**. On gestation day 17 **[plug day not identified]**, the uteri were inspected for resorptions, embryonic death, and live fetuses. Live fetuses were inspected for external abnormalities. Results are shown in Figure 5. The data table did not indicate statistical differences **[superscripts were missing from the table]**, but the text indicates that live fetuses were decreased and resorptions increased at all treatment times. Among live fetuses, abnormalities were said to have been increased with treatment at 6, 9, and 25 h after mating. The specific malformations are reported for the group treated 6 h after mating, which appeared to represent the most sensitive time for the production of resorptions and of abnormal live fetuses. These malformations are given in Table 23 **[some fetuses had more than one abnormality]**.

Strengths/Weaknesses: The idea for the treatment schedule is a good one. Missing aspects of methods are a weakness. The number of fetuses available for evaluation presented in the data table suggests an adequate number of litters were included, as least for the 6-h evaluation. A strength is the testing of a specific critical window in preimplantation development that is not

assessed by standard developmental toxicity tests. A weakness is the use of only a single high dose, but one that can be compared with male dominant lethal data for acute toxicity at critical times.

Utility (Adequacy) for CERHR Evaluation Process: This study shows that a single high dose of acrylamide can produce very early embryo death and malformation, presumably through a mutagenic mechanism. The study provides insight regarding risks of acute high exposures that would be extremely difficult to detect in humans (women attempting conception). The study is not useful for setting safe levels for chronic exposures.

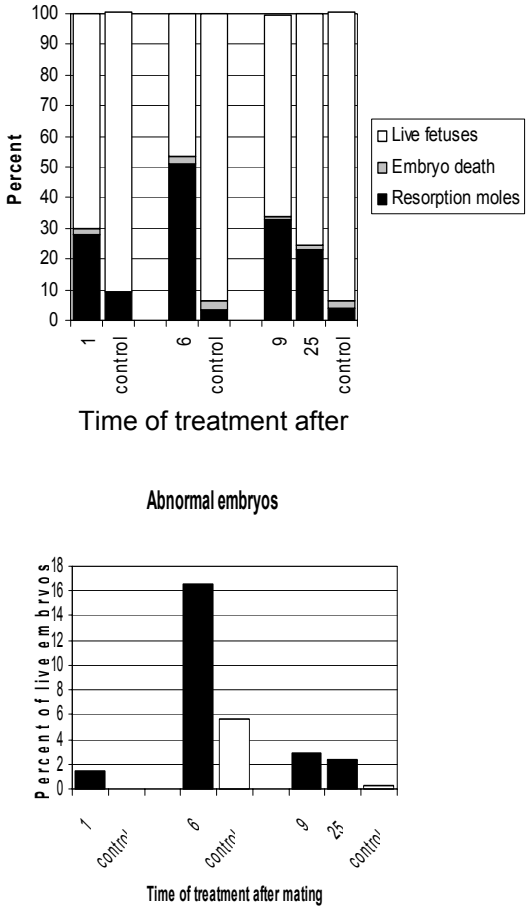


Figure 5. Pregnancy Outcome in Mice as a Percent of Implantations/Female (top) and as a Percent of Live Embryos (bottom) by Number of Hours Between Mating and Treatment with Acrylamide 125 mg/kg. From Data Presented in Rutledge et al. *(114)*

Table 23. Abnormalities in Mouse Fetuses from Dams Treated with 125 mg/kg Acrylamide or Control and Mated 6 h Later, Rutledge et al. *(114)*

	Acrylamide[a]	HBSS (control)[a]
No. live fetus evaluated	203	180
Bent tail	10	3
Bent limbs	8	0
Hydrops	6	0
Eye defect	5	1
Cleft palate	5	0
Abdominal wall defect	3	1
Exencephaly	2	4

[a]Results presented as frequency of fetuses affected

3.2.2 Developmental neurotoxicity—pregnancy dosing

Agrawal and Squibb *(115)* evaluated the effect of gestational acrylamide exposure on dopamine receptor binding in offspring brain. Time-bred pregnant Fisher 344 rats were treated with acrylamide **[purity not specified]** in water at 0 or 20 mg/kg/day by gavage from GD 7–16 **[plug day not defined]**. Animals were apparently permitted to litter and on postnatal day 1 pups were randomized by dose group and reallocated to a control or an acrylamide-exposed dam in a standard cross-fostering manner, yielding four groups: acrylamide-treated dams (n=7) rearing acrylamide-exposed pups, acrylamide-treated dams (n=5) rearing vehicle-exposed pups, vehicle-treated dams (n=5) rearing acrylamide-exposed pups, and vehicle-treated dams (n=6) rearing vehicle-exposed pups. The litters were standardized to 4 males and 4 females. On postnatal days 14 and 21, one male and one female pup were randomly selected from each litter and decapitated. The corpus striatum was dissected from the brain and frozen in liquid nitrogen for analysis at a later date. Striatal tissue was homogenized in 0.32 M sucrose and centrifuged to isolate membranes. Membrane preparations were incubated with radiolabeled spiroperidol to estimate dopaminergic receptors.

Dam body weight and litter size were said not to have been affected by treatment **[data are not shown, and it is not indicated when or how often dams were weighed; a one-way ANOVA was used for dam weight during treatment or following birth at PND 1]**. Pup weights were not different by treatment group at 2 or 3 weeks of age **[pup weight appears to have been analyzed by foster litter rather than by litter of origin. The initial two-factor analyses indicated that fostering was not a significant contributor to overall variance, so it was not considered in the subsequent pairwise analyses of either weight or dopamine receptor end points]**. At two weeks of age, dopamine receptor binding of spiroperidol was decreased by about 20% in male pups that had been antenatally exposed to acrylamide, regardless of whether reared by treated or control dams. For female pups, acrylamide-treated status of the birth dam was associated with a 16–19% decrease in spiroperidol binding, whether the pup was raised by an acrylamide- or a vehicle-treated dam. At three weeks of age, there were no differences among groups in spiroperidol binding. Scatchard analysis of spiroperidol binding was determined using membrane preparations pooled from pups within the same treatment condition **[the age of the pups was not given, but may be assumed to be PND 14, when there were significant decreases in specific binding]** Receptor affinity in acrylamide-exposed pups reared by acrylamide-treated dams was lower than the "control" **[presumably vehicle-exposed pups**

reared by vehicle-treated dams, but "control" may be a reference to prenatal vehicle exposure from two-way ANOVA, regardless of fostering condition]. The K_D values were 0.25 $\times 10^{-9}$ M vs. 0.43 $\times 10^{-9}$ M. "Dosed rats" also had lower receptor density than "control": 18.2 pmol/100 mg protein vs. 31.5 pmol/100 mg protein.

Strengths/Weaknesses: This study did not describe several aspects of methodology and analysis clearly enough to permit any certainty in its interpretation. The neurochemical variables assessed made sense at the time the study was conducted, and specifically relate to the CNS areas/transmitter involved in motor control (CNS signs of acrylamide toxicity in adult rats are motor). The study only included one treated group and used Fisher rats, which is not a strain that has shown sensitivity to some agents that result in CNS malformations. A strength is the evaluation of both sexes.

Utility (Adequacy) for CERHR Evaluation Process: This study can be viewed as suggestive of transient (though not dramatic) changes in the postnatal developing dopaminergic system and is adequate for use on this basis only. The acrylamide dose level used is similar to or higher than some of the other studies reporting developmental neurotoxicity-type findings.

Wise et al. *(116*) from Merck Research Laboratories performed a developmental neurotoxicity evaluation of acrylamide in Sprague-Dawley rats according to then-current EPA guidelines for developmental neurotoxicology studies. After cohabitation, females with copulation plugs in their cage pans or vaginas were considered to be at GD 0. These animals were randomized (balanced for weight) to receive acrylamide (99.9% pure) in deionized water at 0, 5, 10, 15, or 20 mg/kg/day by oral gavage from GD 6 through PND 10. Dams were permitted to litter and rear their own offspring. On PND 3, litters were culled to 5 pups of each sex (based on pups identified randomly on the day of birth). There was a single control litter that had two pups of each sex fostered into it in order to make a litter of five of each sex; the fostered pups were not used in the analysis. One pup of each sex from each litter was killed on PND 11 for evaluation of brain, spinal cord, and peripheral nerve. Pups were removed from their dams during postnatal week 4 and housed 1 or 2 (same sex) to a cage until postnatal week 5–6, when they were singly housed. Behavioral assessments included open-field motor activity, auditory startle habituation, and passive avoidance.

Open-field motor activity was assessed on the same single animal/sex/litter on PND 13, 17, and 21. On one of days 58–60, open field testing was performed on a second animal/sex/litter, that second animal being one previously evaluated for auditory startle habituation on PND 22. Open field testing was performed under red light using ~70 dB background white noise. End points in the open field test included the numbers of beam interruptions in each of six 10-minute periods. Only three 10-minute periods were used for PND 13 animals.

Auditory startle habituation was evaluated on PND 22 and on one day between PND 58–60, using animals at this second time point that had previously undergone open-field testing. After a 3-minute acclimation period during which animals were exposed to 72-dB background white noise, animals were exposed to 60 120-dB 50-msec bursts of white noise with a 5-sec interval between trials. End points included the peak amplitude of each startle movement and the interval between acoustic stimulus and startle movement **[evaluated by deflection of a platform and expressed as millivolts].**

A passive avoidance test was used to assess short-term learning. Animals naïve to the test were evaluated on PND 24 and one of PNDs 58-60 with retesting one week later. The test involved a shuttlebox with a light and a dark compartment. Rats entering the dark compartment received a

1-sec foot shock. Test criteria were met when the animal remained on the lighted side of the shuttlebox for two consecutive 60-sec trials.

Statistical analysis was performed by trend testing. When a significant test for trend was obtained, the top dose group was eliminated and the testing repeated until significance was lost.

Dams in the 20 mg/kg/day group all demonstrated hind-limb splaying, a sign of acrylamide neurotoxicity, on PND 1 through 4, and all dams and pups in this group had died or were euthanized by PND 4 due to excessive pup mortality. In the 15 mg/kg/day group, dams all displayed hind-limb splaying between PND 4 and 9, although some dams appeared to improve after discontinuation of acrylamide treatment on PND 10. There were no clinical signs in the 5 and 10 mg/kg/day dams, although maternal toxicity became apparent in the 10 mg/kg/day dose group during the lactational period, when there was a significant decrease in weight gain compared to control animals.

Pregnancy outcome and neurobehavioral testing results are summarized in Table 24. The authors noted that perinatal mortality was prominent in the 20 mg/kg/day group and that postnatal pup death occurred in the 15 mg/kg/day group. Given the important degree of maternal toxicity in the 15 and 20 mg/kg/day dose groups, the authors suggested that the pup mortality in these groups might not have been a direct effect of acrylamide exposure but rather secondary to the severely compromised condition of the dams. There were no histologic changes in the nervous system tissues in the 15 mg/kg/day group. Behavioral testing was altered only in the 15 mg/kg/day group. Brain weights decreased in the 15 mg/kg/day group, attributed by the authors to a larger decrease in body weight, reflected as an increase in relative brain weight**.** The decrease in offspring weight at exposure levels above 5 mg/kg/day was attributed to maternal toxicity. The decrease in female pup weight in the 5 mg/kg/day group on days 3 and 7 was considered possibly not to have been treatment-related, given the transitory nature of this weight decrease, its confinement to one sex, and to lack of a similar effect on pup weight in the Field et al. study *(113*) at maternal acrylamide doses under 15 mg/kg/day. The authors concluded, "These results suggest that acrylamide is not a selective developmental neurobehavioral toxicant because conventional measures of pup toxicity (i.e., body weight) were observed at dosages lower than those [that] affected behavioral parameters." They identified a NOAEL for developmental toxicity at < 5 mg/kg/day and a NOAEL for developmental neurotoxicity at 10 mg/kg/day. The BMD_{10} calculated on offspring brain weight was 11 mg/kg/day, and the BMDL was 8 mg/kg/day.

Strengths/Weaknesses: This study came close to following EPA developmental neurotoxicity testing guideline (except for pup assignments to behavioral testing). A weakness is the use of the EPA guideline as a rigid protocol design, without more specific assessments that might be predicted (as the authors admit) to be effective in more clearly defining a LOAEL/NOAEL within the study (i.e., their reference in the discussion to grip strength).

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate, based on regulatory guidance. The Panel agrees with bottom line NOAELs and that there was no selective developmental neurotoxicity "within the context of measures assessed" in this study, and that 5 mg/kg/day was a marginal developmental effect/no effect level. The dose-responsive nature of the pup body weight effect in the presence and absence of maternal effect suggests that maternal toxicity probably contributed to (enhanced) the pup effect at the higher doses, but was not totally responsible for the finding at those doses.

Table 24. Summary of Data in the Wise et al. *(116)* Rat Developmental Neurotoxicity Study

Dose, mg/kg/day (n)		0 (10)	5 (9)	10 (8)	15 (12)	20 (12)
Implants/female			↔	↔	↔	Not reported
Live pups/litter	PND 0		↔	↔	↔	↓37%
Pup deaths	PND 1–3		↔	↔	↔	↑33%
	PND 4-21		↔	↔	↑13.3%	Not reported
Pup weight, female	PND 0		↔	↔	↓	↓
	PND 3		↓	↓	↓	↔
	PND 7		↓	↓	↓	killed
	PND 14		↔	↓	↓	
	PND 21		↔	↓	↓	
Pup weight, male	PND 0		↔	↔	↓	↓
	PND 3		↔	↓	↓	↔
	PND 7		↔	↓	↓	killed
	PND 14		↔	↓	↓	
	PND 21		↔	↓	↓	
Weight, week 4–9, female	Average		↔	↓	↓	
	Gain		↔	↔	↓	
Weight, week 4–9, male	Average		↔	↔	↓	
	Gain		↔	↔	↔	
Open field activity, female	PND 13		↔	↔	"↓" (P=0.08)	
	PND 17		↔	↔	↔	
	PND 21		↔	↔	↑	
	adult		↔	↔	↔	
Open field activity, male	PND 13		↔	↔	"↓" (P=0.06)	
	PND 17		↔	↔	↔	
	PND 21		↔	↔	↔	
	adult		↔	↔	↔	
Auditory startle habituation, peak	PND 22, female		↔	↔	↓	
	adult female		↔	↔	↓	
	PND 22, male		↔	↔	↓	
	adult male		↔	↔	↔	
Time to peak (all ages, both sexes)			↔	↔	↔	
Passive avoidance (both sexes)			↔	↔	↔	
Brain weight, absolute	PND 11, female		↔	↔	↓15%	
	PND 11, male		↔	↔	↓15%	
	11 weeks, female		↔	↔	↓ 9%	
	11 weeks, male		↔	↓ 6%	↓12%	
Brain weight, relative	PND 11, female		↔	↑23%	↑65%	
	PND 11, male		↔	↔	↑66%	
	11 weeks, female		↔	↔	↔	
	11 weeks, male		↔	↔	↑17%	

↑,↓ Higher, lower than control group, statistical significance determined, except for brain weight, by trend testing w sequential elimination of highest doses until significance of trend lost. For absolute and relative brain weight, signi not tested in the original paper, but assigned by CERHR based on data presented in the paper using ANOVA with p Dunnett's test.

↔ Statistically not difference from control value. "↓" refers to a statement by the authors that open field activity was reduced at this dose, but lack of formal statistical significance at $P \leq 0.05$.

Walum and Flint *(117)* evaluated the effect of acrylamide **[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 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 acrylamide 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 of gamma-amino butyric acid (GABA) were also decreased by acrylamide exposure **[the text indicates that GABA uptake was "virtually" unaffected; the data table shows a statistically significant 8% reduction in GABA uptake. The Expert Panel agrees that an 8% change in this particular variable is likely within the normal variation of the procedure and unlikely to be biologically meaningful, statistics notwithstanding.]**. The authors concluded that acrylamide may reduce the "differentiation and development of dopaminergic projections" in the developing rat brain.

Strengths/Weaknesses: This study provides an in vitro assessment of a potential mechanism of acrylamide 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.

Utility (Adequacy) for CERHR Evaluation Process: This study is suggestive of a mechanism and is adequate for use in that manner only.

3.2.3 Male-mediated developmental toxicity

Holland et al. *(2)* evaluated morphologic abnormalities in preimplantation embryos, chromatin adducts, and dominant lethality after treatment of male C57Bl/6J mice with acrylamide. The study was funded by grants from the Department of Energy and from NIEHS. The dominant lethal portion of the study appears in Table 12 and is discussed in section 2.3.2.3. Although the premise of the study was that adverse reproductive outcomes were associated with acrylamide genotoxicity, some of the end points were developmental and will be presented here. Acrylamide **[purity not given]** was administered i.p. in sterile PBS. In the first experiment, males were treated with 0, 40, or 50 mg/kg/day acrylamide for five days. There were 8 males in each of the acrylamide-dosed groups and 15 control males **[it appears that controls may have been combined from different experiments].** Untreated C3H/J females were mated with the treated males 4 or 5 times/week for up to 5 weeks. Matings at 1, 2–3, and 4–5 weeks after the exposure were taken to represent acrylamide effects on spermatozoa, spermatids, and spermatocytes, respectively. Males were housed overnight with 3 or 4 females for mating. The presence of a vaginal plug in the morning was assumed to represent pregnancy onset at midnight. Females were killed about 86 to 88 h after the time of assumed pregnancy onset. Corpora lutea were counted and uterine horns were flushed with warm tissue culture medium. Embryos and unfertilized eggs were counted and evaluated for morphologic abnormalities using a dissecting microscope. The frequencies of abnormalities in acrylamide-exposed and control embryos were compared with Fisher's exact test. Logistic regression was used to evaluate the effects of acrylamide dose and time of acrylamide treatment on preimplantation abnormalities. More complex logistic regression models were used to evaluate experiment, female body weight, and number of corpora lutea as well as dose and time. The effect of sire was not significant by multifactorial regression and so embryos within time and dose groups were pooled for analysis.

Acrylamide treatment at the 50 mg/kg/day dose resulted in death of about 10% of males within 24 h of the last injection, but treatment did not affect mating success of surviving males, expressed

as the proportion of females placed with a male that were plug-positive the next morning. The number of corpora lutea per female did not differ among dose groups. The mean (± SD) numbers of embryos recovered per female were 8.1 ± 1.2, 7.4 ± 1.3, and 6.4 ± 1.5 after treatment of males with, respectively, 0, 40, and 50-mg/kg/day acrylamide. These numbers were reported by the authors not to be statistically different from one another **[however, ANOVA by CERHR shows an overall *P* value of 0.0211. Comparing the 50 mg/kg/day group with the control, *P* < 0.05 by post-hoc Dunnett's test. Use of a trend test might have been more powerful in flagging the 40 mg/kg group as significantly different from control. Using the benchmark dose calculation, the BMD_{10} for this end point was 42 mg/kg/day and the BMDL was 21 mg/kg/day]**. There were no differences in mean percent efficiency of recovery (embryos per corpora lutea). Abnormal embryo data are shown in Table 25.

Table 25. Percent Abnormal Embryos (of total number of embryos recovered) by Week of Mating After Treatment of the Male. Experiment 1 from Holland et al. *(2)*.

	Acrylamide dose to the male (mg/kg/day × 5 days)		
Week	0	40	50
1	5.0% (of 40)	61.1% (of 54)[a]	86.2% (29)[a,b]
2	5.0% (of 20)	62.1% (of 37)[a]	69.5% (46)[a]
3	0% (of 32)	59.1% (of 71)[a]	50.0% of 46)[a]
4	no females mated	57.4% (of 54)[a,c]	30.8% (of 26)[a]
5	4.2% (of 24)	22.8% (of 35)[a]	15.1% (of 33)

[a]Different from pooled control, [b]different from 40 mg/kg/day group, and [c]different from 50 mg/kg/day group; all comparisons used Fisher's exact test (embryo-based)

The number of embryos recovered was variable among groups at different weeks, perhaps due in part to the variability in number of mated females, ranging from 0 to 9 per group per week. The proportion of the total embryos that was abnormal appeared highest 1 week after treatment in the 50-mg/kg/day group and decreased thereafter, reaching the control proportion by 5 weeks after treatment. The 40 mg/kg/day group data suggested a plateau effect over weeks 1–4. Embryos were evaluated as either retarded (fewer than 10 blastomeres), lysed (abnormal cell structure), or single cells. The latter were called "unfertilized eggs or zygotes that failed to undergo cleavage."

In a second experiment, Holland et al. *(2)* further explored the dose–response relationship by using 10 (n=10), 25 (n=10), and 50 (n=9) mg/kg/day acrylamide i.p. in male mice for five consecutive days. Males were mated with unexposed females 2 and 3 weeks after treatment, apparently using the same protocol as in the first experiment. Mating success was not found to be affected by treatment. The mean (± SD) numbers of embryos/female in the 0 (pooled controls), 10, 25, and 50 mg/kg/day groups were 8.1 ± 1.2, 7.1 ± 1.4, 7.5 ± 1.1, and 6.4 ± 0.9. There was no reported difference among groups in the number of embryos recovered per female or the number of embryos recovered per corpus luteum **[again, the 50 mg/kg/day group shows a significant decrease in embryos/female by CERHR analysis. Using the benchmark dose approach, the BMD_{10} was 27 mg/kg/day and the BMDL was 18 mg/kg/day. The Expert Panel notes that the data table refers to this experiment as Experiment 3, whereas the text identifies it as the second experiment]**.

The results of the second experiment are shown in Figure 6, taken from a data table in the original paper **[Significant differences in the data table appear to be mismarked with respect to the 10- and 25-mg/kg/day groups at the second week].** There did not appear to be a formal comparison of the 10 mg/kg/day dose to the pooled control, but the implication in the text is that the 10 mg/kg/day dose was inactive **[there is a statement of no difference between the second and third week in the 10 mg/kg/day group, but then "P < 0.01" inexplicably is written]**.

