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Provisional Peer-Reviewed Toxicity Values for

Methacrylonitrile
(CASRN 126-98-7)

Superfund Health Risk Technical Support Center
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Questions regarding the contents of this document may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

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COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMCL	benchmark concentration lower bound 95% confidence interval
BMD	benchmark dose
BMDL	benchmark dose lower bound 95% confidence interval
HEC	human equivalent concentration
HED	human equivalent dose
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
POD	point of departure
p-OSF	provisional oral slope factor
p-RfC	provisional reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
RfC	reference concentration (inhalation)
RfD	reference dose (oral)
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete-to-complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOE	weight of evidence

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR METHACRYLONITRILE (CASRN 126-98-7)

BACKGROUND

A Provisional Peer-Reviewed Toxicity Value (PPRTV) is defined as a toxicity value derived for use in the Superfund Program. PPRTVs are derived after a review of the relevant scientific literature using established Agency guidance on human health toxicity value derivations. All PPRTV assessments receive internal review by a standing panel of National Center for Environment Assessment (NCEA) scientists and an independent external peer review by three scientific experts.

The purpose of this document is to provide support for the hazard and dose-response assessment pertaining to chronic and subchronic exposures to substances of concern, to present the major conclusions reached in the hazard identification and derivation of the PPRTVs, and to characterize the overall confidence in these conclusions and toxicity values. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of this substance.

The PPRTV review process provides needed toxicity values in a quick turnaround timeframe while maintaining scientific quality. PPRTV assessments are updated approximately on a 5-year cycle for new data or methodologies that might impact the toxicity values or characterization of potential for adverse human health effects and are revised as appropriate. It is important to utilize the PPRTV database (http://hhpprtv.ornl.gov) to obtain the current information available. When a final Integrated Risk Information System (IRIS) assessment is made publicly available on the Internet (http://www.epa.gov/iris), the respective PPRTVs are removed from the database.

DISCLAIMERS

The PPRTV document provides toxicity values and information about the adverse effects of the chemical and the evidence on which the value is based, including the strengths and limitations of the data. All users are advised to review the information provided in this document to ensure that the PPRTV used is appropriate for the types of exposures and circumstances at the site in question and the risk management decision that would be supported by the risk assessment.

Other U.S. Environmental Protection Agency (EPA) programs or external parties who may choose to use PPRTVs are advised that Superfund resources will not generally be used to respond to challenges, if any, of PPRTVs used in a context outside of the Superfund program.

QUESTIONS REGARDING PPRTVs

Questions regarding the contents and appropriate use of this PPRTV assessment should be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

INTRODUCTION

Methacrylonitrile is an unsaturated aliphatic nitrile (NTP, 2000, 2001). Also known as methylacrylonitrile or 2-cyanopropene, methacrylonitrile is commonly used in the preparation of homopolymers and copolymers, elastomers, and plastics and as a chemical intermediate in the preparation of acids, amides, amines, esters, and other nitriles (Budavari, 1996). Methacrylonitrile is also used as a replacement for acrylonitrile in the manufacture of an acrylonitrile/butadiene/styrene-like polymer (Considine, 1974). Methacrylonitrile has been identified as a component in unfiltered cigarette smoke (Baker et al., 1984). Table 1 summarizes physicochemical properties for methacrylonitrile. Figure 1 shows the chemical structure of methacrylonitrile.

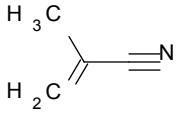


Figure 1. Methacrylonitrile Structure

Table 1. Physical Properties Table for Methacrylonitrile (CASRN 126-98-7)[a]

Property (unit)	Value
Boiling point (ºC)	90.3
Melting point (ºC)	−35.8
Density (g/cm^3)	0.8001
Vapor pressure (mm Hg at 25ºC)	7.12
pH (unitless)	Data not available
Solubility in water (g/100 mL at 25ºC)	2.54
Relative vapor density (air = 1)	2.31
Molecular weight (g/mol)	67.09

[a]Chem ID Plus, 2010 and HSDB, 2009.