Total abnormalities decreased in the 25- and 50-mg/kg/day groups between the second and third week. Arrested cleavage decreased in the 50-mg/kg/day group between the second and third weeks.

In a third experiment, Holland et al. *(2)* treated 42 male mice with 50 mg/kg/day i.p. for five days. Males were mated two and three weeks later and results given by day of mating **[number of males on any given day is not stated]**. Results are shown in Figure 7. Time-dependency was reported to show a significant negative trend.

The results of all three experiments by Holland et al. *(2)* were pooled to evaluate dose- and time-dependency by logistic regression modeling. Dose, time since treatment of the male, and experiment were the most important parameters with exposure of spermatozoa producing the strongest effect. When the three different types of abnormal embryo were evaluated in the model, a significant dose-response relationship was found for zygotes/eggs and for retarded cleavage, but not for blastomere lysis.

Strengths/Weaknesses: This study using i.p. acrylamide at doses comparable to those used in many of the dominant lethal studies permits ready comparison with those studies. The i.p. route of exposure is also a weakness because it is not relevant to human acrylamide exposure. The statistical approach in which sire of origin is disregarded is a weakness. The multifactorial analysis would consider overall sire effect across multiple outcome measurements, but not whether sire had an effect on individual measurements. Statistically, inflation of degrees of freedom probably occurred; however, the values calculated on a per embryo basis and the profile of effects across end points leave little doubt that there was a consistent effect. The lumping of single-cell forms without regard to distinguishing unfertilized oocytes from fertilized eggs/zygotes is a weakness. The presentation of some of the data without SD or SEM precludes calculation of a benchmark dose.

Utility (Adequacy) for CERHR Evaluation Process: This series of studies identifies a male-mediated effect on abnormal preimplantation embryo development, consistent with the dominant lethal studies reviewed in Section 2.3.2.2. Even if all of these effects could be attributed to just a couple of the males in each group, the male-mediated toxicity would have approximately a 20% incidence, which would be a highly meaningful finding, and a genetically based finding at that. The LOAEL is probably 25 mg/kg/day in the four-dose study, although the published report is not consistent on whether 10 mg/kg/day is an effect level. The LOAEL of 25 mg/kg/day is supported by a calculated BMD_{10} of 27 mg/kg/day and BMDL of 18 mg/kg/day.

Nagao *(82)* (support not indicated) treated ICR male mice with acrylamide **[purity not specified]** at 62.5 or 125 mg/kg as single doses or with 5 daily doses of 50 mg/kg/day prior to mating. There were 20 males per dose group with at least two pregnant females per male. Fetuses evaluated on GD 18 had no increase in external malformations compared to concurrent or historical control fetuses. Genetic end points of ths study are discussed in section 2.3.2.3.

Strenghts/Weaknesses: There was little detail on the method of evaluation of fetuses. The results were reported as a frequency within a dose group without regard to litter or sire of origin.

Utility (Adequacy) for CERHR Evaluation Process: This paper is of limited utility due to the absence of visceral or skeletal end points and the lack of detail in the report.

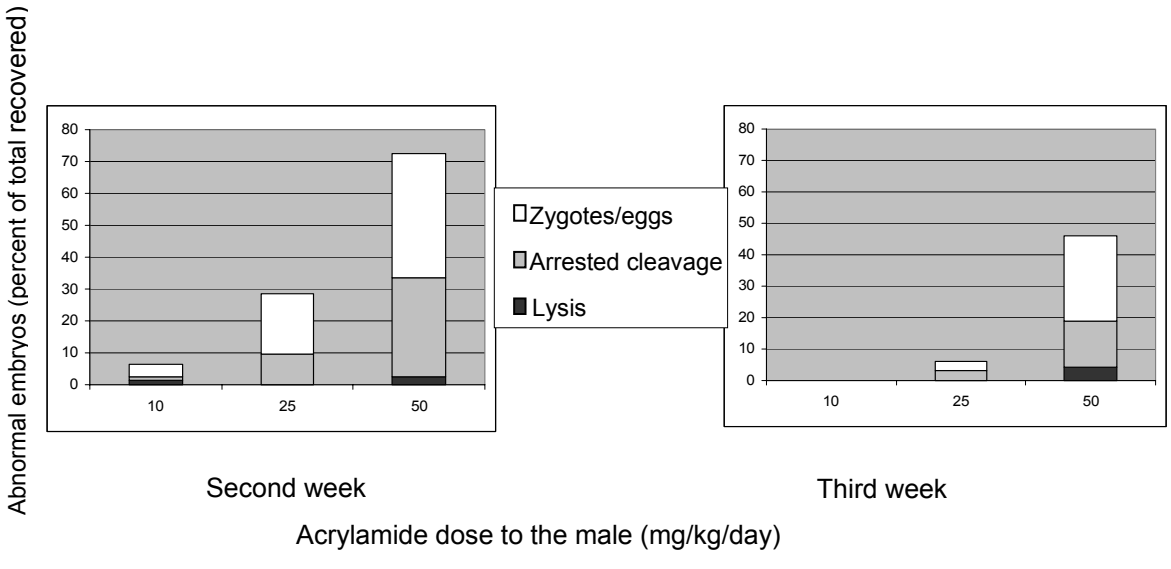


Figure 6. Types of Abnormal Embryos in Experiment 2 of Holland et al. *(2)*. Taken from data table in the original paper.

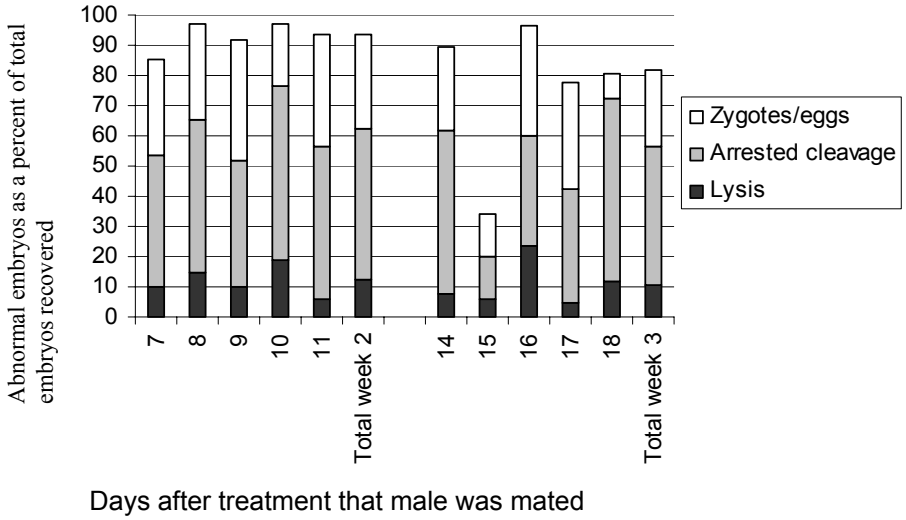


Figure 7. Abnormal Day 4 Embryos Recovered from Female Mice Mated with Males that Had Received Acrylamide 50 mg/kg/day i.p. for Five days. Experiment 3 of Holland et al. *(2)*

3.2.4 Lactation

A study by Husain et al. *(104)*, which included a lactational exposure component, was summarized in Section 2.5.1. That study had found alterations in neurotransmitters in the brains of young rats exposed to acrylamide through milk ingestion.

Friedman et al. *(118)* stated that they were attempting to replicate the findings of Husain et al. *(104)* using the same strain of rat (Wistar) and the same dosing regimen during the lactation period in a GLP-compliant study funded by the Acrylamide Monomer Producers Association. **[The Expert Panel notes that this study used the same exposure paradigm as Husain, but did not include any of the assessments (neurochemical) used in that study]** Acrylamide (99.7% pure) in 0.9% saline was administered to dams at 0 or 25 mg/kg/day by gavage beginning on PND 0 (the day of parturition) and continuing though PND 21 (n=15 dams per dose group). Dams were weighed on PND 0, 4, 7, 14, and 21 and the most recent body weight was used for dose adjustment. Clinical observations were made twice daily, including 2–4 h post-dosing, and dams were evaluated by Functional Observation Battery on PND 7, 14, and 21. The Functional Observational Battery consisted of home cage, handling, open field, and sensory/neuromuscular observations. Maternal grip strength was measured on the same days as the Functional Observational Battery. Litters were reared by their own dams and were not culled. Pups were examined and weighed on PND 4, 7, 14, and 21, when weaned. Female pups were killed at weaning on PND 21 and one female/litter was necropsied. Male offspring were weighed on PND 28 and every 7 days thereafter until PND 91 (13 weeks of age). Grip strength was assessed in 10 males per dose group (from different litters if possible) at PND 30, 60, and 90. Statistical analysis used ANOVA for parametric data (with homogeneous variance by Bartlett's test) and chi-square or Kruskal-Wallis tests for nonparametric data.

Of the 15 dams selected in each dose group, there were 15 and 10 litters remaining at weaning on PND 21 in the 0 and 25 mg/kg/day groups, respectively. One of the dams in the 25 mg/kg/day group delivered a dead litter prior to the onset of acrylamide dosing. One of the dams in this group died after a misdirected dose of acrylamide. One dam lost her entire litter by PND 7. Two dams were killed in moribund condition on PND 18 and 20. Maternal weight was equivalent between dose groups at parturition. All subsequent weights were lower in the 25 mg/kg/day dose group then in the control, and acrylamide-treated dams lost weight over the lactation period. Their feed and water consumption were also significantly less than those in the control group at all time points except PNDs 0–4, when water consumption was equivalent. Acrylamide-exposed dams exhibited clinical signs of toxicity including hindlimb splay and tremors, as well as nonspecific signs of stress. There were differences between the groups in the Functional Observational Battery, with decreases in arousal and increases in indifference as well as neurologic abnormalities in gait and hindlimb splay. There were no histologic abnormalities of the sciatic nerves in the dams.

Litters were comparable in size, weight, and sex ratio on PND 0. The proportion of offspring surviving to PND 21 was reduced in the 25 mg/kg/day group (72.5 ± 11.0%) compared to the control group (98.4 ± 0.9, $P < 0.05$ by Student's *t*-test). Mean pup weight per litter was lower in the acrylamide-treated group from PND 4 until weaning, and remained lower in weaned males from weaning until termination at PND 91. Necropsy of pups that died or were moribund prior to weaning showed no milk in the stomach and intestines distended with air. Male offspring surviving to weaning in the acrylamide-exposed group showed clinical signs of inanition in the early post-wean period, with recovery over time. Fore- and hindlimb grip strength were reduced in post-wean males in the acrylamide group at PND 30 but had recovered by PND 60 in spite of a continued body weight effect. The authors concluded that acrylamide-induced maternal toxicity had compromised the ability of the dam to nourish her pups. Pups that were able to survive to

weaning and were not too weak to eat or drink thrived once they had a food and liquid source other than their dams. The authors considered it unlikely that acrylamide exposure of the offspring occurred through milk. They believed that the alterations in brain amines reported by Husain et al. in *(104)* in pups nursed by acrylamide-exposed dams were confounded by the overall toxicity of acrylamide on the dam with subsequent impairment of lactational competence.

Strengths/Weaknesses: The Husain article does not describe how the number of pups was selected, e.g., whether entire litters were used to make up the samples evaluated. In addition, whole levels of neurotransmitters are relatively uninformative end points. In spite of the failure to report from where the pups came, many of the regional values for activity levels (monoamine oxidase or acetylcholinesterase) did show a developmental trend in the control group, and the majority of major (perhaps biologically meaningful) changes in the acrylamide group occurred early in the study during treatment. The Friedman conclusion that these apparent treatment-related changes may have been due to maternal toxicity (poor nursing/pup nutrition) could account for at least a portion of the body weight effects and apparent developmental delay in several of the neurochemical parameters measured in the Husain study. It is unfortunate that additional doses that did not result in severe toxicity were not included in the either the Husain or Friedman study, so that a characterization of maternal/general developmental/developmental neurotoxicity effects could be made.

Utility (Adequacy) for CERHR Evaluation Process: As both of these studies stand, without a dose-response assessment, there is no way to separate maternal from developmental findings, or direct vs. indirect (maternally mediated) developmental toxicity. What the studies do show together is that with less than optimum study design, both neurochemical and overt functional developmental end points are altered by maternal lactational treatment with this dose of acrylamide.

3.3 Utility of developmental toxicity data

There are no human data on acrylamide developmental effects. The data base is sufficient for an evaluation of acrylamide developmental effects in rats *(78, 113)* and mice *(113)*, although only the study of Field et al. *(113)* included a complete assessment of external, visceral, and skeletal abnormalities in the offspring. The data base includes an adequate developmental neurotoxicity study *(116)*. A study conducted at Bio/dynamics Inc. *(110)* provided supportive information on developmental outcomes not including structural malformations. The data base contains additional studies offering supplemental information. Of particular interest were studies by Rutledge et al. *(114)* demonstrating an increase in abnormal embryos after acrylamide treatment of pregnant mice during preimplantation stages and by Holland et al. *(2)* showing an increase in abnormal preimplantation embryos in pregnancies sired by acrylamide-treated males.

3.4 Summary of developmental toxicity data

3.4.1 Human Data

No human data on acrylamide developmental effects were located.

3.4.2 Experimental Animal Data

Key studies on acrylamide developmental toxicity are summarized in Table 26. The most useful studies were those by Zenick et al. *(78)*, Field et al. *(113)*, and Wise et al. *(116)*

The study by Zenick et al. *(78)* was designed as a female reproductive toxicology study, but produced data relevant to an assessment of developmental toxicity as well. Acrylamide was given in drinking water to female rats at 0, 25, 50, or 100 ppm. After two weeks of treatment,

untreated males were placed overnight with the females for up to 7 days. Acrylamide treated water was withheld during mating but resumed during pregnancy and lactation. Dams delivered their young and litters were culled to 4 males and 4 females on PND 4. End points included maternal weight gain, mating performance, pregnancy rate, pup survival and weight, and day of vaginal patency in female offspring. Neurologic toxicity (hindlimb splay) was seen in dams given 100 ppm acrylamide. Dam body weight and fluid intake were reduced at several time points in the animals given 50 and 100 ppm acrylamide. There were 2 of 15 dams in the 100 ppm group with full or nearly full litter loss. Pup weight was decreased in a dose-dependent manner in the 50 and 100 ppm groups and was transiently depressed in the first week of life in the 25 ppm group. A regression analysis performed without the 100 ppm group, due to incapacity of the dams, showed a significant effect of cumulative acrylamide intake on litter weaning weight. Full litter loss and decreased pup weight may have been due to maternal toxicity. A LOAEL of 25 ppm was selected by the Expert Panel based on transiently decreased pup weight.

Field et al. *(113*) evaluated the developmental toxicity of acrylamide by gavage in Sprague-Dawley rats (29-30/group) and Swiss CD-1 mice (30/group). Rats received acrylamide 0, 2.5, 7.5, or 15 mg/kg/day on GD 6–20. Mice received acrylamide 0, 3, 15, or 45 mg/kg/day on GD 6–17. Maternal weight decreased in a dose-dependent manner in both species. In rats, there was a dose-related increase in the percent fetuses per litter with variations and the percent litters with variations on trend testing but not on pairwise comparison. In mice, fetal weight per litter was decreased 15% in each sex in the highest dose group. There was a significant linear trend for percent fetuses/litter with extra ribs and percent litters with extra ribs, although none of the pairwise comparisons showed a significant increase compared to control. The NOAEL for maternal and fetal toxicity in mice was 15 mg/kg/day. The NOAEL for maternal toxicity in rats was 2.5 mg/kg/day, and the NOAEL for fetal toxicity was 15 mg/kg/day, the highest tested dose **[the increase in variations was not considered an adverse effect]**. Fetal weight reduction in the mouse study was appropriate for benchmark dose calculation. A power model appeared to fit the data better than a linear model. The BMD_{10} for male offspring weight reduction was 42 mg/kg/day with a BMDL of 32 mg/kg/day. The BMD_{10} for female offspring weight reduction was 44 mg/kg/day with a BMDL of 36 mg/kg/day.

Wise et al. *(116*) performed a guideline-compliant (at the time) developmental neurotoxicity evaluation of acrylamide in Sprague-Dawley rats. Animals received acrylamide 0, 5, 10, 15, or 20 mg/kg/day by oral gavage from GD 6 through PND 10. Behavioral assessments included open-field motor activity, auditory startle habituation, and passive avoidance. Dams in the 20 mg/kg/day group all demonstrated hind-limb splaying and all dams and pups in this group died or were euthanized by PND 4 due to excessive pup mortality. In the 15 mg/kg/day group, dams all displayed hind-limb splaying between PND 4 and 9. Maternal toxicity became apparent in the 10 mg/kg/day dose group during the lactational period during which there was a significant decrease in weight gain compared to control animals. Perinatal mortality occurred in the 15 and 20 mg/kg/day groups and was attributed by the authors to the severely compromised condition of the dams. Behavioral testing was altered in the 15 mg/kg/day exposure group. Brain weights decreased in offspring in the 15 mg/kg/day group, attributed by the authors to an even larger decrease in body weight**.** The decrease in offspring weight at exposure levels above 5 mg/kg/day was attributed to maternal toxicity. The decrease in female pup weight in the 5 mg/kg/day group on days 3 and 7 was considered possibly not to have been treatment-related, given the transitory nature of this weight decrease, its confinement to one sex, and to lack of a similar effect on pup weight in the Field et al. study *(113*) at maternal acrylamide doses under 15 mg/kg/day. The authors identified a NOAEL for developmental toxicity at < 5 mg/kg/day and a NOAEL for developmental neurotoxicity at 10 mg/kg/day. The Panel agrees with the NOAELs, and with the conclusions that there was no selective developmental neurotoxicity and that 5 mg/kg/day was a

marginal developmental effect/no effect level. The BMD_{10} calculated on offspring brain weight was 11 mg/kg/day, and the BMDL was 8 mg/kg/day.

Also of use was a study conducted at Bio/dynamics Inc. *(110)* in Sprague-Dawley CD rats. Dietary acrylamide was given to females at 0, 25, or 50 ppm for two weeks prior to mating and from GD 0-19. Acrylamide intake was estimated at 1.75–1.90 and 3.45–3.82 mg/kg bw/day in the 25 and 50 ppm dose groups, respectively. Litters were standardized on PND 4 and pups were examined for postnatal growth and mortality through the lactation period (PND 21). There was a slight but significant reduction in body weight gain in the 50 ppm dams during the premating period. Mating and pregnancy indices were comparable among all treatment groups. There were no differences in mean gestation length, fetal viability at birth, number of live pups at birth, litter size on PND 4, pup weights, and pup survival on PND 4, 14, and 21. On histopathologic evaluation of brain, spinal cord, and sciatic, tibial, and plantar nerves *(111)*, acrylamide-associated changes were confined to scattered nerve fiber degeneration in the sciatic and optic nerves. Details on the incidence and severity of neurological lesions were not provided, preventing interpretation of these findings.

Of some interest was a study by Walden et al. *(112)* in which the activity of five intestinal enzymes was evaluated in the offspring of acrylamide-treated Sprague-Dawley rats. Animals were treated from GD 6–15 with acrylamide 20 mg/kg/day or water by gavage. There were no differences in maternal body weight, or litter averages for pup number, weight, or sex ratio. The Panel found that the data were suggestive of changes in alkaline phosphatase and perhaps a developmental effect on PND 21 on β-glucoronidase. The meaning of such changes to animal status and well-being was not clear.

Two studies addressed a possible relationship between genotoxicity and developmental toxicity. [2] Rutledge et al. *(114)* treated female mice with acrylamide125 mg/kg **[probably i.p.]**1, 6, 9, or 25 h after mating. The intervals represented fertilization, the early pronuclear stage, pronuclear DNA synthesis, and the 2-cell stage, respectively. On GD 17 the uteri were inspected for resorptions, embryonic death, and live fetuses. Live fetuses were inspected for 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 h after mating. In spite of the lack of important details in the paper and a discrepancy between text and table in reporting of the results, this study shows that a single high dose of acrylamide can produce very early embryo death and malformation, presumably through a mutagenic mechanism. Holland et al. *(2)* evaluated morphologic abnormalities in preimplantation embryos conceived at fixed intervals after treatment of male C57Bl/6J mice with acrylamide. This study included evaluation of chromatin adducts and dominant lethality associated with acrylamide treatment, discussed in section 2.3. This study was limited by a combining of single-cell forms, which may have represented both unfertilized oocytes and fertilized eggs/zygotes and by the reporting of data that did not permit ready dose-response modeling. In spite of these drawbacks, the data permitted the conclusion that exposure of spermatids or spermatozoa to acrylamide can

[2] The Expert Panel notes that there are a number of genotoxicity studies with end points that might be considered developmental (e.g., abnormality of conceptuses after parental treatment). Studies that were designed to evaluate genotoxicity were grouped in section 2. These studies include those with dominant lethal (section 2.3.2.3), heritable translocation (section 2.3.2.4), and specific locus mutation (section 2.3.2.5) end points. In addition, the mouse spot test (section 2.3.2.6) involves changes in the offspring after treatment of the pregnant animal, and could be construed as a developmental test. Although these studies are placed for organizational purposes under the heading of genotoxicity rather than developmental or reproductive toxicity, the Expert Panel considers these studies important in evaluating the reproductive and developmental effects of acrylamide.

result in abnormal preimplantation development. In an experiment using 0, 10, 25, and 50 mg/kg/day i.p. × 5 days, the LOAEL for acrylamide developmental effects was probably 25 mg/kg/day. Using the benchmark dose approach, the BMD10 was 27 mg/kg/day and the BMDL was 18 mg/kg/day.

Additional studies were limited in their utility. Edwards *(44)* used single-dose dietary exposures in a series of experiments in pregnant rats; however, the lack of experimental detail and small number of litters prevented this study from being useful. Neuhäuser-Klaus and Schmahl *(88)* performed what they called a teratogenicity study in conjunction with a mouse spot test (discussed in section 2.3). Acrylamide was given i.p. as a single dose or as 3 daily doses, a route and schedule that are not relevant to human exposures. Design and analysis issues prevented this study from being useful in the evaluation, although the findings generally supported other studies. A study by Agrawal and Squibb *(115)* using acrylamide 20 mg/kg/day by gavage from GD 7–16 was suggestive of transient changes in the postnatal developing dopaminergic system, although this study did not describe several aspects of methodology and analysis clearly enough to permit any certainty in its interpretation. A study by Husain et al. *(104)*, and a study by Friedman et al. *(118)* addressed lactation effects of acrylamide 25 mg/kg/day by gavage. Toxicity in dams and pups was prominent and there was no way to separate maternal from developmental findings, or direct from indirect (maternally mediated) developmental toxicity.

The Expert Panel found no data with which to evaluate possible human developmental toxicity of acrylamide. Data are sufficient to conclude that acrylamide can produce developmental toxicity in rats manifested as a decrease in pup weight with maternal drinking water or gavage doses of approximately 4–5 mg/kg/day. The Expert Panel noted that a well-conducted gavage study and a dietary study failed to find this adverse effect at maternal doses of close to 4 mg/kg/day and 15 mg/kg/day, respectively; no explanation for the discrepant findings among studies was apparent. **The Expert Panel concluded that acrylamide can produce developmental neurotoxicity in rats at maternal gavage doses of 15 mg/kg/day. The Expert Panel concluded that acrylamide can produce developmental toxicity in mice as manifest by decreased fetal weight per litter at maternal gavage doses of 45 mg/kg/day. The Expert Panel was unable to separate the effects of acrylamide on rat or mouse offspring from effects that may have been due to maternal toxicity. The Expert Panel concludes that acrylamide treatment of male mice prior to mating can result in developmental toxicity manifested as abnormal preimplantation embryos.** The data are not sufficient to determine the lowest dose at which such an effect might occur, although that dose appears to be in the range of 10–25 mg/kg/day i.p. for five days. The data also are not sufficient to predict whether this male-mediated effect would be manifested as embryonic death (analogous to dominant lethality), fetal malformation, or other manifestations of altered development. The rat and mouse data are assumed relevant to humans.

1 Table 26. Key developmental studies

2

Species/strain	Exposures	Maternal/paternal effect level	Critical developmental effects	Developmental effect level	Reference
Female treatment					
Long-Evans rat	Drinking water 0, 25, 50, 100 ppm, 2 weeks prior to mating through weaning of litter	LOAEL = 50 ppm (6–9 mg/kg/day) (decreased fluid intake) NOAEL = 25 ppm (4 mg/kg/day)	Decreased pup weight	LOAEL = 25 ppm (lowest tested level) (4 mg/kg/day)	Zenick et al. *(78)*
			Decreased litter size	**[BMD_{10} = 98 ppm (10–14 mg/kg/day); BMDL = 40 ppm (4–7 mg/kg/day)]**	
Sprague-Dawley rat	Gavage 0, 2.5, 7.5, 15 mg/kg/day GD 6–20	LOAEL = 7.5 mg/kg/day (decreased body weight gain) NOAEL = 2.5 mg/kg/day	No adverse developmental effects	NOAEL = 15 mg/kg/day (the highest tested dose)	Field *(113)*
Sprague-Dawley rat	Dietary 0, 25, 50 ppm, 2 weeks prior to mating and GD 0–19	LOAEL = 50 ppm (3.45–3.82 mg/kg/day) (decreased body weight gain) NOAEL = 25 ppm (1.75–1.90 mg/kg/day)	No adverse developmental effects (full teratology evaluation not performed)	NOAEL = 50 ppm (3.45–3.82 mg/kg/day)	BIO/DYNAMICS-INC *(110)*
Sprague-Dawley rat	Gavage 0, 5, 10, 15, 20 mg/kg/day, GD 6–PND 10	LOAEL = 10 mg/kg/day (decreased body weight gain in lactation period) NOAEL = 5 mg/kg/day	Transient decrease in female pup weight	Borderline NOAEL/LOAEL = 5 mg/kg/day	Wise *(116)*
			Behavioral testing, brain weight	LOAEL = 15 mg/kg/day NOAEL = 10 mg/kg/day **[BMD_{10} (brain weight) 11 mg/kg/day; BMDL 8 mg/kg/day]**	
Swiss CD-1 mouse	Gavage 0, 3, 15, 45 mg/kg/day, GD 6–17	LOAEL = 45 mg/kg/day (decreased body weight gain) NOAEL = 15 mg/kg/day	Decreased fetal weight per litter	LOAEL = 45 mg/kg/day NOAEL = 15 mg/kg/day **[BMD_{10} 42–44 mg/kg/day; BMDL 32–36 mg/kg/day]**	Field *(113)*
Male treatment					
C57Bl/6J mouse	i.p. 0, 10, 25, 50 mg/kg/day × 5 days	Death in 10% of males at 50 mg/kg/day; other parameters of toxicity not reported	Abnormal preimplantation embryos	LOAEL possibly 25 mg/kg/day; BMD_{10} = 27 mg/kg/day, BMDL = 18 mg/kg/day	Holland *(2)*

LOAEL lowest observed adverse effect level; NOAEL no observed adverse effect level; BMD_{10} Exposure level associated with a 10% response, estimated from a mathematical dose–response model. BMDL Dose associated with the lower bound of the 95% confidence interval around the BMD_{10}. Benchmark doses calculated by CERHR where underlying data permitted modeling.

4.0 REPRODUCTIVE TOXICITY DATA

4.1 Human Data

No human data on acrylamide reproductive effects were located.

4.2 Experimental Animal Data

4.2.1. Female reproduction

In a study by Sakamoto and Hashimoto *(119)*, 24 female ddY mice were exposed to a single concentration of acrylamide in drinking water (1.2 mM, **[85.2 mg/L; 16.1 mg/kg/day based on mean body weight given in the paper and mean water consumption]**) for 4 weeks, then mated (1 male to 3 females) for up to 8 days with an untreated male of the same strain. Half the pregnant mice were killed on GD 13 **[plug day unspecified]** and uterine contents evaluated. The other half were permitted to deliver and rear their young, with observations of weight and behavior for four weeks. **[It is not stated whether there was acrylamide in the drinking water during the cohabitation period or whether pregnant animals were exposed to acrylamide].** Statistical testing was performed using the Fisher exact test or ANOVA followed by Duncan's multiple comparison test. **[It appears from the tables that the treated females were the statistical unit of analysis].** There was said to be a "slight but significant" increase in the number of resorptions per dam at day 13 in the acrylamide-exposed group (1.9 ± 1.5 **[presumed mean ± SD]**) compared to the control (0.1 ± 0.3). The offspring of females that were allowed to litter and raise their young were said not to differ by treatment group with respect to weight gain or behavior over the first four weeks of life **[no data were shown]**.

Strengths/Weaknesses: Weaknesses: a real paucity of specifics about study conduct and findings significantly limits the ability of the Panel to use this study. The study showed that this exposure scenario was not overtly toxic to the dams, and did not cause massive full resorption, but even these tentative conclusions are clouded by the lack of detail.

Utility (Adequacy) for CERHR Evaluation Process: This study was considered to be inadequate for use in the Evaluative Process.

In a study sponsored by the U.S. EPA, Zenick et al. *(78)* conducted a female reproduction study in which acrylamide was given in drinking water to female rats at 0, 25, 50, or 100 ppm. After two weeks of treatment, untreated males were placed overnight with the females for up to 7 days. Untreated water was made available during mating but acrylamide-treatment in drinking water was resumed during pregnancy and lactation. Dams delivered their young and litters were culled to 4 males and 4 females on PND 4. At the end of the lactation period, litters were culled to 2 males and 2 females. End points included maternal weight gain, mating performance, pregnancy rate, pup survival and weight, and day of vaginal patency in female offspring. Neurologic toxicity (hindlimb splay) was seen in dams given 100 ppm acrylamide. Dam body weight and fluid intake were reduced at several time points in the animals given 50 and 100 ppm acrylamide. There were 2 of 15 dams in the 100 ppm group with full or nearly full litter loss. Pup weight was decreased in a dose-dependent manner in the 50 and 100 ppm groups and was transiently depressed in the first week of life in the 25 ppm group. Vaginal opening in female offspring was delayed 2.8 days in the high dose group. A regression analysis performed without the 100 ppm group, due to incapacity of the dams, showed a significant effect of cumulative acrylamide intake on litter weaning weight. This study included considerable information on developmental effects and was discussed in detail in Section 3.2.

Strengths/Weaknesses: Strengths: This study was reasonably comprehensive in that it covered a reasonable pre-mating duration, gestation, and lactation. The Panel has confidence in the quality of the data. Weaknesses: The duration of treatment is not clear, and there was no clear calculation of the dose received by the animals, which is compounded by the fact that dose surely changing dramatically as the animals underwent huge changes in water requirements and body weight. It is not clear just how many animals delivered at each dose. The study would have benefitted from a histologic examination of the treated dams to uncover any pre-functional changes or changes in ovarian dynamics that would only show up several litters into the future. Also missing was a histologic exam of the pups.

Utility (Adequacy) for CERHR Evaluation Process: These data are adequate to conclude that acrylamide exposure at approximately these dose levels showed limited full-litter-loss at the highest concentration (so, no graded reduction in litter size). The full-litter loss and the decrements in pup weight are interpreted by the Expert Panel as developmental toxicity rather than female reproductive toxicivity. While not perfect, this paper contributes to the perception that female reproduction is not vulnerable to doses of acrylamide up to 100 ppm in drinking water.

In a summary paper, Bishop et al. *(120*) reported tests of female "total reproductive capacity" involving 29 chemicals tested over a ten-year period. Female mice were treated with a single intraperitoneal dose of acrylamide **[purity not stated]** in Hank's Balanced Salt Solution at 0 or 125 mg/kg. The following day, females were paired with males for approximately a 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 acrylamide study was not given]** and the total number of offpring produced was used to assess total reproductive capacity. For acrylamide, the female mice were F_1 hybrid SEC × C57BL6 and the males were F_1 hybrid C3H/R1 × C57BL10. There were no differences between the acrylamide and vehicle treated females in number of offspring/female (acrylamide 142.6, control 146.2) or number of litters/female (acrylamide 14.3, control 14.6). The paper lists 34 breeding pairs; it is assumed **[but not stated]** that this number refers to the acrylamide-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 7 animals. **[It is not stated that controls were run concurrently. Standard error or standard deviation are not given].**

Strengths/Weaknesses: The strengths of this paper lie primarily in the size of the effort that it reports: 29 chemicals from a variety of classes, though heavily laden with chemotherapeutics. One strength is that this type of design explicitly evaluates the fertility of future waves of oocytes, a strategy not commonly found in the literature. Weaknesses: Because this is a summary of a large number of studies, the specifics of the acrylamide study are neither available nor presented. Since the paper rightly focuses more space on those compounds that altered total reproductive capacity, it is difficult to ascertain the specifics of the acrylamide experiment, or whether there were any characteristics that might flag the results as unusual or give grounds for caution.

Utility (Adequacy) for CERHR Evaluation Process: This study explicitly evaluates future generations of oocytes, and is useful for evaluating genotoxic potential of compounds in female reproduction. The lack of specifics and details moderate our certainty that acrylamide produces no effect on female reproductive function.

4.2.2 Male reproduction

Shiraishi *(60)*, in a study supported by the Ministry of Education of Japan, treated male ddY mice with acrylamide **[purity not given]** in the diet or by i.p. injection. The purpose of the study was to evaluate chromosome aberrations (results given in **Table 11**); however, testicular weight data were also reported. Mice were 4 weeks old at the start of treatment, with n= 3 or 5 per group. The dietary group was given 500 ppm in the diet for either 1, 2, or 3 weeks before being killed**.** The authors also state that animals were killed 1, 2, or 3 weeks after the end of administration, although only 3 (and not 9) groups are shown in the aberration results. Because food consumption and daily body weights were not measured, the actual dose can only be estimated. **[1000 mg/kg/day based on 0.2 kg/kg food factor (EPA Biological Reference Values, 1988)]** The i.p. group was given 50 or 100 mg/kg and killed 11 or 12 days later. Details of testis harvest and weighing were not provided and statistical methods were not given. The authors state that testis weights were decreased in the dietary group after three weeks on the diet **[a statistically significant 32% reduction in testis weight was confirmed by CERHR using the Student *t*-test]**. The text states that there was a decrease in testis weight 11 and 12 days after i.p. injection of 100 mg/kg **[*t*-test by CERHR shows no significant difference]**, and indeed, the absolute weight of the treated testes remained the same, while the weight of the controls increased over the duration of the dosing period.

Strengths/Weaknesses: This study used mice and gave significant doses of acrylamide (up to half the LD_{50}. Weaknesses: (a) only a small number of animals was used; (b) there was no histology performed, and (c) no statistical treatment of the data. Thus, we can only infer that this exposure paradigm for acrylamide in mice did not cause massive early cell death, and little more.

Utility (Adequacy) for CERHR Evaluation Process: This study is of no utility for the evaluative process.

Hashimoto et al. *(121)* (funding source not stated) treated male mice of the ddY strain with 0 or 0.5 mmol/kg **[35.5 mg/kg]** acrylamide (>95% purity) by gavage twice/week for 8 weeks (n=6/group). This dose was selected as one-third of the LD_{50}. The mice underwent rotarod testing during the dosing period, after which they were killed and testes were harvested. Testes were fixed in 10% neutral formalin and 10-μm sections were stained with hematoxylin and eosin. Body weight was not affected by acrylamide treatment but relative testis weight was decreased 16% (control 0.36 ± 0.051, acrylamide 0.30 ± 0.016; $P < 0.05$). Histopathology was said to be adversely affected with degeneration of germ cells but sparing of Sertoli and interstitial cells **[this study used a number of acrylamide analogs, and histologic findings are summarized for all of them. Low-power photographs were provided but are not helpful in delineating the cell types affected]**. Neurotoxicity was apparent as evidenced by inability to maintain rotarod walking by the end of the treatment period.

An additional 7 animals per dose group were treated with phenobarbital 50 mg/kg/day i.p., 5 days/week for one week prior to and throughout a 10-week dosing period using acrylamide at 0 or 0.5 mmol/kg **[35.5 mg/kg]** twice/week. Both neurotoxicity and decreased relative testicular weight were prevented in acrylamide-treated mice by phenobarbital cotreatment, leading the authors to propose that the induction of hepatic microsomal enzymes had fostered the transformation of acrylamide to nontoxic metabolites. **[The authors report in the Discussion that "testicular damage was completely prevented" by phenobarbital, but it is not apparent from their Methods or Results section that histopathologic examination of the testes was performed in phenobarbital co-treated animals]**.

Strengths/Weaknesses: Strengths: (a) One strength is that the dosing was by gavage, thus more closely controlling the exposure; (b) testis architecture was evaluated, and (c) the epithelium was thinner and less populated in the acrylamide-treated mice, which is concordant with the reduced testis weight. Weaknesses: (a) This study used a small number of animals per group; (b) the fixative and other methods used conspire to produce uninterpretable histologic sections. While only small photomicrographs are presented, it is clear that if these are representative. The images cannot be used to support the conclusions the authors draw about the Sertoli or Leydig cells being unaffected; (c) since only a single time-point was evaluated, the authors cannot reach a conclusion about the target cell type; they can only conclude which cell populations appear to be present in reduced numbers, which is a different thing; (d) because only body and organ weight data are presented for the phenobarbital co-administration study, the authors over-interpret their data when they state in the Discussion that "The testicular damage was completely prevented by [phenobarbital]…" No cell counts were performed, and only a single duration of exposure was evaluated. Additionally, (e) the reader has no idea how the metabolite profile of acrylamide is changed by phenobarbital, which limits the confidence we can put in the conclusion of this experiment.

Utility (Adequacy) for CERHR Evaluation Process: This paper provides marginal improvements over previous papers, in that some histology is performed and reported, and the reported effects are consistent with the moderate weight reductions. This study is almost adequate to conclude that 35 mg/kg/d, twice/week for 8 weeks, reduces cellularity in murine testes.

Sakamoto et al. *(122*) administered acrylamide (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, and 10 days after dosing. Testes were fixed in Bouin's fluid for 1 h, cut, and then further fixed in formalin. Sections were stained with periodic acid-Schiff's 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 half the 30-day-old and 65% of the 60-day-old mice. In the prepubertal mice, body weight was significantly decreased at 1 and 5 days after dosing with 150 mg/kg acrylamide. The numeric values for mean body weight at 2 and 3 days after dosing were similar to the 1- and 5-day values, but the larger standard deviation prevented identification of statistical significance. In the adult mice, body weight was significantly reduced 1, 2, and 3 days after dosing with 150 mg/kg acrylamide. There were no significant alterations in testicular weight at either dose of acrylamide. There were no deaths and no significant effects on body weight at 100 mg/kg acrylamide in either age group. Histologic abnormalities in the testes of prepubertal animals treated with 150 mg/kg acrylamide appeared in spermatids, particularly round spermatids (Golgi and cap phase) one day after treatment. Nuclear vacuolization and swelling were the most common lesions in the spermatids. Degeneration of spermatocytes and spermatogonia were 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 acrylamide-induced cell death than did spermatids.

Strengths/Weaknesses: A significant strength of this paper is that it used appropriate methods to analyze testis microscopic structure. The single-dose paradigm effectively uncovered round spermatids as a vulnerable population, although it is still possible that these effects were mediated through initial changes in the Sertoli cell. A weakness is that a slightly lower dose was not also used, since as dose increases, the window of vulnerability opens wider, and more cells become

affected. A minor weakness is the modest number of animals per treatment group (n=4), which is only reported in a table footnote.

Utility (Adequacy) for CERHR Evaluation Process: This study has utility for the evaluative process in that it histologically identifies a vulnerable testis cell population in mice, and based on the methods used, the Expert Panel has reasonable confidence that this information is correct.

Lähdetie et al. *(67)* in a germ cell genotoxicity study sponsored by the Commission of European Communities used flow cytometry to characterize testicular cell population proportions 18 days after acrylamide **[purity not specified]** was administered i.p. at 50 or 100 mg/kg in Sprague-Dawley rats. Stage I tubules were isolated for evaluation. Two tubule segments at Stage I were planned from each testis (4 segments per male) of 5 or 6 males per dose group, compared to a saline-injected control. In actuality, 5 control males contributed 28 segments, and the 6 and 5 males of the 50 and 100-mg/kg acrylamide groups contributed 21 and 12 segments, respectively. ANOVA with post-hoc Tukey test was used. The number of cells at each DNA peak was said to be altered significantly only by 100 mg/kg acrylamide, which was associated with a 35% reduction in the number of stem cell spermatogonia **[CERHR performed an ANOVA with post-hoc Tukey test and identified a significant 30% reduction in stem cell spermatogonia at 50 mg/kg as well as 100 mg/kg]**.

Strengths/Weaknesses: Weaknesses: (a) a lack of detail of the methods used (it would be impossible to reproduce the studies here based on the methods given in this paper); (b) uncertainty whether the controls were evaluated for statistical difference before being pooled; (c) uncertainties surrounding the purity of the compound and composition of the dosing solution; (d) the certainty that the cell populations identified in their table of flow cytometry data are truly limited to those cells (i.e., there are many other 2C cells in the testis that could be present in the "stem cell spermatogonia" population); (e) no internal quality control indicators that the stages intended to be segregated were actually the ones recovered. Strengths: (a) the use of coded slides for the analysis; (b) direct analysis of the tissue of interest; (c) appropriate timing and intelligent design. The Panel's moderate confidence in these data is supported by the expertise and experience of the authors in performing these techniques, but reduced somewhat by the lack of methodologic detail and absence of data showing that the intended stages and cells were really the ones that were captured and analyzed. It is not clear that 18 or 19 days of treatment affects only the early spermatocytes, as this 19-day treatment period also targets the long meiotic prophase and meiosis itself. From these data, one may reasonably conclude that acrylamide causes micronuclei in male germ cells, although the precise cell type affected cannot be stated with absolute confidence.

Utility (Adequacy) for CERHR Evaluation Process:

Pacchierotti et al. *(84)* in a study supported by the Commission of the European Community administered acrylamide **[purity not given]** in HBSS i.p. to male B6C3F_1 mice at single doses of 0, 75, or 125 mg/kg, or 50 mg/kg/d × 5 doses for a total of 250 mg/kg. Males were mated to untreated females seven days after the last acrylamide treatment, following which subsets of the males were killed at 7, 14, 21, 28, and 35 days **[following the end of treatment]** for flow cytometric analysis of testicular cell populations (at least five mice per time sample per group). Additional mice were killed three days after the treatments (at least six per dose group), and additional groups of six mice each were given 100 and 150 mg/kg and killed 35 days later. Testes were minced, treated with pepsin, and filtered through a 37 µm-pore nylon mesh to produce single cell suspensions. Suspensions were fixed in ethanol and frozen at –20°C for up to

several weeks. For flow cytometry, DNA was stained with 4',6-diamidino-2-phenylindole (DAPI).

The mating portion of the study was used to generate zygotes for evaluation of chromosome aberrations (discussed in section 2.3.2.3). There was a decrease in percent mated females seven days after the last acrylamide treatment that was not dose-related. The percent mated (plug-positive) was 86.7, 57.1, 54.1, and 61.0 in the 0-, 75-, 125-, and 5 × 50-mg/kg/day groups, respectively. When males in the 125-mg/kg group were cohabited with females 28 days after treatment, 86.7% of females showed evidence of mating, a result identical to that in the control group. When metaphases were evaluated as part of the chromosome aberration portion of the study, among cells scored from mated females with 5 × 50-mg/kg/day consorts, there was a 31.7% incidence of second meiotic metaphases. In the other treatment groups and in the control, the incidence of second meiotic metaphases ranged from 0.5 to 1.6% of cells scored. This finding was taken as evidence that sperm from the high-dose males failed to fertilize these oocytes.