The IRIS database (U.S. EPA, 1987a) lists a Reference Dose (RfD) of 1×10^{-4} mg/kg-day for methacrylonitrile based on a subchronic inhalation study in dogs with increased SGOT (serum glutamic oxaloacetic transaminase, also known as aspartate aminotransferase [AST]) and SGPT (serum glutamic pyruvic transaminase, also known as alanine aminotransferase [ALT]) levels where the lowest-observed-adverse-effect level (LOAEL) was noted at 8.8 ppm, and the no-observed-adverse-effect level (NOAEL) was noted at 3.2 ppm (Pozzani et al., 1968). The NOAEL was used as the point of departure to calculate the RfD. Neither a Reference Concentration (RfC) nor a cancer assessment for methacrylonitrile are listed in the IRIS database (U.S. EPA, 1987a). Methacrylonitrile is not included on the Drinking Water Standards and Health Advisories List (U.S. EPA, 2006) or on the Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1994). A subchronic RfD of 0.001 mg/kg-day is reported in the Health Effects Assessment Summary Tables (HEAST)

(U.S. EPA, 2010) for increased SGPT based on the study by Pozzani et al. (1968). In addition, both a subchronic RfC of 0.007 mg/m^3 and a chronic RfC of 0.0007 mg/m^3 for increased SGPT levels based on Pozzani et al. (1968) are reported in the HEAST. HEAST also cites, for methacrylonitrile, a Health and Environmental Effects Document (HEED) for selected nitriles (U.S. EPA, 1987b). The toxicity of methacrylonitrile has not been reviewed by the Agency for Toxic Substances and Disease Registry (ATSDR, 2010) or the World Health Organization (WHO, 2010). The California Environmental Protection Agency (CalEPA, 2008) has not derived toxicity values for exposure to methacrylonitrile. The American Conference of Governmental Industrial Hygienists (ACGIH, 2005) has established a threshold limit value (TLV) of 1 ppm for a time-weighted-average (TWA) with a "skin" notation (indicating possible skin absorption). The National Institute of Occupational Safety and Health (NIOSH, 2010) has also established a recommended exposure limit (REL) of 1-ppm (3 mg/m^3) TWA for methacrylonitrile, with a "skin" notation. No occupational exposure limit for methacrylonitrile has been derived by the Occupational Safety and Health Administration (OSHA, 2010).

HEAST does not report any carcinogenicity values for methacrylonitrile (U.S. EPA, 2010). The International Agency for Research on Cancer (IARC, 2010) has not reviewed the carcinogenic potential of methacrylonitrile. Methacrylonitrile is not included in the *12th Report on Carcinogens* (NTP, 2011). CalEPA (2008) has not derived a quantitative estimate of the carcinogenic potential of methacrylonitrile. The National Toxicology Program (NTP) conducted a 2-year carcinogenicity study of methacrylonitrile (gavage exposure) in rats and mice and concluded that there was no evidence of carcinogenic activity in either sex of either species (NTP, 2001).

Literature searches were conducted on sources published from 1900 through March 7, 2012, for studies relevant to the derivation of provisional toxicity values for methacrylonitrile, CASRN 126-98-7. Searches were conducted using EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: AGRICOLA; American Chemical Society; BioOne; Cochrane Library; DOE: Energy Information Administration, Information Bridge, and Energy Citations Database; EBSCO: Academic Search Complete; GeoRef Preview; GPO: Government Printing Office; Informaworld; IngentaConnect; J-STAGE: Japan Science & Technology; JSTOR: Mathematics & Statistics and Life Sciences; NSCEP/NEPIS (EPA publications available through the National Service Center for Environmental Publications [NSCEP] and National Environmental Publications Internet Site [NEPIS] database); PubMed: MEDLINE and CANCERLIT databases; SAGE; Science Direct; Scirus; Scitopia; SpringerLink; TOXNET (Toxicology Data Network): ANEUPL, CCRIS, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HEEP, HMTC, HSDB, IRIS, ITER, LactMed, Multi-Database Search, NIOSH, NTIS, PESTAB, PPBIB, RISKLINE, TRI, and TSCATS; Virtual Health Library; Web of Science (searches Current Content database among others); World Health Organization; and Worldwide Science. The following databases outside of HERO were searched for health information: ACGIH, ATSDR, CalEPA, EPA IRIS, EPA HEAST, EPA HEEP, EPA OW, EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)

Table 2 provides an overview of the relevant database for methacrylonitrile and includes all potentially relevant repeat-dose short-term-, subchronic-, and chronic-duration studies. Principal studies are identified. Entries for the principal studies are bolded.