There were no changes in proportional testicular cell populations 2, 3, and 4 weeks after treatment **[data were not shown, and no comment was made concerning proportions 1 week after treatment. It is possible that the Methods section indication that males were killed "after mating at sequential time intervals (7, 14, 21, 28 and 35 days)" refers to time intervals after mating, and hence 14, 21, 28, 35, and 42 days after treatment. If so, the 42-day data are missing].** Data are reported for the 3 and 35-day post-treatment time points. Statistically significant findings at 3 days include about a 25% decrease in 4C cells (said to represent primary spermatocytes and G_2 spermatogonia) after 75 and 125 mg/kg acrylamide, a 50% increase in elongating/elongated diploid spermatids after 5 × 50 mg/kg/day acrylamide, and a 10–11% increase in elongated and round spermatids after 125 mg/kg acrylamide. Statistically significant findings 35 days after treatment with 100, 125, or 150 mg/kg acrylamide included a 30% decrease in elongated spermatids at the top dose and about a 50–100% increase (not dose-related) in diploid spermatids at all doses. This latter finding caused the authors to speculate that acrylamide impairs chromosome segregation during mitosis in spermatogonia. **[The Expert Panel finds this to be the only plausible explanation]**.

Strengths/Weaknesses: Strengths of this paper are: (a) the expertise of the authors; (b) the appropriate methods and statistics; (c) the concordance between these findings and others reported on this issue. Weaknesses include (a) some inconsistencies in reporting the data (i.e., where are the 42 day data?); and (b) the fact that the reductions that are seen at day 3 are not also present at higher doses and longer times.

Utility (Adequacy) for CERHR Evaluation Process: These data are adequate for the evaluative process, and sufficient to conclude that there are transient genotoxic effects that occur in specific cell populations in the testis. The confidence in this conclusion is only slightly reduced by the ad hoc nature of some of the doses and experiments, and by occasional data gaps. The chromosomal aberrations data at the high dose (125 mg/kg, 7 days) are supportive of other dominant lethal data *(1, 74*), and of an effect on fertility *(73*), as evidenced by the increase in unfertilized eggs. Like many acute genetic toxicology studies for this compound, the dosing paradigm does not lend itself to extrapolation to long-term exposures, but the effects described are consistent with other reports in the literature, and provide some mechanistic underpinnings for some of these other effects.

Costa et al. *(123*) examined the role of acrylamide and glycidamide in causing reproductive and neurotoxic effects in male Sprague-Dawley rats. The study was conducted at the University of Washington but sponsorship is not known. In the reproductive toxicity portion of the study, 8

sexually mature rats/group (350 g) were treated with 50 mg/kg bw/day acrylamide **[purity not specified]** for 7 days or 50 mg/kg bw/day glycidamide for 14 days. A control group was treated with the distilled water vehicle. **[Based on the protocol description for the neurotoxicity portion of this study, it appears that rats were treated by the i.p. route.]** The rats were examined for reproductive organ weight, testicular protein content, and sperm count and viability. Statistical significance of results was analyzed by one-way ANOVA followed by Fischer exact test for comparison between groups. Body weights of the glycidamide-treated rats were significantly lower compared to controls but were not different from the acrylamide-treated rats. Neither compound affected testicular weight but glycidamide significantly reduced epididymal weight. Treatment with glycidamide also resulted in significant reductions in testis protein content, sperm count, and sperm viability. **[Although the results section states that acrylamide treatment did not result in any of these effects on testes or sperm, Figure 3 in the study indicates that sperm counts were significantly reduced in the acrylamide treated rats.]** Results were compared to the neurotoxicity test in which a separate group of rats were i.p. injected with acrylamide or glycidamide for eight days. Results included impaired rotarod performance at 50 mg/kg bw/day acrylamide and 100 mg/kg bw/day glycidamide and hindlimb splay at ≥25 mg/kg bw/day acrylamide. The study authors concluded that glycidamide is involved in reproductive toxicity but not neurotoxicity associated with acrylamide exposure.

Strengths/Weaknesses: Weaknesses: (a) the doses of glycidamide were not based on any sort of molar conversion from acrylamide; molar equivalence would make the data more readily interpretable; (b) there is no rationale in this paper for the measures of reproductive "toxicity" that were chosen, and the relationship of protein content to toxicity is loose and unspecified, and there is no precedent in the literature for showing that acrylamide reduces sperm viability; (c) it would have been insightful if the authors had measured glycidamide levels in the neurons, reproductive organs, and hemoglobin of all animals in this study, which would have allowed a more meaningful link between exposure and effect to be drawn; (d) no histopathology was examined; (e) there is no indication that these durations of exposure were sufficient to show an effect on testis weight if one were to occur at these doses; (f) insufficient specifics are provided on the methods of analysis for the protein and viability; (g) the text and figures are inconsistent in reporting sperm count effects. Strengths: the concept is interesting, and the statistics appear to be appropriate.

Utility (Adequacy) for CERHR Evaluation Process: From these data, we can conclude that glycidamide produces some effect on the testis and on sperm content in the vas, but we are uncertain of the nature and degree of these effects. These data are not very useful for the Evaluative Process, due to the superficial nature of the exploration, the uncertainty of the relationship between what was measured and what was concluded, the apparent lack of correlation between the chosen doses and times for acrylamide and glycidamide, and the lack of histology.

Marchetti et al. *(3*), supported by DOE and NIEHS) gave acrylamide **[purity not specified]** to male B6C3F1 mice at 50 mg/kg/day for 5 consecutive days as part of a study to assess chromosome exchanges in the male pronucleus (discussed in section 2.3.2.3). This study was performed to assess a new method of detecting multiple cytogenetic abnormalities, and was not intended to be used to identify a lowest effective dose. This purpose also led to unique designs, and a recognition that some details important in Guideline-driven studies were not a focus here (such as details of numbers of animals, or analysis of dosing solutions). Mice were mated with untreated females of the same strain at 2.5, 6.5, 9.5, 12.5, 20.5, 27.5, 41.5, and 48.5 days after the last acrylamide injection, to produce fertilization by sperm that had been exposed to acrylamide at the epididymal spermatozoon, early spermatozoon, elongated spermatid, mid-spermatid, round

spermatid, pachytene spermatocyte, differentiating spermatogonium, and stem cell stages, respectively. Females were superovulated with pregnant mare's serum followed by hCG, then mated. Plugged females were given colchicine 24 h after hCG to arrest zygote development at metaphase of the first cleavage. Females were killed 6 h after colchicine and zygotes harvested. The proportion of fertilized eggs (of all eggs harvested) was decreased by treatment at all time points except 48.5 days prior to mating. The proportion of zygotes (of all fertilized eggs) was decreased in the 2.5–12.5 day period, that is, during the mid-spermatid through epididymal spermatozoon stage, which the authors point out as repair-deficient stages. **[The proportions were analyzed by chi-square, which takes the harvested cell as the statistical unit. The sire of origin was not indicated; in fact, the number of males is not stated except that matings were 1:1 and "[z]ygotes harvested from 10–15 females were pooled…"]**.

Strengths/Weaknesses: Strengths are: (a) the expertise and experience of the authors in these techniques; (b) other data in this study, which provide information about mechanism and vulnerable stage of spermatogenesis; (c) appropriate statistics. Weaknesses are: (a) unique design does not allow for usual determination of effects on specific males (which was, in fact, quite beside the point of the paper); and (b) lack of analysis of the dosing solution or neat agent.

Utility (Adequacy) for CERHR Evaluation Process: From a reproductive perspective, this study adds to our understanding of precisely which stages of germ cell development are affected by 50 mg/kg/day × 5 day exposure to acrylamide. It confirms that fertilization is a vulnerable process, and that testicular spermatids are a vulnerable stage, and adds a cell-cycle-delay effect not previously identified.

Adler et al. *(77)* (financial support not stated) treated male mice with acrylamide **[purity not given]** 125 mg/kg i.p. with or without 1-aminobenzotriazole, an inhibitor of hepatic and renal P-450. The 1-aminobenzotriazole was given at 50 mg/kg i.p. daily for three days with acrylamide given on the fourth day. Control animals for each treatment received vehicle, which was saline for 1-aminobenzotriazole and double-distilled water for acrylamide, and an additional control group received daily injections of saline for three days and an injection of double-distilled water on the fourth day. The inhibition of P-450 was believed to prevent metabolism of acrylamide to glycidamide. Two dominant lethal studies were performed using (102/E1 × C3H/E1)F_1 males and females. The dominant lethal results are summarized in Table 12. The second dominant lethal study included four males per group from which epididymides were obtained 1 week after completion of the 4-day treatment regimen. Both caudae were incised and sperm allowed to diffuse or swim out in fetal calf serum for 1 h. Sperm concentration was determined using a hemocytometer. Motility was estimated by light microscopy as percent fast, slow, non-progresssive, and "immobile". Morphology was also determined **[method of determination not given except by reference to the 1992 WHO manual, which is a manual for evaluation of human sperm]**. Sperm concentration and percent normal forms were not affected by any treatment. Motility was decreased by acrylamide with the mean percentage of immotile sperm increasing from 38.8 ± 8.9 in the control to 76.8 ± 4.2 in the acrylamide-treated group. Percent immotile sperm in the two groups given 1-aminobenzotriazole were intermediate, without a significant impact of acrylamide (1-aminobenzotriazole + acrylamide: 66.3 ± 4.2%; 1-aminobenzotriazole + water: 60.0 ± 10.0%; *P* NS).

Strengths/Weaknesses: Strengths are: (a) the study (and results) were repeated, which gives significant credibility to the results; (b) the data are provided in detail in extensive tables; (c) sperm data were collected from some males, to eliminate (or simply explore) possible effects on sperm count or motility; (d) there was a sufficient number of animals used. Weaknesses are: (a) there was no independent determination that 1-aminobenzotriazole actually inhibited P450

activity in these animals, and there was no measuresment of glycidamide levels in the acrylamide-and-1-aminobenzotriazole-treated mice, so one does not know that the intended mechanism was truly at work. Also, there is no reference to support using 1-aminobenzotriazole for such an effect; (b) inappropriate statistics seem to have been performed; (c) the figures are not clear, lack any indication of variance, and are very poorly described; (d) the sperm methods are unique and described in insufficient detail (i.e., no criteria are given for 'fast' or 'slow' sperm, or for malformed sperm; this study cannot be repreated using only this description);

Utility (Adequacy) for CERHR Evaluation Process: If the P450 inhibition by 1-aminobenztriazole is taken at face value, and if we assume that such an inhibition occurred in these animals, then we have an insight into mechanism and into the active metabolite that is responsible for the dominant lethality in mice. Together with data from Hashimoto et al. (who showed lower toxicity after pre-administration of phenobarbital) and Costa et al. (who dosed with glycidamide and found effects on testis weight and protein levels), we have the implication of metabolism. At face value, it would seem that these Hashimoto et al. and Adler et al. are contradictory. However, since neither study confirmed the alterations in P450 metabolic profiles, we can only assume that metabolism is important. Indeed, all three studies leave us with only the imputed involvement of glycidamide as the active intermediate; this hypothesis awaits a rigorous proof. Once proven in animals, glycidamide could become a biomarker of exposure in humans, with consequent increased confidence that toxicity is or is not likely in humans given exposure to X amount of acrylamide (which produced Y amount of glycidamide). The reduced proportion of "fast" sperm is not consistent with the increased beat/cross frequency noted by Tyl et al. (discussed below), although this inconsistency might be partially explained by the subjectivity of the current measurement methods and the different species involved. Invoking sperm tail motor proteins as a target for acrylamide (or glycidamide) is premature, since the effect was not demonstrated in sperm treated in vitro. While this effect could be due to effects on the epididymis, prostate, seminal vesicles, or coagulating gland, a direct effect on sperm is admittedly reasonable, given the timing of exposure and measurement. This is assuming that an effect truly exists, which is uncertain, given these methods. These data imply that metabolism is important, but these data alone do not prove it.

Sakamoto and Hashimoto *(119)* gave male mice (ddY strain) acrylamide in drinking water at 0.3, 0.6, 0.9, and 1.2 mM **[21.3, 42.6, 64.0, and 85.2 mg/L, respectively]**. Water intake was not influenced by treatment and the mean water consumption in the top dose group was reported as 6.2 g/animal/day. **[Using the mean body weight at the end of the dosing period in the top dose group (35.4 g), water consumption would have averaged 214 mL/kg/d. The drinking water concentrations would produce acrylamide doses of 4.6, 9.1, 13.7, and 18.2 mg/kg/day.]** There were 9 males each in the three lowest acrylamide doses and 14 males each in the control and high dose groups. Animals in the top dose group were described as having "very slight hindlimb weakness." Males were given the treated water for 4 weeks, following which half the males in each group were mated 1:3 for up to 8 days with untreated females of the same strain. **[It is not stated whether there was acrylamide in the drinking water during the cohabitation period].** Half the pregnant mice were killed on GD 13 **[plug day unspecified]** and uterine contents evaluated. The other half were permitted to deliver and rear their young, with observations of body weight and behavior for four weeks. The remaining males were killed at the end of the treatment period and used for evaluation of liver and reproductive organ weight and evaluation of epididymal sperm, obtained by mincing the tissue in 10% neutral buffered formalin. Sperm concentration was evaluated in a hemocytometer and morphology was evaluated in Eosin Y-stained smears. Statistical testing was performed using the Fisher exact test or ANOVA followed by Duncan's multiple comparison test. **[It appears from the tables that the treated male was the statistical unit of analysis].** Results of these studies are summarized in Table 27

[with calculations of BMD_{10} and BMDL values where the underlying data permitted. The number of males was not given for each determination. Since males were said to have been mated 1:3 with females, the number of females divided by 3 was taken as the number of males for benchmark dose calculations. There appeared to have been 3 or 4 males used per dose group in the mating studies]. The highest two concentrations of acrylamide appeared active with dose-related decreases in the number of fetuses/dam. Other reproductive end points were adversely affected at the high dose. The offspring of females that were allowed to litter and raise their young were said not to differ by treatment group with respect to weight gain or behavior over the first four weeks of life **[no data were shown]**.

Strengths/Weaknesses: This study used mice, a species relatively resistant to neurologic damage. The study is weakened by a lack of detail about exposure levels. The unusual method of preparing sperm for analysis is a minor weakness (as mincing can mis-shape the sperm, and formalin fixation of the epididymis during sperm extraction might reduce the efficiency of extraction, although this reduced efficiency would apply to all groups). Other minor weaknesses are the modest number of animals and the lack of histopathology (although histopathology was evaluated and reported in a previous study). A strength is allowing half the high-dose animals to deliver their young; a weakness is that this procedure reduced the number of animals available for any analysis, and details of the observations made on the young are omitted.

Utility (Adequacy) for CERHR Evaluation Process: These data are adequate to allow a conclusion that in mice, 4-6 weeks of exposure to about 13 mg/kg/d acrylamide reduces litter size from treated males, while about 18 mg/kg/d also reduces the impregnation rate and sperm count, and increases resorptions. Using the benchmark dose approach and the authors' estimates of acrylamide intake at the stated water concentrations, the 10% effect level may be as low as 3 mg/kg/day for resorptions/dam after paternal treatment. Our certainty about the strength of these effects and the effective doses is reduced because of the relatively small numbers of animals examined.

Table 27. Pregnancy Outcome in Females Mated to Male ddY Mice After Exposure of Males to Acrylamide in Drinking Water for Four Weeks. From Sakamoto and Hashimoto *(119)*

	Concentration of acrylamide in drinking water (mM) **[mg/L]**			
	0.3 **[21.3]**	0.6 **[42.6]**	0.9 **[64.0]**	1.2 **[85.2]**
Estimated dose (mg/kg/day)	4.6	9.1	13.7	18.2
Pregnant/mated females (GD 13)	↔	↔	↔	↓67%
Fetuses/dam (GD 13)	↔	↔	↓31%	↓78%
BMD_{10} = 0.6 mM **[45 mg/L]**				
BMDL = 0.3 mM **[22 mg/L]**				
Resorptions/dam (GD 13)[a]	↔	↔	↔	↑10-fold
BMD_{10} = 0.2 mM **[14 mg/L]**				
BMDL = 0.04 mM **[3 mg/L]**				
Pregnant/mated females (at term)	not reported	not reported	not reported	↓60%
Offspring/dam (at term)	not reported	not reported	not reported	↓67%
Offspring birth weight	not reported	not reported	not reported	↔
Relative liver weight	↔	↔	↔	↔
Relative testis weight	↔	↔	↔	↔
Relative seminal vesicle weight	↔	↔	↔	↔
Epididymal sperm				
Count (per g epididymis)	↔	↑1.2-fold	↑1.3-fold	↓35%
BMD_{10} = 1.1 mM **[78 mg/L]**				
BMDL = 1.0 mM **[71 mg/L]**				
Percent abnormal forms	↔	↔	↔	↑2.2-fold
BMD_{10} = 0.9 mM **[61 mg/L]**				
BMDL = 0.1 mM **[8 mg/L]**				

↑,↓ Statistically significant increase, decrease compared to control. ↔ Not statistically different from control.
[a]Standard deviations were larger than means, suggesting that ANOVA was not the preferred statistical option.
BMD_{10} Exposure level associated with a 10% response, estimated from a mathematical dose–response model.
BMDL Dose associated with the lower bound of the 95% confidence interval around the BMD_{10}.

In a study supported by the US EPA, Zenick et al. *(78)* gave acrylamide **[purity not specified]** in drinking water to male Long-Evans hooded rats at 0, 50, 100, or 200 ppm for up to 10 weeks **[based on graphs of mean water intake and body weight, CERHR estimates the mean acrylamide intake at the beginning of exposure to have been about 5, 7, and 12 mg/kg/day, and at the end of the study (10 weeks in the 50 and 100 ppm groups, 6 week in the 200 ppm group) to have been 5, 8, and 12 mg/kg/day in the low, medium, and high exposure groups, respectively. The authors assessed cumulative acrylamide intake as 544 and 547 mg/kg in the 100 and 200 ppm groups. Assuming the cumulative intake refers to intake over 10 and 6 weeks, respectively, for these two dose groups, the mean acrylamide intake over the study period was 7.8 and 13.0 mg/kg/day in the middle and high dose groups]**. Males (70 days old) were acclimated to a reversed 14:10 light:dark photoperiod with lights on at 10 pm and were exposed to ovariectomized hormonally primed females for mating experience. This acclimation period lasted three weeks. During the study period, a female was presented each week. Mating was monitored visually every two weeks. At baseline and during exposure week 9, females were killed after mating and the ejaculate was recovered from the genital tract **[the method of recovery was not given]**. The recovered ejaculate was evaluated for copulatory plug weight and sperm count, motility, and morphology. Assignment to exposure group was balanced for baseline body weight, sperm count, and latency to ejaculation. During week 10, males in the 0 and 100 ppm groups were mated with intact estrous females. Females were killed on GD 17 **[plug day**

not specified, but in another experiment in this paper, plug day was GD 1]. Fetuses and implantations were counted **[staining for implantations is not mentioned].** Males were killed at week 11 for histologic evaluation of one testis and epididymal fluid **[fixed in Bouin's fluid, stain not indicated].** The other testis was homogenized for spermatid count and the other epididymis was minced for sperm count **[detailed methods not given]**. Repeated measures ANOVA was used for most analyses; ratios for fertility and post-implantation loss were evaluated by chi-square. Duncan's post-hoc test was used after some of the ANOVAs. The unit of analysis appears to have been the male or the female **[based on the number of females reported in the tables, it appears that mating was 1:1, making the female equivalent to the male as the statistical unit for mating data]**.

The data tables show 14 males in the 0 ppm group, although initial group size was 15. In the 200 ppm group, hind-limb splaying occurred by week 4. Three of 15 males in this group died or were killed in moribund condition by week 5 and the remaining 12 males were killed at week 6. The 200-ppm males showed a decrease in body weight and water intake compared to the 0-ppm controls. There was less severe hind-limb splaying in "some" of the 100-ppm males. Body weight was numerically lower at all time points in the 100-ppm males compared to controls but there were reportedly no significant differences compared to the 0-ppm controls **[standard errors or standard deviations were not shown]**.

Prior to the onset of hind-limb splaying, males in the 100- and 200-ppm groups showed an increased number of mounts during cohabitation with prepared females. The authors indicated that they could not tell if some of these mounts were incomplete intromissions. The authors also indicated an increase in mounts in the 50-ppm group during the last week of observation (week 9), although this putative increase was not statistically significant **[the square root transformation of mount data are shown without error bars]**. Intromissions were increased in the 200-ppm group at week 4 and in the 100-ppm group at week 9. Mount latency was not affected by treatment. During the final week of assessment (week 6 in the 200-ppm group and week 9 in the 100-ppm group), only 4/12 and 11/15 males in the high and middle-dose groups, respectively, ejaculated in the 30-minute mating period. Of those animals ejaculating, ejaculation latency was not affected by treatment. Among the 11 females mated with 100-ppm males that ejaculated, sperm were recovered from only one uterus **[the text says semen, but surely sperm is meant]**. Sperm were identified in all vaginae of these 11 females but sperm counts were significantly lower than in the vaginae of females mated with 0-ppm males ($14 \pm 20 \times 10^6$ vs. $56 \pm 18 \times 10^6$; $P \leq 0.01$). Motility and morphology could not be assessed in the vaginal sperm from females mated to males of the 100-ppm group; there was no effect of acrylamide treatment on these parameters in sperm from females mated to 50-ppm males. Copulatory plug weights were not different among females mated to 50- or 100-ppm males compared to controls. After cohabitation with intact estrous females, all 14 control and all 15 100-ppm males produced evidence of mating within 5 days; however, only 5/15 females (33%) mated to 100-ppm males showed evidence of pregnancy compared to 11/14 females (79%) mated to 0-ppm males ($P < 0.01$ according to the authors **[$P = 0.025$ by Fisher test performed by CERHR]**). Post-implantation loss in the females mated to 100-ppm males was $31.7 \pm 3.8\%$ compared to $8.0 \pm 1.1\%$ in females mated to 0-ppm males ($P < 0.01$). Individual post-implantation losses in the five females mated to 100-ppm males were 0, 21, 38, 50, and 50% **[which gives a mean ± SEM of 31.8 ± 9.6% in contrast to the mean and SEM given by the authors]**. By contrast, of the 11 pregnant females in the control group, two litters showed post-implantation losses above 10% and eight had 0 post-implantation losses **[these data are given in the Discussion section of the paper]**.

At week 6, the 12 surviving males from the 200-ppm treatment group were killed along with 3 untreated males from the same initial shipment. There were no differences between the treated and untreated males in body weight, absolute or relative organ weights, or sperm parameters **[data were not shown]**. At week 11, all remaining males were killed and comparisons were made between acrylamide treated groups and 0-ppm controls. There were no differences in body weight, organ weights (including separate prostate+seminal vesicle, vas deferens, and epididymis weights), sperm per gram cauda epididymis, and spermatids per g testis. Testicular histology was comparable in the 100- and 0-ppm groups. The authors interpreted the results as consistent with impaired fertility due to disturbances in copulation. They believed that the abnormalities of mounting and intromission may have reflected neurologic dysfunction, albeit prior to the appearance of hind-limb splay. The abnormal copulatory function on the part of the male may have resulted in insufficient stimulation of the female genital tract for normal facilitation of sperm transport into the uterus, accounting for uterine sperm in only one of the 11 females with vaginal sperm after mating with 100-ppm treated males, and pregnancy in only 33% of intact females successfully mated (by copulatory plug criteria) with males in the same dose group.

Strengths/Weaknesses: The thoroughness of the analysis of male reproductive function is a big strength for this paper, as are the larger numbers of animals used, and appropriate statistical analyses. Good histology is a strength, but the numerous cell counts are even better, and allow a good "dissection" of site of effect. Recovering ejaculate from the females' tract is a unique strength in that it assesses that which was delivered to the female, not what was captured in the male at necropsy, which may differ. While other explanations may account for the fertility differences they saw (such as impaired sperm discharge during ejaculation due to nerve damage), these other explanations do not reduce the fundamental strengths of this paper. Significant strengths are methods of testis fixation and cell counting on the contralateral side. Imprecision in exposure assessment is a weakness, as is imprecision in reporting the results (e.g., numbers of affected males, variance around the means).

Utility (Adequacy) for CERHR Evaluation Process: The uncertainties in exposure in this paper only moderately reduce the fundamental utility of this paper for the evaluative process. From this work, we can conclude that male rats consuming 7–8 mg/kg/d will have reduced fertility not because of a lesion in sperm production, but because of a defect in the delivery of sperm to the female. These data do not exclude the possibility that genetic damage is partly responsible for the reduction in fertility. These data show that a defect in delivery occurs at doses lower than those that affect spermatogenesis, leaving the conclusion that other reports of a lesion in spermatogenesis also imply an existing occult defect in delivery in those same animals.

Sublet et al. *(73)* in a study sponsored by the US EPA exposed male Long-Evans hooded rats to acrylamide (>99% pure). The study included a dominant lethal design plus an evaluation of mating and fertilization success to investigate what proportion of apparent dominant lethality and preimplantation loss might be attributable to impaired fertility as opposed to death of the conceptus prior to implantation. The dose range was selected to avoid neurotoxicity (hind limb incoordination). In the first study, males received acrylamide in distilled water by gavage at 0, 30, 45, or 60 mg/kg/day for 5 days and were mated during weeks 1, 2, 3, 4, 7, and 10 after treatment. In the second study, males received acrylamide in distilled water by gavage at 0, 5, or 15 mg/kg/day for 5 days and were mated during weeks 1–4. Sperm in the vaginal lavage of the female was taken as GD 0. Females were killed on GD 15. Corpora lutea, implantations, and live and dead fetuses were counted. Uteri that appeared nonpregnant were stained with 10% ammonium sulfide. The results, summarized in Table 12, showed an increase in pre- and post-implantation loss with mating 1–3 weeks after treatment of males with acrylamide 15 mg/kg/day or greater for five days.

Because previous work *(78)* suggested that subchronic acrylamide treatment of male rats produced copulatory disturbances and impaired transport of sperm to the uterine lumen, the current study evaluated similar parameters after the acute, 5-day gavage exposure. For this study, male rats were kept in a 14:10 reverse light:dark cycle with lights off at 10 am and were treated with acrylamide 0, 15, or 45 mg/kg/day for 5 days by gavage (n=10 males/dose group). Hormonally-primed ovariectomized females were introduced for mating beginning two days after the last acrylamide dose and continuing for 4 weeks. Each female was killed 15 minutes after ejaculation **[the Results section says 10–15 minutes]** and the uterus and vagina opened at laparotomy. Fluid was aspirated from the uterus and examined for sperm by light microscopy **[Microscopy of vaginal fluid is not described but must also have occurred given that results are given for vaginal sperm]**. No abnormalities of male copulatory behavior were observed at any dose **[data not shown]**. All females had sperm in the vagina at all time points after treatment of males with any dose of acrylamide. One week after treatment of males with vehicle, 10/10 females had sperm in the uterus. Comparable figures after treatment of males with 15 mg/kg/day and 45 mg/kg/day were 6/10 and 2/10, the latter proportion being statistically different from the control. There were no differences in the proportions of females with uterine sperm 2, 3, or 4 weeks after treatment of males with either dose of acrylamide.

An additional 10 males per dose group were treated with acrylamide in distilled water at 0 or 45 mg/gk/day for five days by gavage and mated with hormonally-primed ovariectomized females each week for four weeks, as above. Females were killed 15 minutes after copulation and uterine fluid aspirated at laparotomy. Sperm concentration, percent motility, curvilinear and straight-line velocity, and linearity were evaluated using a computer-assisted sperm analysis system. There were no differences among these parameters at any time in any dose group except for a decrease in motile sperm and curvilinear velocity in females three weeks after treatment of males with acrylamide 45 mg/kg/day for five days (control motility 75%, acrylamide-treated motility 58%, $P < 0.05$; control curvilinear velocity 132.12 ± 7.02 μm/sec, acrylamide-treated curvilinear velocity 122.06 ± 3.32 μm/sec, $P < 0.05$) **[The Panel notes that there were 20 comparisons (5 sperm parameters at 4 time points each), without attention to a possible multiple comparison problem]**.

An estimate of fertilization was made in a final study in which males were treated with acrylamide in distilled water at 0, 15, or 45 mg/kg/day for five days by gavage. Two days after treatment and every week for a total of three weeks, males were housed with proestrous females from 2 pm until the following morning. Sperm on vaginal lavage was taken as evidence of pregnancy and females were killed at 10 am, about 10–14 h after the estimated time of mating. Ovaries, oviducts, and uteri were dissected and cumulus masses recovered from the oviducts. Hyaluronidase was used to disperse the cumulus cells and oocytes were viewed using Nomarski optics. The oocyte was assessed as having been fertilized if either a sperm head and tail or two pronuclei were seen within the oocyte. The experiment was replicated for week 3. The percent oocytes fertilized per female was reduced in a dose-dependent manner in Week 1 (0, 15, and 45 mg/kg/day fertilization rates were 84, 41, and 29%, respectively in 14, 13, and 16 females, respectively). A decrease in fertilization was also seen during week 3 in the 45 mg/kg/day group (control 65% (n=8), treated 12% (n=15) in the first replicate; control 92%, treated 9% in the second replicate **[n not given]**).

The authors suggested that impaired fertilizing ability of acrylamide-exposed sperm may play a role in the apparent preimplantation loss rate seen after treatment. They proposed that although copulatory behavior appeared normal in their experiments, and acrylamide doses were below those associated with gross neurologic impairment, there might be subtle abnormalities of

copulation resulting in a failure to deposit the ejaculate appropriately against the cervix or in a failure of adequate stimulation of the female to result in facilitation of sperm transport. They noted that in some instances, the implantation rates appeared higher than fertilization rates under the same conditions, and proposed that there might be an acrylamide-associated delay in fertilization such that at 10–14 h after the estimated time of mating, sperm might not yet have fertilized but would ultimately have done so.

Strengths/Weaknesses: Thoroughness of design and evaluation is a plus, as is the analysis of the acrylamide at the beginning of the experiment. Pilot studies and adequate numbers of animals are additional strengths, as are the sperm transport and fertilization studies. Indeed, the fact that this paper reports a series of related experiments that successively drill further down into a mechanism of effect makes it uniquely valuable. The consistency of the effects, and the inter-relatedness of the findings, give credence to the conclusions. Weakness would be inappropriate statistics for at least some of the end points, and the absence of histology in the treated males, to verify that the later fertility reduction might be due to reduced sperm output from the testis, secondary to a testis lesion similar to that seen by Sakamoto et al. The Panel has high confidence in the quality of these data.

Utility (Adequacy) for CERHR Evaluation Process: This study has significant value for the evaluative process in that it further dissects the fertilization and mating processes, and identifies these processes as likely targets of acrylamide action in rats at 15 and 45 mg/kg/d for 5 days of exposure.

Tyl et al. *(124)*, sponsored by the Acrylamide Monomer Producers Association, extended the study design of Sublet et al. *(73)* to include neurologic assessments. The stated purpose of the study was "to confirm the potential of oral (gavage) exposure o[f] male Long–Evans rats to acrylamide monomer for 5 days to produce reproductive and/or dominant lethal effects in the males detected by mating exposed males to unexposed females, and to determine whether there was a relationship between reproductive toxicity and neurotoxicity…"

The species, strain, and dosing parameters were identical to those used by Sublet et al. *(73)* and the study design matched the first week of the Sublet et al. *(73)* study. Virgin male Long–Evans rats were exposed by daily gavage (5 mL/kg) for five consecutive days to acrylamide (>99.7% pure) at 0, 5, 15, 30, 45, or 60 mg/kg/day. Dosing solutions were made in sterile distilled water and were 90–100% of target concentrations. There were 25 animals in each dose group. Males were 11 weeks old and were randomized by weight. On the third day after the last acrylamide dose, males were paired 1:1 with virgin proestrous or estrous female rats, also randomized by weight. Males and females were paired at 2 PM and females evaluated the next morning for vaginal sperm or a copulation plug. The morning of vaginal examination was designated GD 0.

Following the overnight mating, males underwent grip testing after which they were killed. Five males/group were perfusion-fixed with glutaraldehyde; the remaining 20 males/group were used for evaluation of cauda epididymal sperm, including computerized automated sperm analysis (CASA) parameters. Sciatic nerves from perfusion-fixed males were evaluated with hematoxylin and eosin and with Holm's Silver/Luxol Fast Blue (to differentiate axons and myelin sheath). Females were killed on GD 15 for evaluation of uterine contents including the status of each implantation. Uteri without apparent implantations were stained with 10% ammonium sulfide for evaluation of possible implantation sites. Females without evidence of successful mating were handled in a similar manner.

Statistical analysis of data meeting Bartlett's test for homogeneity of variances was performed by general linear model procedures to evaluate trends, with ANOVA followed by Dunnett's multiple comparison test for evaluating differences of acrylamide dose group data from control data. Nonparametric tests included the Kruskal-Wallis test followed by post-hoc Mann-Whitney *U* test, Jonckheere's test for dose-response trends of continuous data, chi-square (followed by Fisher test) for frequency data, and the Cochran-Armitage test for linear trend on proportions.

Data from this study are summarized in Table 28. Males receiving acrylamide at doses more than 5 mg/kg/day gained less weight or lost weight during the dosing interval. Some weight recovery occurred during the three-day postdosing interval, but overall weight gain during the study (Days 1–8) was less in males treated with acrylamide at 15 mg/kg/day or above. During the entire 8-day study period, males at the highest dose lost about 25 g compared to about a 30-g gain in weight in the controls **[estimated from figure].** Hind-limb grip strength was decreased in the top dose group but in no other groups, and there were no histologic alterations in the sciatic nerve at any dose. Reproductive data were presented in two ways: considering only confirmed-pregnant females as pregnant or, as in the study of Sublet et al. *(73*), considering sperm-positive females as pregnant. In the latter analysis, a mated female with no implantation sites would be considered to have 100% preimplantation loss, while in the former analysis, a mated female with no implantation sites would be considered nonpregnant, i.e., as having failed to undergo fertilization. The number of males that mated during the single overnight cohabitation was 16/25 in the control group and 9/24 in the 60 mg/kg/day acrylamide group. Among the control males, 13 produced a litter, while only 2 of the 60-mg/kg/day acrylamide-treated males produced a litter.

A decrease in live implants per litter and an increase in resorptions/litter were identified in the top two dose groups on pairwise statistical testing. In their discussion, the authors consider the 45 and 60 mg/kg/day doses to be effect levels based on the pairwise comparisons, although they write that 15 mg/kg/day and above were arguably active doses based on the trend testing results. There is no other discussion of the significance of trend testing; however, if the authors' data on live implants/sperm-positive female or post-implantation loss/litter are graphed using a linear model (Figure 8), it is not obvious that a NOAEL has been identified based on pairwise testing. Using these linear models, a benchmark dose approach is shown in Table 29.

The authors consider whether acrylamide is a "primary reproductive toxicant" or whether the reproductive toxicity was likely to have been due to systemic toxicity, specifically neurotoxicity. In one part of the discussion, the changes in body weight at 15 mg/kg/day and above were considered to be unrelated to the reproductive effects, based on a cited reference that indicated no adverse male reproductive effects with a 30% weight reduction from feed restriction. In another part of the discussion, the weight data are used to identify 15–60 mg/kg/day as being associated with acute systemic toxicity. The authors write, "In this study, statistically significant indicators of acute systemic toxicity occurred at 45 to 60 mg/kg/day, and statistically significant indicators of reproductive toxicity occurred at 45 to 60 mg/kg/day, and arguably at 15 to 30 mg/kg/day based on trend testing, which is at least consistent with the systemic toxicity, specifically neurotoxicity, being causative (or contributory) to the observed reproductive toxicity." **[It is not clear why the authors refer specifically to neurotoxicity, which was demonstrated only at the 60 mg/kg/day dose].**

Although the authors called attention to the clastogenicity of acrylamide, they considered a nongenotoxic mechanism to be the most likely explanation for the decrease in the mating index inasmuch as the fertilizing sperm were probably in the epididymis at the time of acrylamide treatment. **[It is not clear what the authors mean here: the mating index is the proportion of paired males that mated, whereas the epididymal effects cited would bear on fertility rather**

than mating. It is possible that they envision a fertility effect that results in mating without the deposition of vaginal sperm or a copulatory plug, but they cite Sublet et al. *(73*) that vaginal sperm are not decreased in spite of the decrease in uterine sperm].

Strengths/Weaknesses: Strengths of this study: (a) sufficient numbers of animals and dose levels; (b) analysis of dosing solutions; (c) apparently correct statistical analysis; (d) the thoroughness with which the effects were analyzed. Alert technicians noticing differences in sperm motility during necropsy, and prompting a more widespread and thorough analysis of this finding, is one example of the quality of science demonstrated in this report. Weaknesses: (a) the relatively poor mating performance of these animals (it might have been useful to mate 1 male with 2 females); and (b) Large variances for the post-implantation loss data make the benchmark dose modeling less useful due to lack of confidence in the mathematical model.

Utility (Adequacy) for CERHR Evaluation Process: These data are very useful for the Evaluative Process, given the confidence that the Panel has in the quality of the data and the analysis. The fact that these data effectively replicate the effects noted by Sublet et al. validates the effects. A benchmark dose approach would seem the best way to utilize all these data , as there clearly are trends of effect in the dataset at doses lower than those asterisked in the pairwise comparison. While the authors were unable to separate unambiguously the reproductive from the dominant lethal effects, they did a thorough job of evaluating this relationshiop, and we can be confident that doses for these effects overlap considerably.

Table 28. Results from Tyl et al. *(124)* Study of Male Reproductive Toxicity and Neurotoxicity of Acrylamide.

			5	15	30	45	60
Male body weight	On study day 5 (prior to last dose)		↔	↔	↓	↓	↓
	On study day 8 (prior to mating)		↔	↔	↓	↓	↓
	Δ Study day 1-5 (dosing interval)		↔	↓	↓	↓	↓
	Δ Study day 5-8 (postdosing, premating)		upward trend (P < 0.001); no pairwise differences				
	Δ Study day 1-8		↔	↓	↓	↓	↓
Grip strength		Forelimb	↔	↔	↔	↔	↔
		Hindlimb	↔	↔	↔	↔	↓21.4 %
Males that mated/males that were paired			↔	↔	↔	↔	↓41%
Males siring litters/males that mated[a]			↔	downward trend; no pairwise differences			
Implantation sites/sperm-positive female			downward trend (P < 0.01); no pairwise differences				
Implantation sites/confirmed pregnant female			↔	↔	↔	↔	↔
Live implants/sperm-positive female			downward trend (P < 0.001)			↓54%	↓86%
Live implants/confirmed pregnant female			downward trend (P < 0.05); no pairwise differences				
Preimplantation loss/confirmed pregnant female			↔	↔	↔	↔	↔
Resorptions[b]/confirmed pregnant female			upward trend (P < 0.01)			↑ 9-fold	↑ 15-fold
Sperm parameters	Concentration		↔	↔	↔	↔	↔
	Percent motile		↔	↔	↔	↔	↔
	Percent progressively motile		downward trend; no pairwise differences				
	Beat cross frequency		upward trend (P < 0.001)				↑36%

[a]This parameter, called the fertility index, is equivalent in this study to the pregnancy index (Pregnant females/males that mated). [b]Postimpantation loss/confirmed pregnant female. [c]There were 25 males per group except at 60 mg/kg/day (n=24, 1 male removed for fractured tibia).

Figure 8. Results of Tyl et al. *(124)* Plotted by CERHR using U.S. EPA Benchmark Dose Software. BMD: Exposure level associated with a 10% response, estimated from a mathematical dose–response model. BMDL: Dose associated with lower 95% confidence interval around the BMD. Data are expressed as mean ± SD, n = 25/dose group except n=24 for the 60 mg/kg/day group

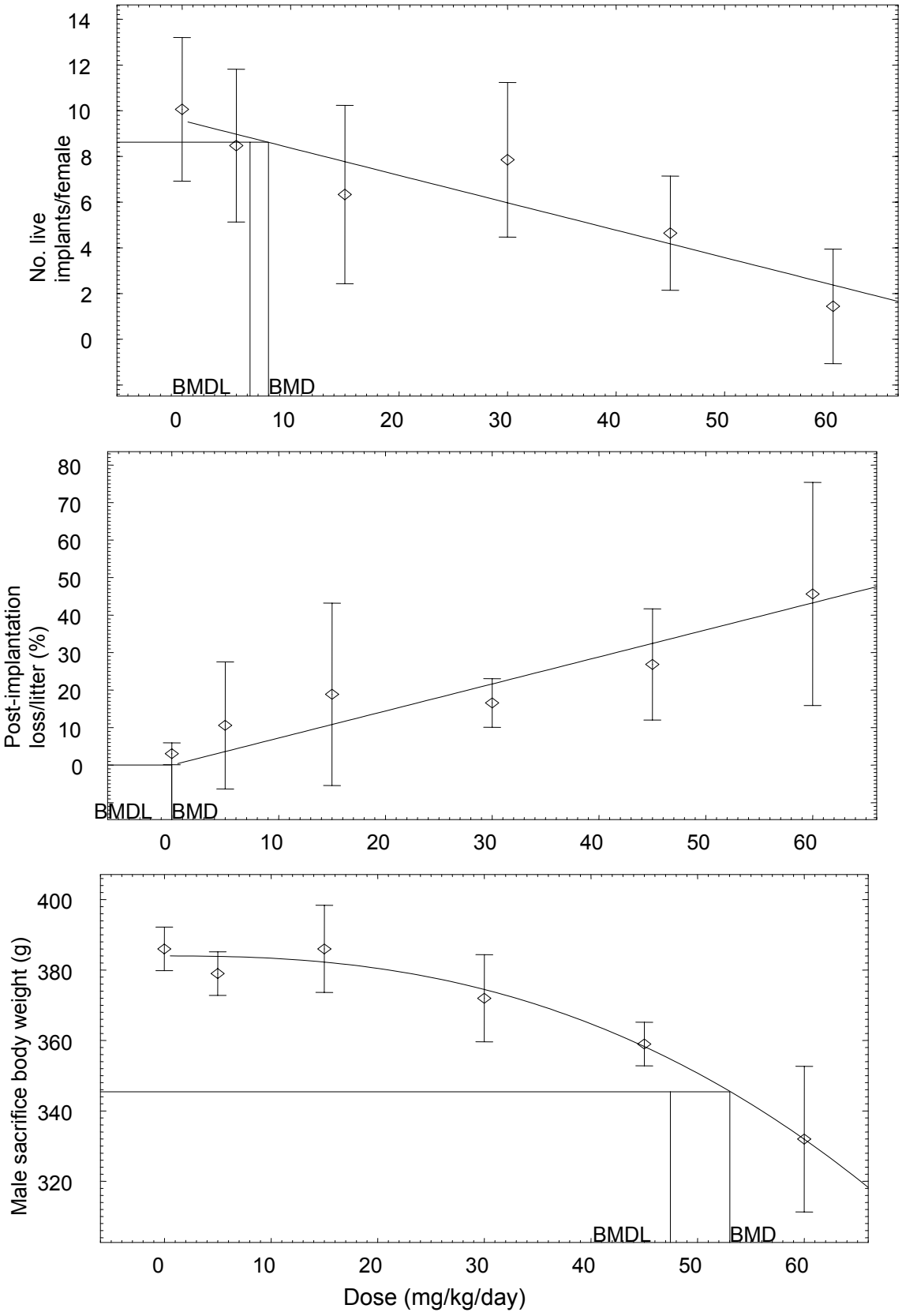


Table 29. Benchmark Doses (mg/kg/day, po gavage) Calculated by CERHR Based on Linear Model for Reproductive End points and Power Model for Weight Using Data from Tyl et al. *(124)*

End point	BMD_{10}	BMDL
Live implants/sperm-positive female	8	6
Post-implantation loss/litter	<<1	<<1
Male sacrifice body weight (estimated from graph)	53	47

BMD_{10} Exposure level associated with a 10% response, estimated from a mathematical dose–response model.
BMDL Dose associated with the lower bound of the 95% confidence interval around the BMD_{10}

4.2.3 Continuous breeding or multigeneration design

Chapin et al. *(125)* performed a Reproductive Assessment by Continuous Breeding (RACB) on acrylamide and three analogs (*N,N'*-methylenenisacrylamide, methacrylamide, and *N*-(hydroxymethyl)acrylamide) under the sponsorship of the National Toxicology Program. The study animal was the Swiss mouse. The RACB protocol consisted of four tasks. The first task was a 28-day dose-range finding study. Task 2 started with a 7-day dosing period followed by 98 days of continued dosing with animals housed together as mating pairs. During the continuous cohabitation phase, pups were removed from their dams after birth and destroyed. At the end of the 98 days, the male and female were separated and continued to be dosed for a six week holding period. During this time, pregnant females were allowed to deliver and raise their last litters until weaning. The dams continued to be dosed during pregnancy and lactation and pups were dosed with the same drinking water solutions after weaning. Task 3 was a cross-over mating trial during which high-dose treated animals were cohabited with untreated control animals to identify which sex was affected. Treatment was withheld during this 1-week mating trial. Task 4 was a fertility study using the F_1 animals that arose from the last litter in the 98-day cohabitation phase. These animals were tested at 74 ± 10 days of age, having been dosed since weaning. A dominant lethal test was also conducted in some high dose males at the end of the 98-day cohabitation phase. Each male was mated with three untreated females for up to four nights, and the females allowed to carry their pregnancies until GD 16, at which time they were killed and uteri examined for live, dead, and resorbing implants.