HUMAN STUDIES

Oral Exposures

No studies investigating the effects of oral exposure to methacrylonitrile in humans have been identified.

Inhalation Exposures

The effects of inhalation exposure on humans to methacrylonitrile were evaluated in two short-term studies (both identified as Pozzani et al., 1968). No other studies have been located regarding the effects of inhalation exposure to methacrylonitrile in humans.

Short-term Studies

Pozzani et al. (1968) performed a peer-reviewed study involving 8−9 volunteers (ages 22−57, sex not specified) whereby subjects were exposed to methacrylonitrile vapor at concentrations of 0, 2, 7, 14, or 24 ppm (equivalent to 0, 5.5, 19.2, 38.4, and 65.9 mg/m^3). Subjects were placed in a glass-lined room and exposed to a series of methacrylonitrile concentrations in the following order: 24, 14, 0, 7, 14, 24, 7, 2, 0, and 2 ppm. Each of the methacrylonitrile concentrations was administered for 1 minute, and after the series of 10 varying exposure concentrations, the study authors repeated the experiment with a minimum time lapse of 45 minutes between experiments. The subjects were unaware of the order in which they were exposed to the concentrations. Throughout the exposure, the subjects responded to simple questions regarding odor detection and throat-, eye-, and nose irritation. At 24 and 14 ppm, most subjects could detect an odor initially, but only half the subjects could detect the 7-ppm concentration of methacrylonitrile. None of the subjects were able to distinguish 0 ppm from 2 ppm. Subjects reported irritation of the throat (17% of subjects), eye (22% of subjects), and nose (6% of subjects) after exposure to the 24-ppm concentration. No subjects reported irritation at any other concentration. Based on the limited details in this study, the LOAEL is identified as 24 ppm (65.9 mg/m^3) with a NOAEL of 14 ppm (38.4 mg/m^3) based on irritation of mucous membranes.

Another experiment by Pozzani et al. (1968) exposed a group of nine volunteers (sex and age not provided) to 2-ppm (equivalent to 5.5 mg/m^3) methacrylonitrile vapor for 10 minutes. In the same experiment, the study authors exposed another group of seven subjects (sex and age not provided) to 14 ppm (equivalent to 38.4 mg/m^3) for 10 minutes. Occurrence of throat-, eye-, and nose irritation was recorded after each minute. Lacrimation as a result of exposure was also measured after each minute, as well as the number of subjects able to detect an odor. Lacrimation and nose-, throat-, and eye irritation were reported in at least 1−2 subjects over the 10-minute time interval at 2 ppm, and in at least one subject at 14 ppm. No other details were provided. The details in this study were too limited to assign a NOAEL or LOAEL.

Table 2. Summary of Potentially Relevant Data for Methacrylonitrile (CASRN 126-98-7)