Acrylamide (97–99% pure) was diluted in deionized filtered water and supplied to animals as their sole drinking water source. Concentrations were 100 ± 10% of target except for two isolated occasions. Based on the dose-range finding results, concentrations selected for Task 2 were 3, 10, and 30 ppm. The limiting toxicity in the dose-range finding study was neurologic with decreases in fore- and hindlimb grip strength. Based on water consumption in female mice, the acrylamide doses were estimated as 0.81, 3.19, and 7.22 mg/kg/day. Water consumption in males was highly variable and was considered unreliable due to presumed palatability-related manipulation of sipper tubes by the males.

There were no effects of treatment on the proportion of fertile pairs (pairs delivering at least one litter), percentage of pairs delivering each litter, number of litters per pair (4.8 to 5 litters/pair), or proportion of pups born alive. There were no alterations in live pup weight, pup weight adjusted for litter size, or time to litter. When litters were considered individually by their order, there was an 8–15% decrease in the number of live pups per litter for litters 2 and 3 in the top dose compared to the control but no significant alteration in live pups per litter for litters 1, 4, or 5, causing the authors to propose that the lack of progressive reproductive toxicity made the biologic importance of the finding "suspect. Considering all litters together in each dose group, the authors indicated that there was a dose related trend for decreased live pups/litter, with a significant decrease on pairwise testing between the high dose group and the

control: mean pups/litter ± SEM (n) for the 0, 3, 10, and 30 ppm groups were 13.6 ± 0.5 (39), 13.9 ± 0.4 (20), 13.6 ± 0.5 (20), and 12.2 ± 0.5 (19), respectively **[Analysis by CERHR did not confirm a statistically significant test for trend]**. On crossover testing, there was no difference between the high dose male × control female matings or the high dose female × control male matings and the control male × control female matings with respect to any parameter, including live pups/litter. Numerically, however, the high dose male × control female matings produced 9.4 pups/litter, compared to 11.4 and 11.5 pups/litter for the control × control and high dose female × control male matings, respectively. The authors noted that this 18% apparent decrease in pups/litter from treated males was similar in magnitude to the 10% decrease in pups/litter at the high dose when all litters were considered in the continuous cohabitation phase. They proposed that the lack of significant decrease in live pups/litter may have been due to the lower power of the single-litter crossover trial.

On dominant lethal testing, there were significant increases in early resorptions (+102%) and total post-implantation losses (+99%) in the 30 ppm group compared to the control group, with dose-related tests for trend across the full dose range. There was an 8% decrease in live fetuses in the 30 ppm group.

After use in the 98-day cohabitation study plus six-week holding period, and after the crossover trials, adults were killed and reproductive tracts evaluated. There were no alterations in gross or histologic examination of male or female reproductive tissues except for a 10–12% decrease in spermatids/g testis in the middle and high dose group. There were no alterations in total spermatids/testis, however, and no changes in epididymal sperm concentration, motility, or frequency of abnormal forms. Estrous cyclicity was not altered by treatment.

There were no alterations in postnatal survival or body weight at weaning in the F_1 generation. Female body weight at the time of the Task 4 mating showed a dose-related decreasing trend with a significant 8% decrease from the control value in the top dose group on pairwise testing. There were no effects in the F_1 matings on percentage of females with a vaginal plug, percentage of plugged females delivering offspring, or pup weight. The number of live pups in the top dose group was decreased by 47% compared to control and a dose-related trend was shown across dose groups in live pups/litter **[Benchmark dose calculation by CERHR was performed on live pups/litter in the F_1 generation. The BMD_{10} was 17 ppm and the BMDL 8 ppm].** When F_1 animals were killed, there were no treatment effects on reproductive organ weight or histology except for a decrease in absolute, but not relative, prostate weight in the high dose group and testicular degeneration in 1 of 10 animals each in the middle and high dose groups. There were no alterations in this generation in spermatid heads per gram testis. Based on water consumption as adults, exposure to acrylamide in the F_1 generation was estimated as 0.86, 2.9, and 7.7 mg/kg/day in the 3, 10, and 30 ppm groups, respectively. **[These estimates give a BMD_{10} of about 5 mg/kg/day and a BMDL of about 2 mg/kg/day based on the reduction in live pups/litter]**.

Grip strength decreases were identified in high-dose treated animals. For the F_0 (continuously cohabited) animals, decreased grip was shown in the forelimbs of males and females exposed to 30 ppm acrylamide and in the forelimbs of females exposed to 10 ppm acrylamide. Male hindlimb grip was decreased in the 30 ppm group. In the F_1 animals, only male forelimb grip strength was reduced significantly. Grip strength was evaluated differently in the two generations: in the parental generation, grip strength was assessed as the percent difference between weeks 0 and 17, while in the F_1 generation, grip strength was assessed at week 10. The authors interpreted the decreases in grip strength in the parental generation as minimal, and they believed that the F_1 generation reduction only in male forelimbs at a single time point may have represented "some degree of biological 'noise.'"

The authors concluded that based on the findings in the dominant lethal study and the lack of effects of acrylamide on female reproductive parameters, "the primary site for the reproductive toxicity of

[acrylamide] is the male, that this can be entirely accounted for by the dominant-lethal activity of [acrylamide], and that it occurs independently of changes in any epididymal sperm measure."

Strengths/Weaknesses: This study is careful and exhaustive and is one of the few studies that independently monitored acrylamide concentration and purity throughout the course of the study. The authors conclude that dominant lethal effects can explain the male reproductive toxicity of acrylamide. It is not known if this experiment would have revealed an increase in the number of sterile and semi-sterile F_1 males. In other words, it is not known if heritable translocations influenced the depression of litter size at the high dose group of F_1 males. Other weaknesses are: (a) the use of mice, a species commonly used for genetic toxicity testing, but perhaps less suitable in comparing reproductive toxicity to neurotoxicity for compounds that depend on a greater length of motor neurons to express their toxicity. Rats would have been the better choice for these studies; (b) the relative paucity of genetic/reproductive investigations (i.e., the authors only evaluated dominant lethality, and not the mechanisms underlying any possible effects). A strength is the explicit simultaneous comparison of reproductive and neurotoxicity across several different congeners of acrylamide with suspected (or known) variations in reproductive toxicity, although of little relevance to the CERHR process.

Utility (Adequacy) for CERHR Evaluation Process: A higher dose of acrylamide would have perhaps made a sharper distinction between reproductive toxicity and neurotoxicity (based on grip strength in F_0 males and females) and between 10 and 30 for reproductive toxicity (based on reductions in litter size in the crossover mating trial, and resorptions and post-implantation loss in the dominant lethal trial). The choice of congeners for this study; however, does not appear to have been made to further illuminate the mechanism of toxicity of acrylamide, and little information can be taken from the results of the congener studies. The study shows that reproductive effects may occur in the absence of, or before, neurologic symptoms appear.

Tyl et al. *(83)* performed a two-generation reproduction and dominant lethal study sponsored by the Acrylamide Producers Association/SOCMA (Synthetic Organic Chemical Manufacturers Association). Male and female Fisher 344 rats were given acrylamide (>99.9% pure) in drinking water. Drinking water concentrations were adjusted weekly based on animal body weight and water consumption to provide doses of 0, 0.5, 2.0, or 5.0 mg/kg/day with 30 animals per dose group. Concentrations in water were 84-112% of the nominal concentrations.

F_0 animals were obtained at 28 days of age, and then begun on test for a 10-week prebreeding exposure period. Following this period, males and females in the same dose group were cohabited 1:1 for up to 7 days. Females with a copulation plug or vaginal sperm were considered to be at GD 0 and were housed singly. Females without evidence of mating after seven days were moved to the cage of a male that had successfully mated with a another female, and were cohabited for up to an additional 7 days. During cohabitation, the acrylamide concentration in the water bottle was based on the female's weight and water consumption prior to mating, likely resulting in underdosing of the males during this time period. This concentration was maintained during gestation and the first week of lactation without adjustment. During the second and third week of lactation, the concentration of acrylamide in the drinking water was reduced to ½ and ⅓, respectively, of the prebreeding concentration, to allow for increased water consumption during lactation and to avoid overtreating offspring that were beginning to drink the water. During the fourth week of lactation, the ½ concentration was used to acclimate the offspring. Until all F_1 offspring were weaned, weanlings were given water with the same acrylamide concentration as had been given to the F_0 animals during their first week of treatment. When the last F_1 litter reached 35 days of age, 30 male and 30 female pups were randomly selected to produce the F_2 generation and were maintained for a total of 11 weeks on acrylamide at the dose used for their parents. **[It is assumed that the 11 weeks started at 35 days of age. It is not stated whether selection of F_1 animals was balanced across**

litters]. The procedures for mating of F_1 animals were identical to the procedures used for the F_0 animals.

After F_0 males had been used for mating, dosing was continued for up to an additional 64 days on acrylamide in case the animals were needed for additional mating. They were then removed from acrylamide treatment for two days prior to being used in the dominant lethal study. In the dominant lethal study, males were paired 1:2 with untreated females. Females were killed on GD 14 (plug or sperm = GD 0) and ovaries and uteri inspected for corpora lutea and implantation sites, respectively. Uteri that appeared nongravid were stained with ammonium sulfide to identify early resorption sites.

Statistical testing was performed using ANOVA and *t*-tests for parametric data and Kruskal-Wallis followed by the Mann-Whitneu *U*-test for nonparametric data. Frequency data were compared using the Fisher exact test, according to the methods section, although some of the tables make reference to chi-square testing.

Selected data for F_0 animals are summarized in Table 30. Reductions in weight gains were seen at all acrylamide doses during at least some of the study period. The text states that all acrylamide-exposed F_0 males exhibited a higher incidence of head tilt and leg splay than did control males; however, the data table gives the incidence of head tilt as 2/30 in the control group, and 6/30, 2/30, and 6/30 in the 0.5, 2.0, and 5.0 mg/kg/day groups. The incidence of foot splay was 3/30, 10/30, 7/30, and 10/30 in the 0, 0.5, 2.0, and 5.0 mg/kg/day dose groups. **[The table does not indicate statistical significance for any comparisons with the control, and Fisher exact test performed by CERHR confirms a lack of statistical significance for these comparisons].** None of the females in any group had head tilt. The incidence of foot splay was 1/30, 2/30, 6/30, and 6/30 in the 0, 0.5, 2.0, and 5.0 mg/kg/day groups, also not statistically significant.

Rates of mating and pregnancy were similar among dose groups in the F_0 generation. The authors note that an unexpectedly low proportion of control males (17 of 30) produced pregnancies, but that after providing a second week of mating with a proven male, 25 of 30 control females became pregnant. The proportion of mated females that became pregnant was not altered by acrylamide treatment, and gestation length was similar among groups. The number of live pups/litter was reduced in the top dose group, and post-implantation loss was increased in this group (Table 31). Necropsy of F_0 adults showed no gross lesions.

F_1 pups did not differ in body weight by treatment group with the exception of male pups in the high-dose group during the last half of the lactation period. The decrease in pup survival during the first four days of life was attributed to the loss of single-pup litters. **[The data table in the paper mentions Day 4 pre- and post-cull data; however, no cull is described in the methods until day 28].** Necropsy of the F_1 offspring that were not selected to reproduce did not show gross lesions or histologic changes in reproductive organs or nervous system tissues. Head tilt was found in 4/30 males in the 5.0 mg/kg/day group but in no other dose groups and in no females. This proportion (4/30) was statistically different from 0/30. Foot splay was not identified in any F_1 animals. Selected F_1 parameters are given in Table 32. A decrease in weight gain was seen in the middle and top dose groups. As in the F_0 matings, there was a decrease in live pups/litter. A five-fold increase in the numerical value for post-implantation loss in the 5.0 mg/kg/day dose group compared to the control group was not statistically significant, likely because of large variance **[the standard deviation for each dose group is greater than the mean, suggesting that mean ± SD will not give an accurate picture of the central tendency and distribution. According to the methods section, post-implantation loss was expressed as an index, [(No. of implantations–no. of live pups)/No. of implantations)] × 100 and evaluated using the Fisher exact test; however, the expression of mean ± SD in the table does not provide confidence that a**

nonparametric test was used]. The proportion of F_1 males impregnating F_1 females and the proportion of mated females that became pregnant did not differ by dose group.

Dominant lethal results for F_0 males are summarized in Table 33 and are consistent with the post-implantation loss data reported for the F_0 matings **[consistent also with the numerical (but statisitically-non-significant) increase in post-implantation loss in the F_1 matings]**. The dominant lethal data also appear in Table 12.

Strengths/Weaknesses: The quantification of and care associated with knowing the administered dose is a major strength of this study (including analysis of dosing solutions), as is the expertise with which doses were chosen (based on the moderate but significant weight effects that were seen, and the absence of mortality or overwhelming neurotoxicity symptoms). Other strengths are the use of very robust numbers of animals, appropriate statistics (although clouded slightly by different statistics mentioned in the Methods section and some tables), the evaluation of a large number of relevant end points (signs of neurotoxicity and behavioral/carriage alterations in both generations, and high-quality neuropathology, for example) and the testing of dominant lethality and reproductive capability in the same design, using the same males. The consistency of effects across the generations lends added weight to their being real. There are no meaningful weaknesses in the study design, or the carrying out of this design or the interpretation of the data. A modest weakness is that sperm parameters were not evaluated; it would lend weight to the conclusion of a primary effect of dominant lethality if the authors had shown by cell counting and motility evaluation that these measures were unaffected. However, the study was performed at a time when such end points were not routinely measured or expected in Guideline studies, so this is not necessarily a fault of the authors or sponsors. Similarly, in retrospect, it would have been useful to explicitly evaluate female fertility, which is inferred as a target based on the slightly greater effects in the F_0 and F_1 matings compared to the male dominant lethal mating, but one cannot fault the authors for a cloudy crystal ball.

Utility (Adequacy) for CERHR Evaluation Process: These data are of high quality, and are adequate and informative for the Evaluative Process. They are adequate to identify a male dominant lethal dose (ca. 5 mg/kg/d), to show that the neurotoxicity seems to occur at doses similar to those that affected fertility, and to infer that female fertility might be affected as well. These data shed no light on mechanism, but were not designed to.

Table 30. Selected F_0 Data from Tyl et al. *(83)*

			Acrylamide dose (mg/kg/day)		
			0.5	2.0	5.0
Male body weight, by week	prebreeding	Day 0	↔	↔	↔
		7	↔	↔	↔
		14	↔	↔	↔
		21	↔	↔	↔
		28	↔	↓	↓
		35	↔	↓	↓
		42	↓	↓	↓
		49	↓	↓	↓
		56	↓	↓	↓
		63	↓	↓	↓
		0-70	↓6%	↓7%	↓10%
	mating	70	↓	↓	↓
		77	↓	↓	↓
		84	↔	↔	↔
Female body weight, by week	prebreeding	Day 0	↔	↔	↔
		7	↔	↔	↔
		14	↔	↓	↓
		21	↔	↔	↔
		28	↔	↓	↓
		35	↔	↔	↔
		42	↔	↔	↔
		49	↔	↓	↓
		56	↓	↓	↓
		63	↔	↓	↓
		0-70	↓4%	↓9%	↓36%
	mating	70	↔	↓	↓
	gestation	Day 0	↔	↔	↔
		7	↔	↔	↔
		14	↔	↔	↔
		21	↔	↓	↓
		0-20	↔	↔	↓28%
	lactation	Day 1	↔	↔	↔
		4	↔	↔	↓
		7	↔	↔	↓
		14	↔	↔	↓
		21	↔	↔	↓
		28	↔	↔	↔
		1-28	↔	↔	↑41%
Plug/sperm positive females			↔	↔	↔
Implantations/dam			↔	↔	↓35%
Live pups/litter			↔	↔	↓54%
Post-implantation loss			↔	↔	↑4.4-fold

↑,↓ Statistically increased, decreased compared to control value. ↔ Not statistically difference from control value.

Table 31. Selected F_1 Litter Parameters During Lactation from Tyl et al. *(83)*. Mean ± SD.

		Acrylamide dose (mg/kg/day)			
		0	0.5	2.0	5.0
Litters delivered (n)		20	24	26	17
Live litter size (n)		9.8 ± 3.1	9.8 ± 3.5	9.7 ± 2.4	4.5 ± 2.6**
4-day survival (%)		99.5 ± 2.2	94.8 ±20.6	99.4 ± 2.2	86.2 ± 34.0*
Pup weight, male					
	Postnatal day 1	↔	↔	↔	↔
	4	↔	↔	↔	↔
	7	↔	↔	↔	↔
	14	↔	↔	↔	↓11%
	21	↔	↔	↔	↓10%
	28	↔	↔	↔	↓8%
Pup weight, female ?					
	Postnatal day 1	↔	↔	↔	↔
	4	↔	↔	↔	↔
	7	↔	↔	↔	↔
	14	↔	↔	↔	↔
	21	↔	↔	↔	↔
	28	↔	↔	↔	↔

*,**$P \leq 0.01$, 0.001 compared to control value. ↑,↓ Statistically increased, decreased compared to control value.
↔ Not statistically difference from control value.

Table 32. Selected F_1 Data from Tyl et al. *(83)*

			Acrylamide dose (mg/kg/day)		
			0.5	2.0	5.0
Male body weight, by week	prebreeding	Day 0	↔	↔	↓
		7	↔	↔	↔
		14	↔	↓	↓
		21	↔	↔	↓
		28	↔	↓	↓
		35	↔	↓	↓
		42	↔	↓	↓
		49	↔	↓	↓
		56	↔	↓	↓
		63	↔	↓	↓
		70	↔	↓	↓
		0-77	↔	↔	↔
	mating	77	↔	↓	↓
		84	↔	↓	↓
		91	↔	↓	↓
Female body weight, by week	prebreeding	Day 0	↔	↔	↔
		7	↔	↔	↔
		14	↔	↔	↔
		21	↔	↓	↔
		28	↔	↓	↓
		35	↔	↓	↓
		42	↔	↓	↓
		49	↔	↓	↓
		56	↔	↓	↓
		63	↔	↓	↓
		70	↔	↓	↓
		0-77	↔	↔	↓12%
	mating	Day 77	↔	↓	↓
	gestation	Day 0	↔	↔	↔
		7	↔	↔	↔
		14	↔	↔	↓
		21	↔	↓	↓
		0-20	↔	↓14%	↓35%
	lactation	Day 1	↔	↔	↔
		4	↔	↓	↓
		7	↔	↔	↓
		14	↔	↔	↓
		21	↔	↔	↔
		28	↔	↔	↔
		1-28	↔	↔	↑49%
Plug/sperm positive females			↔	↔	↔
Implantations/dam			↔	↔	↓14%
Live implants/litter			↔	↔	↓20%
Post-implantation loss			↔	↔	↔

↑,↓ Statistically increased, decreased compared to control value. ↔ Not statistically difference from control value.

Table 33. Dominant Lethal Results from Tyl et al. *(83)*

	Acrylamide dose (mg/kg/day)		
	0.5	2.0	5.0
Plug/sperm positive females	↔	↔	↔
Implantations/dam	↔	↔	↓14%
Live implants/litter	↔	↔	↓20%
Post-implantation loss	↓40%	↔	↑2.3-fold

↑,↓ Statistically increased, decreased compared to control value. ↔ Not statistically difference from control value.