Category	Number of Male/Female, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference/Comments	Notes[b]
Human								
			1. Oral (mg/kg-d)[a]					
None								
			2. Inhalation (mg/m^3)[a]					
Short-term	8−9 subjects (sex not specified), case report, 10 exposures for 1 min, repeated with 45 min or longer intervals in between	0, 2, 7, 14, 24 ppm (0, 5.5, 19.2, 38.4, 65.9)	Nose- (6 %), throat- (17 %), or eye irritation (22 %) experienced in the high-dose group.	38.4	Not run	65.9	Pozzani et al. (1968)	
	16 subjects (sex and age not specified), case report, 10 min	2 ppm or 14 ppm (5.5 [9 subjects] or 38.4 [7 subjects])	Nose-, throat-, and eye irritation in at least 1−2 subjects in both dose groups.	Not determined	Not run	Not determined	Pozzani et al. (1968)	
Animal								
			1. Oral (mg/kg-d)[a]					
Subchronic	**10/10, F344/N rat, gavage, 5 d/wk, 13 wk**	**0, 7.5, 15, 30, 60, 120 (0, 5.36, 10.7, 21.4, 42.9, 85.7[c])**	**Statistically significant increases (>10%) in absolute and relative liver and lung weights in males at 30 mg/kg-d. Statistically significant increases in olfactory epithelium metaplasia in males at 60 mg/kg-d and females at 60 and 120 mg/kg-d.**	**10.7**	**5.06**	**21.4**	**NTP (2000)**	**PS p-RfD$_S$**
	12/12, Sprague-Dawley rat, daily gavage, 39−51 d	0, 7.5, 15, 30	Anemia in males at 21 mg/kg-d.	15	Not run	30	MHLW (2001)	

Table 2. Summary of Potentially Relevant Data for Methacrylonitrile (CASRN 126-98-7)

Category	Number of Male/Female, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference/Comments	Notes[b]
	10−16/0, Sprague-Dawley rat, gavage, 5 d/wk, 12 wk	0, 50, 70, 90 (0, 36, 50, 64[c])	Death of two rats at 50 mg/kg-d and 8 rats at 90 mg/kg-d. No changes in electrophysiological parameters.	None	Not run	None (FEL = 36)	Gagnaire et al. (1998)	
	10/10, B6C3F$_1$ mouse, gavage, 5 d/wk, 13 wk	0, 0.75, 1.5, 3, 6, 12 (0, 0.54, 1.1, 2.1, 4.3, 8.6[c])	No adverse treatment-related effects at any dose. No effects on reproductive organ weights or sperm motility patterns.	8.6	Not run	None	NTP (2000)	
Chronic	50/50, F344/N rat, gavage, 5 d/wk, 2 yr	0, 3, 10, 30 (0, 2.14, 7.14, 21.4[c])	Increased incidences of cytoplasmic vacuolization in liver in males at 30 mg/kg-d and in females at ≥3 mg/kg-d.	None	Not run	2.14	NTP (2001)	
	50/50, B6C3F$_1$ mouse, gavage, 5 d/wk, 2 yr	0, 1.5, 3, 6 (0, 1.07, 2.14, 4.29[c])	No nonneoplastic effects observed at any dose.	4.29	Not run	None	NTP (2001)	
Developmental	0/26, Sprague-Dawley rat, gavage, GDs 6−15	0, 5, 25, 50	No effect on number of live fetuses, fetal body weight, or morphological development.	50 (develop-mental), 50 (maternal)	Not run	None (develop-mental), None (maternal)	NTP (1993a); George et al. (1996)	
	0/17−22, New Zealand White rabbit, gavage, GDs 6−19	0, 1, 3, 5	1 death at 3 mg/k-d, 1 death at 5 mg/kg-d in maternal rabbits (unexplained). No adverse effect on postimplantation loss or fetal body weight. No effect on litter size or malformations.	5 (develop-mental), 5 (maternal)	Not run	None (develop-mental), None (maternal)	NTP (1993b); George et al. (1996)	
	0/6 Sprague-Dawley rat, gavage, 1st or 2nd wk of gestation	50 (1st wk of gestation), 50, 100 (2nd wk of gestation)	Ataxia, decreased body weights, edema in fallopian tubes, effects on fertility.	None	Not run	50	Farooqui and Villarreal (1992)	

Table 2. Summary of Potentially Relevant Data for Methacrylonitrile (CASRN 126-98-7)