4.3 Utility of data

Based on the studies by Zenick et al. *(78)*, Sublet et al. *(73)*, and Tyl et al. *(83,124)*, the Expert Panel determined that there are sufficient data to evaluate male and female reproductive toxicity in rats exposed to acrylamide in drinking water or by gavage using litter size, viability, and pup weight as traditional end points as well as using less traditional end points involving the delivery of sperm to the female reproductive tract. The studies of Sakamoto and Hashimoto *(119)* and of Chapin et al. *(125)* are judged adequate to permit the evaluation of the effects on male and female reproduction in mice of acrylamide in drinking water using traditional reproductive end points. In addition to these studies, there are several studies using i.p. dosing that provide supplemental information on possible genotoxic mechanisms of acrylamide toxicity.

4.4 Summary

4.4.1 Human data

No human data on acrylamide reproductive effects were identified.

4.4.2 Experimental animal data

The key reproductive toxicology studies are summarized in Table 34.

4.4.2.1 Female reproduction

The key study for the assessment of female reproduction was Zenick et al. *(78)* in which acrylamide was given in drinking water to female rats at 0, 25, 50, or 100 ppm. After two weeks of treatment, untreated males were placed overnight with the females for up to 7 days. End points included maternal weight gain, mating performance, pregnancy rate, pup survival and weight, and day of vaginal patency in female offspring. Neurologic toxicity (hindlimb splay) was seen in dams given 100 ppm acrylamide. There were 2 of 15 dams in the 100 ppm group with full or nearly full litter loss. Pup weight was decreased in a dose-dependent manner in the 50 and 100 ppm groups and was transiently depressed in the first week of life in the 25 ppm group. Full litter loss and decreased pup weight were considered by the Expert Panel to represent developmental toxicity rather than female reproductive toxicity.

Other studies that included evaluations of female reproductive function *(119, 120)* were reported with insufficient detail to be used in the evaluation.

4.4.2.2 Male reproduction

The Expert Panel notes that there are a number of genotoxicity studies with end points that might be considered reproductive (e.g., pre-imlantation loss after parental treatment). Studies that were designed to evaluate genotoxicity were grouped in section 2. These studies include those with dominant lethal (section 2.3.2.3), heritable translocation (section 2.3.2.4), and specific locus mutation (section 2.3.2.5) end points. Although these studies are placed for organizational purposes under the heading of genotoxicity rather than developmental or reproductive toxicity, the Expert Panel considers these studies important in evaluating the reproductive and developmental effects of acrylamide. Because genotoxicity studies were reviewed and summarized in section 2, attention in this section is focused on studies the primary end points of which were not genotoxic, although some reproductive outcomes from genotoxicity studies are repeated here.

Three studies of adequate design were reported in suffient detail to permit an evaluation of acrylamide effects on male reproduction *(73, 78, 124*). Zenick et al. *(78*) gave acrylamide in drinking water to male rats at 0, 50, 100, or 200 ppm for up to 10 weeks. Mating with hormone-primed ovariectomized females was visually monitored and copulatory plugs were retrieved for weight, and sperm count, motility, and morphology during week 9. Sperm in the uterus were also assessed. During week 10, males in the 0 and 100 ppm acrylamide groups were mated with intact estrous females, which were killed on GD 17 for evaluation of intrauterine contents. Males were killed at week 11 for histology of one testis and epididymal fluid, and homogenization resistant spermatid count determination from the other testis. Three of 15 males in the 200 ppm group died or were killed in moribund condition and the remainder were killed by week 6. This dose was associated with neurological toxicity (hindlimb splay). Fewer males in the 100 and 200 ppm group demonstrated ejaculation compared with the control group. There was a decrease in vaginal sperm in females mated to 100 ppm males compared to control males and only a single female (of 15) mated to a 100-ppm male had sperm in the uterus. There was a decrease in the proportion of 100 ppm males establishing a pregnancy (5/15) compared to the control group (11/14), and post-implantation loss was higher in females that were mated to 100 ppm sires (31.7 ± 3.8%) compared to 0 ppm sires (8.0 ± 1.1%). Testicular histology did not show abnormalities associated with 100 ppm acrylamide treatment. The authors concluded that the male reproductive toxicity of acrylamide at this dose, which corresponded to about 7–8 mg/kg/day was due to abnormalities of copulatory behavior and delivery of sperm to the female genital tract.

Sublet et al. *(73*) used a dominant lethal design plus an evaluation of mating and fertilization success in rats to investigate what proportion of apparent dominant lethality and preimplantation loss might be attributable to impaired fertility as opposed to death of the conceptus prior to implantation. The dominant lethal study (reviewed in Section 2.3.2.3) showed an increase in pre- and post-implantation loss with mating 1–3 weeks after treatment of males with acrylamide 15 mg/kg/day or greater for five days. To confirm and extend the work of Zenick et al. *(78*), male rats were treated with acrylamide 0, 15, or 45 mg/kg/day for 5 days by gavage and mated to untreated hormonally-primed ovariectomized females. Females were killed 10–15 minutes after ejaculation and the uterine and vaginal fluids examined for sperm by light microscopy. No abnormalities of male copulatory behavior were observed at any dose, and all females had sperm in the vagina, but there was a significant decrease in the proportion of females with uterine sperm after mating with males in the 45 mg/kg/day group 1 week after treatment. There were no differences in the proportions of females with uterine sperm 2, 3, or 4 weeks after treatment of males with either dose of acrylamide. Uterine sperm were evaluated by computer-assisted sperm analysis after females mated with 0 and 45 mg/kg/day acrylamide-treated males. There were no differences in sperm parameters at any time in either dose group except for a decrease in motile

sperm and curvilinear velocity in females three weeks after treatment of males with acrylamide 45 mg/kg/day for five days (control motility 75%, acrylamide-treated motility 58%, $P < 0.05$; control curvilinear velocity 132.12 ± 7.02 μm/sec, acrylamide-treated curvilinear velocity 122.06 ± 3.32 μm/sec, $P < 0.05$). An estimate of fertilization was made by recovering cumulus masses from oviducts 10–14 h after mating females with males that had been treated with acrylamide 0, 15, or 45 mg/kg/day for five days by gavage. Oocyte fertilization was demonstrated by identification of either a sperm head and tail or two pronuclei. The percent oocytes fertilized per female was reduced in a dose-dependent manner in Week 1 (both acrylamide doses) and 3 (45 mg/kg/day acrylamide). The authors suggested that impaired fertilizing ability of acrylamide-exposed sperm may play a role in the apparent preimplantation loss rate seen after treatment. They proposed that although copulatory behavior appeared normal in their experiments, and acrylamide doses were below those associated with gross neurologic impairment, there might be subtle abnormalities of copulation resulting in a failure to deposit the ejaculate appropriately against the cervix or in a failure of adequate stimulation of the female to result in facilitation of sperm transport.

Tyl et al. *(124)* repeated a portion of the study of Sublet et al. *(73)*, using a design that matched the first week of the Sublet et al. study. Male rats were exposed by daily gavage for five consecutive days to acrylamide at 0, 5, 15, 30, 45, or 60 mg/kg/day, 25 animals/dose group. Three days after the treatment period, males were cohabited with untreated females for a single overnight period, then males underwent grip testing and were killed for evaluation of cauda epididymal sperm parameters (using computer-assisted methods). Sciatic nerves from some males were evaluated histologically. Mated females were killed on GD 15 for evaluation of uterine contents. Hindlimb grip strength was decreased in males that received the 60 mg/kg/day dose. Sciatic nerve histology was not affected by any dose. There were fewer males in the 60 mg/kg/day group than the control group that mated or produced litters. Pairwise comparison showed statistically significant reproductive effects at 45 and 60 mg/kg/day and statistically significant decreases in weight gain at 15 mg/kg/day and above. The authors argued that systemic toxicity was "causative (or contributory)" to the reproductive toxicity. **[Benchmark dose calculations by CERHR suggest reproductive effects were more sensitive than systemic effects as manifest by reductions in body weight gain].**

Other studies provided supporting information. Hashimoto et al. *(121)* treated male ddY mice by gavage with acrylamide 35.5 mg/kg twice/week for 8 weeks and demonstrated a decrease in relative testicular weight. Sakamoto et al. *(122)* administered acrylamide (95% purity) to ddY mice as a single oral dose of 100 or 150 mg/kg at age 30 days (prepubertal) or 60 days (adult) and found the round spermatid (Golgi and cap phase) to be the cell type most sensitive to acrylamide toxicity. Pacchierotti et al. *(84)* treated male $B6C3F_1$ mice with single i.p. acrylamide doses of 0, 75 , or 125 mg/kg or with 5 daily i.p. doses of 50 mg/kg. Males were mated to untreated females 7 days after the last acrylamide dose. Males were killed at weekly intervals for flow cytometric analysis of testicular cell populations. The percent females showing evidence of mating after cohabitation with treated males was reduced in all acrylamide groups without regard to acrylamide dose. The reduction in mating success resolved by 28 days after treatment in the 125 mg/kg group. Three days after treatment, there was a decrease in testicular 4C cells with 75 or 125 mg/kg acrylamide, an increase in elongated/elongating spermatids with 5 × 50 mg/kg/day acrylamide, and an increase in elongated and round spermatids with 125 mg/kg acrylamide. Thirty-five days after i.p. treatment with acrylamide 100, 125, or 150 mg/kg, there was a decrease in elongated spermatids at the top dose and a decrease in diploid spermatids at all doses, causing the authors to propose that acrylamide impairs chromosome segregation during spermatogonial mitosis. Marchetti et al. *(3)* gave i.p. acrylamide to male $B6C3F_1$ mice at 50 mg/kg/day for 5 consecutive days as part of a study to assess chromosome exchanges in the male pronucleus

(discussed in section 2.3). Treated males were mated with untreated females at different time intervals to characterize acrylamide effects on specific germ cell stages. Acrylamide treatment was associated with a decrease in fertilization except with treatment of males at the stem cell stage. The proportion of fertilized eggs that developed to zygotes was decreased with treatment from the mid-spermatid to epididymal stages. Sakamoto and Hashimoto *(119*) gave male ddY mice acrylamide in drinking water at 0.3, 0.6, 0.9, and 1.2 mM **[21.3, 42.6, 64.0, and 85.2 mg/L, respectively]** for 4 weeks prior to mating or prior to evaluation of reproductive organ weight and epididymal sperm. There were dose related decreases in the number of fetuses/dam in the top two dose groups and decreases at the top dose in the proportion of mated dams that were pregnant, resorptions per dam, and offspring per dam. Epididymal sperm count was decreased and abnormal sperm forms increased at the top dose. Using the benchmark dose approach, the 10% effect level may be as low as 3 mg/kg/day for resorptions/dam after paternal treatment; however, the small sample size (3 or 4 males per dose group for some determinations) reduced confidence in the estimates of effective dose.

Two studies (Costa et al. *(123*), *(77*)) provided evidence that the male reproductive toxicity of acrylamide is mediated by glycidamide; however, these studies did not provide conclusive evidence to establish glycidamide as the proximate reproductive toxicant.

4.4.2.3 Continuous breeding or multigeneration designs

Chapin et al. *(125*) performed a Reproductive Assessment by Continuous Breeding in Swiss mice on acrylamide or one of three analogs. Acrylamide was given in drinking water at 0, 3, 10, and 30 ppm, Doses in females were estimated to be 0, 0.81, 3.19, and 7.22 mg/kg/day. Doses in males could not be estimated due to highly variable water intake. There were no effects of treatment on the proportion of fertile pairs (pairs delivering at least one litter), percentage of pairs delivering each litter, number of litters per pair (4.8 to 5 litters/pair), or proportion of pups born alive. There were no alterations in live pup weight, pup weight adjusted for litter size, or time to litter. There was a decrease in live pups per litter at the top dose in litters 2 and 3 (of a total of 4 or 5 litters per mating pair). Consider all litters together, there was a significant decrease at the high dose in mean pups/litter. Early resorptions and post-implantation loss were increased on dominant lethal testing at the top dose as well. Cross-over matings suggested that the acrylamide effect on mean pups/litter was mediated through the treated male. Sperm parameters, estrous cyclicity, and reproductive tract histology were not altered by treatment except for a decrease in spermatids/g testis in the 10 and 30 ppm groups. Reproduction in F_1 mice that were exposed during gestation and again from weaning was not impaired except for a decrease in live pups/litter that was significant on pairwise comparison in the top dose group and that showed a dose-related trend across all dose groups. Absolute, but not relative, prostate weight was decreased at the high dose, but there was no alteration in spermatids/g testis as there had been in the F_0 generation. Estimated adult acrylamide intake in the F_1 generation was 0, 0.86, 2.9, and 7.7 mg/kg/day. The authors interpreted the reproductive effects of acrylamide in males as attributable to dominant lethal activity.

Tyl et al. *(83*) performed a two-generation reproduction and dominant lethal study in Fisher 344 rats given acrylamide in drinking water. Drinking water concentrations were adjusted weekly based on animal body weight and water consumption to provide doses of 0, 0.5, 2.0, or 5.0 mg/kg/day with 30 animals per dose group. Animals were mated after a 10-week exposure period. Drinking water concentrations were adjusted during lactation to allow for increased water consumption and to avoid over-treating pups when they began to drink water. Offspring were weaned to the same acrylamide exposure groups as their dams. Offspring were mated (30/sex/treatment group) to produce the F_2 generation. Some of the F_0 males were continued in a dominant lethal study. The number of live pups/litter was decreased and post-implantation loss

was increased at the top dose for both F_0 and F_1 matings, although statistical confirmation was not obtained for post-implantation loss in the F_1.

The Expert Panel found no data with which to evaluate possible human reproductive toxicity of acrylamide. Data are sufficient to conclude that acrylamide is not a female reproductive toxicant in rats at exposures up to 100 ppm in drinking water (about 11 mg/kg/day). Data are sufficient to conclude that acrylamide produces reproductive toxicity in male mice manifest as a decrease in litter size with drinking water exposures of 64 ppm (about 13 mg/kg/day). There is a possible interactive effect of treatment of male and female mice with acrylamide with a decrease in live litter size at drinking water concentrations of 30 ppm (5–7 mg/kg/day), although the Expert Panel recognizes that male effects appear to predominate. **Data are sufficient to conclude that acrylamide is a reproductive toxicant in male rats manifest as impaired delivery of sperm to the female genital tract with a reduction in the establishment of viable pregnancies at exposure levels of about 7–8 mg/kg/day in drinking water or 15 mg/kg/day by gavage.** As in mice, there is a possible interactive effect in rats when the male and female are treated with decreased live litter size and increased post-implantation loss at parental exposure levels of 5 mg/kg/day in drinking water. **The data from rodent studies are assumed relevant to humans.** The Expert Panel found the data suggestive that the male reproductive toxicity of acrylamide in rodents is due to both impairment of mating ability and abnormalities of sperm involving sperm chromatin and possibly sperm functional capabilities such as motility. The rat and mouse data are assumed relevant to assessment of human reproductive risk.

Table 34. Key Reproductive Studies

Sex/species/strain	Exposures	Critical effects	Effect level	Reference
Female Long-Evans rats	Drinking water 0, 25, 50, 100 ppm	Decreased pup weight[a]	LOAEL = 25 ppm (lowest tested level) (4 mg/kg/day)	Zenick et al. *(78)*
		Decreased litter size[a]	**[BMD_{10} = 98 ppm (10–14 mg/kg/day); BMDL = 40 ppm (4–7 mg/kg/day)]**	
Male ddY mice	Drinking water 21.3, 42.6, 64.0, 85.2 ppm **[converted from mM]**	Decreased litter size	LOAEL = 64.0 ppm (~13 mg/kg/day). **[BMD_{10} = 45 ppm; BMDL = 22 ppm]**	Sakamoto *(119)*
		Decreased pregnancy rate and increased resorptions	LOAEL = 85.2 ppm (~18 mg/kg/day) **[BMD_{10} = 14 ppm; BMDL = 3 ppm]**	
Male Long-Evans rats	Drinking water 0, 50, 100, 200 ppm	Impaired ejaculation, decreased vaginal sperm and pregnancy rates and increased post-implantation loss in cohabited females	LOAEL = 100 ppm (about 7–8 mg/kg/day)	Zenick et al. *(78)*
Male Long-Evans rats	Gavage 0, 0, 5, 15, 30, 45, 60 mg/kg/day × 5 days	Increased pre- and post-imlantation loss	LOAEL = 15 mg/kg/day	Sublet *(73)*
	Gavage 0, 15, 45 mg/kg/day × 5 days	Decreased uterine sperm in cohabited females, decreased uterine sperm motility	LOAEL = 45 mg/kg/day	
		Decreased fertilization of oocytes	LOAEL = 15 mg/kg/day (lowest tested level)	
Male Long-Evans rats	Gavage 0, 0, 5, 15, 30, 45, 60 mg/kg/day × 5 days	Decreased mating; fewer litters produced	LOAEL = 45 mg/kg/day **[BMD_{10} = 8 mg/kg/day and BMDL = 6 mg/kg/day for live implants/sperm-positive female; benchmark doses << 1 for post-implantation loss/litter. Large variances render the benchmark approach of questionable utility]**	Tyl *(124)*
Swiss mice, cohabiting pairs	Drinking water 0, 3, 10, 30 ppm	Decreased live litter size in two generations	LOAEL = 30 ppm (5–7 mg/kg/day)	Chapin *(125)*
Fisher 344 rats, 2-generation	Drinking water 0, 0.5, 2.0, 5.0 mg/kg/day	Decreased live litter size and increased post implantation loss in two generations	LOAEL = 5 mg/kg/day	Tyl *(83)*

LOAEL lowest observed adverse effect level; NOAEL no observed adverse effect level; BMD_{10} Exposure level associated with a 10% response, estimated from a mathematical dose–response model. BMDL Dose associated with the lower bound of the 95% confidence interval around the BMD_{10}. Benchmark doses calculated by CERHR where underlying data permitted modeling.

[a]These end points were considered by the Expert Panel to represent developmental toxicity rather than reproductive toxicity.

5.0 SUMMARIES, CONCLUSIONS, and CRITICAL DATA NEEDS

Start text here.

5.1 Summary and Conclusions of Reproductive and Developmental Hazards

Start text here.

5.2 Summary of Human Exposure

5.3 Overall Conclusions

5.4 Critical Data Needs

6.0 REFERENCES

References

1. Shelby, M. D., Cain, K. T., Hughes, L. A., Braden, P. W. and Generoso, W. M. Dominant lethal effects of acrylamide in male mice. Mutat Res 1986; 173: 35-40.
2. Holland, N., Ahlborn, T., Turteltaub, K., Markee, C., Moore, D., 2nd, Wyrobek, A. J. and Smith, M. T. Acrylamide causes preimplantation abnormalities in embryos and induces chromatin-adducts in male germ cells of mice. Reprod Toxicol 1999; 13: 167-78.
3. Marchetti, F., Lowe, X., Bishop, J. and Wyrobek, A. J. Induction of chromosomal aberrations in mouse zygotes by acrylamide treatment of male germ cells and their correlation with dominant lethality and heritable translocations. Environmental And Molecular Mutagenesis 1997; 30: 410-417.
4. Sega, G. A., Alcota, R. P. V., Tancongco, C. P. and Brimer, P. A. Acrylamide binding to the DNA and protamine of spermiogenic stages in the mouse and its relationship to genetic damage. Mutat Res 1989; 216: 221-230.
5. Chemfinder. Acrylamide. Available at: http://chemfinder.cambridgesoft.com/result.asp. 2003;
6. HSDB. Acrylamide. 2003.
7. IPCS. Acrylamide Environmental Health Criteria 49 (http://www.inchem.org/documents/ehc/ehc/ehc49.htm). 1985.
8. European-Union. European Union Risk Assessment Report: Acrylamide. Institute for Health and Consumer Protection; 2002.
9. Mallinckrodt. Acrylamide Material Safety Data Sheet. Mallinckrodt Science Product Division. 1987.
10. IPCS. Poisons Information Monograph 652: Acrylamide (http://www.inchem.org/documents/pims/chemical/pim652.htm). 1996.
11. IARC. Acrylamide. TA:IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans PG 1994; 60.
12. NTP. Report on Carcinogens, Tenth Edition. ed. US Department of Health and Human Services, Public Health Service; 2002.
13. EPA. Chemical summary for acrylamide. Office of Pollution Prevention and Toxics; 1994.
14. Friedman, M. Chemistry, Biochemistry, and Safety of Acrylamide. A Review. J Agric Food Chem 2003; 51: 4504-26.
15. FDA. Acrylamide: Questions & Answers. Center for Food Safety and Applied Nutrition. 2003.
16. NLM. Household Products Database: Polyacrylamide. NIH National Library of Medicine Specialized Information Services. 2001.
17. FAO/WHO. Health implications of acrylamide in food (http://www.who.int/fsf/Acrylamide/Acrylamide_index.htm). Geneva, Switzerland: 2002.
18. FDA. FDA draft action plan for acrylamide in food - February 24, 2003 Update. Center for Food Safety and Applied Nutrition, Office of Plant & Dairy Foods & Beverages. 2003.
19. JIFSAN/NCFST. Exposure and biomarkers white paper. In: ed.^eds. Acrylamide in food workshop: scientific issues, uncertainties, and research strategies. ed. 2002:
20. JIFSAN/NCFST. Mechanisms of formation of acrylamide in food summary report. In: ed.^eds. Acrylamide in food workshop: scientific issues, uncertainties, and research strategies. ed. 2002:
21. EPA. National primary drinking water regulations: Treatment techniques for acrylamide and epichlorohydrin. 2002.
22. EPA. Consumer Factsheet on: Acrylamide. 2002.
23. Smith, C. J., Perfetti, T. A., Rumple, M. A., Rodgman, A. and Doolittle, D. J. "IARC group 2A Carcinogens" reported in cigarette mainstream smoke. Food Chem Toxicol 2000; 38: 371-83.
24. EPA. TRI Calculation Results. 2000.
25. European_Commision. Opinion of the scientific committee on food on new findings regarding the presence of acrylamide in food (http://europa.eu.int/comm/food/fs/sc/scf/out131_en.pdf). Brussels, Belgium: 2002.
26. Sorgel, F., Weissenbacher, R., Kinzig-Schippers, M., Hofmann, A., Illauer, M., Skott, A. and Landersdorfer, C. Acrylamide: increased concentrations in homemade food and first evidence of its variable absorption from food, variable metabolism and placental and breast milk transfer in humans. Chemotherapy 2002; 48: 267-74.

27. Calleman, C. J. The metabolism and pharmacokinetics of acrylamide: implications for mechanisms of toxicity and human risk estimation. Drug Metab Rev 1996; 28: 527-90.
28. NIOSH. National Occupational Exposure Survey (1981-1983). 2003.
29. ACGIH. Acrylamide. TA:ACGIH. Documentation of the threshold limit values and biological exposure indices PG 2001; 3:
30. ACGIH. Acrylamide. Documentation of the threshold limit values and biological exposure indices 2003; 12.
31. NIOSH. Acrylamide: NIOSH Pocket Guide. 2003.
32. OSHA. Air contaminants. 29 CFR 1910.1000 2001;
33. Pantusa, V. P., Stock, T. H., Morandi, M. T., Harrist, R. B. and Afshar, M. Inhalation exposures to acrylamide in biomedical laboratories. AIHA J (Fairfax, Va) 2002; 63: 468-73.
34. Schettgen, T., Broding, H. C., Angerer, J. and Drexler, H. Hemoglobin adducts of ethylene oxide, propylene oxide, acrylonitrile and acrylamide-biomarkers in occupational and environmental medicine. Toxicol Lett 2002; 134: 65-70.
35. Schettgen, T., Weiss, T., Drexler, H. and Angerer, J. A first approach to estimate the internal exposure to acrylamide in smoking and non-smoking adults from Germany. Int J Hyg Environ Health 2003; 206: 9-14.
36. Fennell, T. R., MacNeela, J. P., Morris, R. W., Watson, M., Thompson, C. L. and Bell, D. A. Hemoglobin adducts from acrylonitrile and ethylene oxide in cigarette smokers: effects of glutathione S-transferase T1-null and M1-null genotypes. Cancer Epidemiol Biomarkers Prev 2000; 9: 705-12.
37. Hagmar, L., Tornqvist, M., Nordander, C., Rosen, I., Bruze, M., Kautiainen, A., Magnusson, A. L., Malmberg, B., Aprea, P., Granath, F. and Axmon, A. Health effects of occupational exposure to acrylamide using hemoglobin adducts as biomarkers of internal dose. Scand J Work Environ Health 2001; 27: 219-26.
38. Molak, V. NIOH and NIOSH Basis for an Occupational Health Standard. Acrylamide: A Review of the Literature. Atlanta, Georgia: U.S. Department of Health and Human Services; 1991.
39. JIFSAN/NCFST. Overview of acrylamide toxicity and metabolism. In: ed.^eds. Acrylamide in food workshop: scientific issues, uncertainties, and research strategies. ed. 2002:
40. Sumner, S. C., Williams, C. C., Snyder, R. W., Krol, W. L., Asgharian, B. and Fennell, T. R. Acrylamide: a comparison of metabolism and hemoglobin adducts in rodents following dermal, intraperitoneal, oral, or inhalation exposure. Toxicol Sci 2003; 75: 260-70.
41. Barber, D. S., Hunt, J. R., Ehrich, M. F., Lehning, E. J. and LoPachin, R. M. Metabolism, toxicokinetics and hemoglobin adduct formation in rats following subacute and subchronic acrylamide dosing. Neurotoxicology 2001; 22: 341-53.
42. Miller, M. J., Carter, D. E. and Sipes, I. G. Pharmacokinetics of Acrylamide in Fisher-334 Rats. Toxicol Appl Pharmacol 1982; 63: 36-44.
43. Schettgen, T., Kutting, B., Hornig, M., Beckmann, M. W., Weiss, T., Drexler, H. and Angerer, J. Trans-placental exposure of neonates to acrylamide-a pilot study. Int Arch Occup Environ Health 2004; 74:
44. Edwards, P. M. The insensitivity of the developing rat foetus to the toxic effects of acrylamide. Chem Biol Interact 1976; 12: 13-18.
45. Marlowe, C., Clark, M. J., Mast, R. W., Friedman, M. A. and Waddell, W. J. The distribution of [14C]acrylamide in male and pregnant Swiss-Webster mice studied by whole-body autoradiography. Toxicol Appl Pharmacol 1986; 86: 457-65.
46. Ikeda, G. J., Miller, E., Sapienza, P. P., Michel, T. C., King, M. T. and Sager, A. O. Maternal-foetal distribution studies in late pregnancy. II. Distribution of [1-14C]acrylamide in tissues of beagle dogs and miniature pigs. Food Chem Toxicol 1985; 23: 757-61.
47. Ikeda, G. J., Miller, E., Sapienza, P. P., Michel, T. C., King, M. T., Turner, V. A., Blumenthal, H., Jackson, W. E., 3rd and Levin, S. Distribution of 14C-labelled acrylamide and betaine in foetuses of rats, rabbits, beagle dogs and miniature pigs. Food Chem Toxicol 1983; 21: 49-58.
48. Kirman, C. R., Gargas, M. L., Deskin, R., Tonner-Navarro, L. and Andersen, M. E. A physiologically based pharmacokinetic model for acrylamide and its metabolite, glycidamide, in the rat. J Toxicol Environ Health A 2003; 66: 253-74.

49. Sumner, S. C., Fennell, T. R., Moore, T. A., Chanas, B., Gonzalez, F. and Ghanayem, B. I. Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. Chemical Research In Toxicology 1999; 12: 1110-1116.
50. Bergmark, E., Calleman, C. J. and Costa, L. G. Formation of hemoglobin adducts of acrylamide and its epoxide metabolite glycidamide in the rat. Toxicol Appl Pharmacol 1991; 111: 352-363.
51. Igisu, H., Goto, I., Kawamura, Y., Kato, M. and Izumi, K. Acrylamide encephaloneuropathy due to well water pollution. J Neurol Neurosurg Psychiatry 1975; 38: 581-4.
52. NICNAS. Acrylamide: Priority existing chemical assessment report No. 23. 2002.
53. He, F. S., Zhang, S. L., Wang, H. L., Li, G., Zhang, Z. M., Li, F. L., Dong, X. M. and Hu, F. R. Neurological and electroneuromyographic assessment of the adverse effects of acrylamide on occupationally exposed workers. Scand J Work Environ Health 1989; 15: 125-9.
54. NTP. NTP Chemical Repository: Acrylamide. 2001.
55. Burek, J. D., Albee, R. R., Beyer, J. E., Bell, T. J., Carreon, R. M., Morden, D. C., Wade, C. E., Hermann, E. A. and Gorzinski, S. J. Subchronic toxicity of acrylamide administered to rats in the drinking water followed by up to 144 days of recovery. J Environ Pathol Toxicol 1980; 4: 157-82.
56. EPA. IRIS Acrylamide File. 2001.
57. Johnson, K. A., Gorzinski, S. J., Bodner, K. M., Campbell, R. A., Wolf, C. H., Friedman, M. A. and Mast, R. W. Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. Toxicol Appl Pharmacol 1986; 85: 154-68.
58. Dearfield, K. L., Douglas, G. R., Ehling, U. H., Moore, M. M., Sega, G. A. and Brusick, D. J. Acrylamide: a review of its genotoxicity and an assessment of heritable genetic risk. Mutat Res 1995; 330: 71-99.
59. Besaratinia, A. and Pfeifer, G. P. Weak yet distinct mutagenicity of acrylamide in mammalian cells. J Natl Cancer Inst 2003; 95: 889-96.
60. Shiraishi, Y. Chromosome aberrations induced by monomeric acrylamide in bone marrow and germ cells of mice. Mutat Res 1978; 57: 313-24.
61. Xiao, Y. and Tates, A. D. Increased frequencies of micronuclei in early spermatids of rats following exposure of young primary spermatocytes to acrylamide. Mutat Res 1994; 309: 245-53.
62. Dobrzynska, M. M. and Gajewski, A. K. Induction of micronuclei in bone marrow and sperm head abnormalities after combined exposure of mice to low doses of X-rays and acrylamide. Teratog Carcinog Mutagen 2000; 20: 133-40.
63. Backer, L. C., Dearfield, K. L., Erexson, G. L., Campbell, J. A., Westbrook-Collins, B. and Allen, J. W. The effects of acrylamide on mouse germ-line and somatic cell chromosomes. Environ Mol Mutagen 1989; 13: 218-26.
64. Adler, I. D. Clastogenic Effects of Acrylamide in Different Germ-Cell Stages of Male Mice. Banbury Report 1990; 34: 115-131.
65. Collins, B. W., Howard, D. R. and Allen, J. W. Kinetochore-staining of spermatid micronuclei: studies of mice treated with X-radiation or acrylamide. Mutat Res 1992; 281: 287-94.
66. Russo, A., Gabbani, G. and Simoncini, B. Weak genotoxicity of acrylamide on premeiotic and somatic cells of the mouse. Mutat Res 1994; 309: 263-72.
67. Lahdetie, J., Suutari, A. and Sjoblom, T. The spermatid micronucleus test with the dissection technique detects the germ cell mutagenicity of acrylamide in rat meiotic cells. Mutation Research 1994; 309: 255-262.
68. Gassner, P. and Adler, I. D. Induction of hypoploidy and cell cycle delay by acrylamide in somatic and germinal cells of male mice. Mutation Research 1996; 367: 195-202.
69. Bjorge, C., Brunborg, G., Wiger, R., Holme, J. A., Scholz, T., Dybing, E. and Soderlund, E. J. A comparative study of chemically induced DNA damage in isolated human and rat testicular cells. Reprod Toxicol 1996; 10: 509-19.
70. Schmid, T. E., Xu, W. and Adler, I. D. Detection of aneuploidy by multicolor FISH in mouse sperm after in vivo treatment with acrylamide, colchicine, diazepam or thiabendazole. Mutagenesis 1999; 14: 173-179.
71. Gassner, P. and Adler, I. D. Analysis of chemically induced spindle aberrations in male mouse germ cells: comparison of differential and immunofluorescent staining procedures. Mutagenesis 1995; 10: 243-52.

72. Smith, M. K., Zenick, H., Preston, R. J., George, E. L. and Lon, R. E. Dominant Lethal Effects Of Subchronic Acrylamide Administration In The Male Long-Evans Rat. Mutation Research 1986; 173: 273-277.
73. Sublet, V. H., Zenick, H. and Smith, M. K. Factors associated with reduced fertility and implantation rates in females mated to acrylamide-treated rats. Toxicology 1989; 55: 53-67.
74. Ehling, U. H. and Neuhaeuser-Klaus, A. Reevaluation of the induction of specific-locus mutations in spermatogonia of the mouse by acrylamide. Mutat Res 1992; 283: 185-191.
75. Gutierrez-Espeleta, G. A., Hughes, L. A., Piegorsch, W. W., Shelby, M. D. and Generoso, W. M. Acrylamide: dermal exposure produces genetic damage in male mouse germ cells. Fundam Appl Toxicol 1992; 18: 189-92.
76. NTP. Final report on the reproductive toxicity of acrylamide (ACRL) (CAS no. 79-06-1) in CD-1 Swiss mice. NTIS Technical Report 1993;
77. Adler, I. D., Baumgartner, A., Gonda, H., Friedman, M. A. and Skerhut, M. 1-Aminobenzotriazole inhibits acrylamide-induced dominant lethal effects in spermatids of male mice. Mutagenesis 2000; 15: 133-6.
78. Zenick, H., Hope, E. and Smith, M. K. Reproductive toxicity associated with acrylamide treatment in male and female rats. J Toxicol Environ Health 1986; 17: 457-72.
79. Working, P. K., Bentley, K. S., Hurtt, M. E. and Mohr, K. L. Comparison of the dominant lethal effects of acrylonitrile and acrylamide in male Fischer 344 rats. Mutagenesis 1987; 2: 215-20.
80. Shelby, M. D., Cain, K. T., Cornett, C. V. and Generoso, W. M. Acrylamide: induction of heritable translocation in male mice. Environ Mutagen 1987; 9: 363-8.
81. Dobrzynska, M., Lenarczyk, M. and Gajewski, A. K. Induction of dominant lethal mutations by combined X-ray-acrylamide treatment in male mice. Mutat Res 1990; 232: 209-15.
82. Nagao, T. Developmental abnormalities due to exposure of mouse paternal germ cells, preimplantation embryos, and organogenic embryos to acrylamide. Congenital Anomalies 1994; 34: 35-46.
83. Tyl, R. W., Friedman, M. A., Losco, P. E., Fisher, L. C., Johnson, K. A., Strother, D. E. and Wolf, C. H. Rat two-generation reproduction and dominant lethal study of acrylamide in drinking water. Reprod Toxicol 2000; 14: 385-401.
84. Pacchierotti, F., Tiveron, C., D'Archivio, M., Bassani, B., Cordelli, E., Leter, G. and Spano, M. Acrylamide-induced chromosomal damage in male mouse germ cells detected by cytogenetic analysis of one-cell zygotes. Mutat Res 1994; 309: 273-84.
85. Titenko-Holland, N., Ahlborn, T., Lowe, X., Shang, N., Smith, M. T. and Wyrobek, A. J. Micronuclei and developmental abnormalities in 4-day mouse embryos after paternal treatment with acrylamide. Environmental And Molecular Mutagenesis 1998; 31: 206-217.
86. Adler, I. D., Reitmeir, P., Schmoller, R. and Schriever-Schwemmer, G. Dose response for heritable translocations induced by acrylamide in spermatids of mice. Mutat Res 1994; 309: 285-91.
87. Russell, L. B., Hunsicker, P. R., Cacheiro, N. L. and Generoso, W. M. Induction of specific-locus mutations in male germ cells of the mouse by acrylamide monomer. Mutat Res 1991; 262: 101-107.
88. Neuhauser-Klaus, A. and Schmahl, W. Mutagenic and teratogenic effects of acrylamide in the mammalian spot test. Mutat Res 1989; 226: 157-62.
89. Sega, G. A. Adducts in sperm protamine and DNA vs. mutation frequency. Prog Clin Biol Res 1991; 372: 521-30.
90. Sega, G. A. and Generoso, E. E. Measurement of DNA breakage in specific germ-cell stages of male mice exposed to acrylamide, using an alkaline-elution procedure. Mutat Res 1990; 242: 79-87.
91. Sega, G. A., Generoso, E. E. and Brimer, P. A. 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 1990; 16: 137-142.
92. Generoso, W. M., Sega, G. A., Lockhart, A. M., Hughes, L. A., Cain, K. T., Cacheiro, N. L. A. and Shelby, M. D. Dominant Lethal Mutations, Heritable Translocations, and Unscheduled DNA Synthesis Induced in Male Mouse Germ Cells by Glycidamide, a Metabolite of Acrylamide. Mutation Research 1996; 3: 175-183.

93. Collins, J. J., Swaen, G. M., Marsh, G. M., Utidjian, H. M., Caporossi, J. C. and Lucas, L. J. Mortality patterns among workers exposed to acrylamide. J Occup Med 1989; 31: 614-7.
94. Sobel, W., Bond, G. G., Parsons, T. W. and Brenner, F. E. Acrylamide cohort mortality study. Br J Ind Med 1986; 43: 785-8.
95. Marsh, G. M., Lucas, L. J., Youk, A. O. and Schall, L. C. Mortality patterns among workers exposed to acrylamide: 1994 follow up. Occupational And Environmental Medicine 1999; 56: 181-190.
96. Pelucchi, C., Franceschi, S., Levi, F., Trichopoulos, D., Bosetti, C., Negri, E. and La Vecchia, C. Fried potatoes and human cancer. Int J Cancer 2003; 105: 558-60.
97. Mucci, L. A., Dickman, P. W., Steineck, G., Adami, H. O. and Augustsson, K. 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 2003; 88: 84-9.
98. Friedman, M. A., Dulak, L. H. and Stedham, M. A. A lifetime oncogenicity study in rats with acrylamide. Fundam Appl Toxicol 1995; 27: 95-105.
99. Damjanov, I. and Friedman, M. A. Mesotheliomas of tunica vaginalis testis of Fischer 344 (F344) rats treated with acrylamide: a light and electron microscopy study. In Vivo 1998; 12: 495-502.
100. Fullerton, P. M. and Barnes, J. M. Peripheral neuropathy in rats produced by acrylamide. Br J Ind Med 1966; 23: 210-21.
101. Kaplan, M. L. and Murphy, S. D. Effect of acrylamide on rotarod performance and sciatic nerve beta-glucuronidase activity of rats. Toxicol Appl Pharmacol 1972; 22: 259-68.
102. Suzuki, K. and Pfaff, L. D. Acrylamide neuropathy in rats. An electron microscopic study of degeneration and regeneration. Acta Neuropathol (Berl) 1973; 24: 197-213.
103. Ko, M. H., Chen, W. P., Lin-Shiau, S. Y. and Hsieh, S. T. Age-dependent acrylamide neurotoxicity in mice: morphology, physiology, and function. Exp Neurol 1999; 158: 37-46.
104. Husain, R., Dixit, R., Das, M. and Seth, P. K. 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 1987; 25: 19-28.
105. Dixit, R., Husain, R., Mukhtar, H. and Seth, P. K. Acrylamide induced inhibition of hepatic glutathione-S-transferase activity in rats. Toxicol Lett 1981; 7: 207-10.
106. McCarver, D. G. and Hines, R. N. The ontogeny of human drug-metabolizing enzymes: phase II conjugation enzymes and regulatory mechanisms. J Pharmacol Exp Ther 2002; 300: 361-6.
107. Hayes, J. D. and Pulford, D. J. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 1995; 30: 445-600.
108. Hines, R. N. and McCarver, D. G. The ontogeny of human drug-metabolizing enzymes: phase I oxidative enzymes. J Pharmacol Exp Ther 2002; 300: 355-60.
109. Anzenbacher, P. and Anzenbacherova, E. Cytochromes P450 and metabolism of xenobiotics. Cell Mol Life Sci 2001; 58: 737-47.
110. BIO/DYNAMICS-INC. A Fetal Toxicity Study of Acrylamide in Rats. EPA/OTS; Doc #878211679; NTIS/OTS0206055 1979;
111. ALBERT-EINSTEIN-CLG-OF-MEDICIN. A Fetal Toxicity Study of Acrylamide in Rats. Wayne, NJ: 1980.
112. Walden, R., Squibb, R. E. and Schiller, C. M. Effects of prenatal and lactational exposure to acrylamide on the development of intestinal enzymes in the rat. Toxicol Appl Pharmacol 1981; 58: 363-9.
113. Field, E. A., Price, C. J., Sleet, R. B., Marr, M. C., Schwetz, B. A. and Morrissey, R. E. Developmental toxicity evaluation of acrylamide in rats and mice. Fundam Appl Toxicol 1990; 14: 502-12.
114. Rutledge, J. C., Generoso, W. M., Shourbaji, A., Cain, K. T., Gans, M. and Oliva, J. Developmental anomalies derived from exposure of zygotes and first-cleavage embryos to mutagens. Mutat Res 1992; 296: 167-177.
115. Agrawal, A. K. and Squibb, R. E. Effects of acrylamide given during gestation on dopamine receptor binding in rat pups. Toxicol Lett 1981; 7: 233-8.
116. Wise, L. D., Gordon, L. R., Soper, K. A., Duchai, D. M. and Morrissey, R. E. Developmental neurotoxicity evaluation of acrylamide in Sprague-Dawley rats. Neurotoxicology And Teratology 1995; 17: 189-198.

117. Walum, E. and Flint, O. P. Selective Effects of Acrylamide, Metylene Bisacrylamide, and Haloperidol on Neuronal Development in Rat Embryo Midbrain Micromass Cultures. In Vitro Toxicology. A Journal of Molecular and Cellular Toxicology 1993; 6: 125-134.
118. Friedman, M. A., Tyl, R. W., Marr, M. C., Myers, C. B., Gerling, F. S. and Ross, W. P. Effects of lactational administration of acrylamide on rat dams and offspring. Reprod Toxicol 1999; 13: 511-20.
119. Sakamoto, J. and Hashimoto, K. Reproductive toxicity of acrylamide and related compounds in mice--effects on fertility and sperm morphology. Arch Toxicol 1986; 59: 201-5.
120. Bishop, J. B., Morris, R. W., Seely, J. C., Hughes, L. A., Cain, K. T. and Generoso, W. M. Alterations in the reproductive patterns of female mice exposed to xenobiotics. Fundamental And Applied Toxicology 1997; 40: 191-204.
121. Hashimoto, K., Sakamoto, J. and Tanii, H. Neurotoxicity of acrylamide and related compounds and their effects on male gonads in mice. Arch Toxicol 1981; 47: 179-89.
122. Sakamoto, J., Kurosaka, Y. and Hashimoto, K. Histological changes of acrylamide-induced testicular lesions in mice. Exp Mol Pathol 1988; 48: 324-34.
123. Costa, L. G., Deng, H., Gregotti, C., Manzo, L., Faustman, E. M., Bergmark, E. and Calleman, C. J. Comparative studies on the neuro- and reproductive toxicity of acrylamide and its epoxide metabolite glycidamide in the rat. Neurotoxicology 1992; 13: 219-24.
124. Tyl, R. W., Marr, M. C., Myers, C. B., Ross, W. P. and Friedman, M. A. Relationship between acrylamide reproductive and neurotoxicity in male rats. Reprod Toxicol 2000; 14: 147-57.
125. Chapin, R. E., Fail, P. A., George, J. D., Grizzle, T. B., Heindel, J. J., Harry, G. J., Collins, B. J. and Teague, J. The reproductive and neural toxicities of acrylamide and three analogues in Swiss mice, evaluated using the continuous breeding protocol. Fundamental And Applied Toxicology 1995; 27: 9-24.