Category	Number of Male/Female, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference/Comments	Notes[b]
	Female Sprague-Dawley rat (number. not provided), oral (no further details provided), 1st or 2nd wk of gestation	50 (1st wk of gestation), 100 (2nd wk of gestation)	Litters aborted at 50 mg/kg-d.	None	Not run	None (FEL = 50)	Villarreal et al. (1988)	
Reproductive	20/20, Sprague-Dawley rat, daily oral gavage, Reproductive Assessment by Continuous Breeding study, F0 exposed from Day 1 for approximately 15 wk, F1 exposed from weaning to approximately 80 d old.	F0: 0, 2, 7, 20 F1: 0, 20	In F1 rats, 19% decrease in epididymal sperm density and organ weight changes at 20 mg/kg-d but sperm morphology not changed.	7 (F0)	9.7	20 (F1)	NTP (1997)	
	12/12, Sprague-Dawley rat, daily gavage, 46 d (m), 14 d before mating to Day 4 of lactation (f)	0, 7.5, 15, 30	No effects on reproductive performance such as mating, fertility, delivery, and lactation in both sexes of all treated rats.	30	Not run	None	MHLW (2001)	
Carcinogenic	50/50, F344/N rat, gavage, 5 d/wk, 2 yr	0, 3, 10, 30 (0, 2, 7, 21[c])	No increase in neoplastic effects at any dose.	None	Not run	None	NTP (2001)	
	50/50, B6C3F$_1$ mouse, 5 d/wk, gavage, 2 yr	0, 1.5, 3, 6 (0, 1, 2, 4[c])	No increase in neoplastic effects at any dose.	None	Not run	None	NTP (2001)	

Table 2. Summary of Potentially Relevant Data for Methacrylonitrile (CASRN 126-98-7)

Category	Number of Male/Female, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference/Comments	Notes[b]
2. Inhalation (mg/m^3)[a]								
Short-term	6/6, Harlan-Wistar rat, 0.47, 0.93, 1.88, 3.75, 7.5, 14 min	234,270[d]	Death of 6/6 rats at 3.75-, 7.5-, and 14-min exposures.	None	Not run	None (FEL = 234,270)	Pozzani et al. (1968)	
	6/6, Harlan-Wistar rat, LC_{50}, 4 h	Not provided	LC_{50} observed to be 899 mg/m^3 (m), and 1359 and 1918 mg/m^3 (f).	None	Not run	None	Pozzani et al. (1968)	
	6/0, A/J strain mice, LC_{50}, 4 h	Not provided	LC_{50} observed to be 99 mg/m^3.	None	Not run	None	Pozzani et al. (1968)	
	6/0, Albino guinea pig, LC_{50}, 4 h	Not provided	LC_{50} observed to be 241 mg/m^3.	None	Not run	None	Pozzani et al. (1968)	
	4/0, Albino rabbit, LC_{50}, 4 h	Not provided	LC_{50} observed to be 100 mg/m^3.	None	Not run	None	Pozzani et al. (1968)	
	0/3 dogs (2 mongrels and 1 cocker spaniel), LC_{50}, 4 h	Not provided	LC_{50} not determined.	None	Not run	None	Pozzani et al. (1968)	
Subchronic	**12/12, Harlan-Wistar rat, 7 h/d, 5 d/wk, 91 d**	**0, 19.3, 52.6, 109.3 ppm (0, 11.0, 30.1, 62.5 mg/m^3 HEC)[e]**	**At 62.5 mg/m^3, statistically significantly higher (>10%) relative liver weights in males and females.**	**30.1**	**Not run**	**62.5**	**Pozzani et al. (1968)**	**PS**
	3/0, Beagle dog, 7 h/d, 5 d/wk, 90 d	**0, 3.2, 8.8, 13.5 ppm (0, 1.8, 4.9, 7.6 mg/m^3)[e]**	**Transient increases in SGOT and SGPT in 1/3 dogs at 4.9 mg/m^3.**	**1.8**	**Not run**	**4.9**	**Pozzani et al. (1968). IRIS (U.S. EPA, 1987a) derived an oral RfD based on this study, converting from inhalation exposure to oral exposure (method no longer used)**	**PS**

Table 2. Summary of Potentially Relevant Data for Methacrylonitrile (CASRN 126-98-7)

Category	Number of Male/Female, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference/Comments	Notes[b]
Chronic	None							
Developmental	0/20−23, Sprague-Dawley rat, 6 h/d, GDs 6−20	0, 32.9, 68.5, 137, 274[d]	Statistically significantly reduced fetal body weights at 274 mg/m^3.	137	Not run	274	Saillenfait et al. (1993)	
Reproductive	None							
Carcinogenic	None							

[a]Dosimetry: NOAEL, BMDL/BMCL, and LOAEL values are converted to an adjusted daily dose (ADD in mg/kg-d) for oral noncancer effects and a human equivalent concentration (HEC in mg/m^3) for inhalation noncancer effects. Values are converted to a human equivalent dose (HED in mg/kg-d) for oral carcinogenic effects. All long-term exposure values (4 wk and longer) are converted from a discontinuous to a continuous (weekly) exposure. Values from animal developmental studies are not adjusted to a continuous exposure.

[b]Notes: IRIS = Utilized by IRIS, date of last update; PS = Principal study, NPR = Not peer reviewed.

[c]Converted from a discontinuous (5 d/wk) to a continuous exposure (7 d/wk) by multiplying by 5 ÷ 7.

[d]Not converted to HEC exposure because short-term study. Converted to mg/m^3 equivalent concentrations as follows: ppm to mg/m^3: mg/m^3 = ppm × MW ÷ 24.45; MW methacrylonitrile = 67.09.

[e]Converted to HEC exposure as follows: dose × MW ÷ 24.45 × (h/d ÷ 24) × (d dosed ÷ total d) × blood gas partition coefficient for extrarespiratory effect = ppm × 67.09 g/mole ÷ 24.45 × (7 h ÷ 24 h) × (65 d ÷ 91 d) × 1 = ppm × 0.57166.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to methacrylonitrile have been evaluated in three subchronic (NTP, 2000; MHLW, 2001; Gagnaire et al., 1998), one chronic and carcinogenic (NTP, 2001), two developmental (NTP, 1993a,b; George et al., 1996), and four reproductive (NTP, 1997; MHLW, 2001; Farooqui and Villarreal, 1992; Villarreal et al., 1988) studies.

Short-term Studies

No studies could be located regarding the effects of short-term oral exposure of animals to methacrylonitrile.

Subchronic Studies

NTP, 2000; rat study

NTP (2000) is selected as the principal study for derivation of the subchronic p-RfD. As part of a peer-reviewed subchronic study of methacrylonitrile, NTP (2000) performed gavage studies in rats. This study was performed according to Good Laboratory Practice (GLP) standards. Groups of 20 male and 20 female F344/N rats were administered doses of 0-, 7.5-, 15-, 30-, 60-, or 120-mg/kg-day methacrylonitrile (99.9% purity), for 5 days/week (calculated to be equivalent to 0, 5.36, 10.7, 21.4, 42.9, and 85.7 mg/kg-day) by gavage in deionized, purified water, for up to 13 weeks. Ten males and 10 females from each dose group were preselected for interim evaluations at 32 days. The remaining 10 males and 10 females from each group were dosed 5 days/week, for 13 weeks. At the end of the dosing period (either 32 days or 13 weeks), the rats were euthanized and examined. All animals were necropsied, and the weight of the heart, right kidney, liver, lung, stomach (without contents), right testis, and thymus were recorded. Additionally, a clinical pathology evaluation, including hematology and clinical chemistry analyses, was performed on interim evaluation rats on Day 4 (hematology and clinical chemistry only) and on Day 32 on core study rats at the end of the 13-week study. A complete histopathological examination was conducted on all control rats, male rats in the 60-mg/kg-day dose group, female rats in the 120-mg/kg-day dose group, and all rats that died before scheduled evaluations. Tissues that exhibited lesions at gross examination were also examined microscopically in rats that received lower doses of methacrylonitrile. In addition, the nasal cavity was identified as a target organ and examined in all lower dose groups. Statistical data analysis was performed using the Fisher's Exact test, the parametric comparison procedures of Williams and Dunnett, the nonparametric multiple comparison methods of Shirley and Dunn, Jonckheere's test, and the Mann-Whitney U test.

At the 32-day interim evaluation, 9/10 male rats in the 120-mg/kg-day dose group died during the first week of study, while all the female rats in the 120-mg/kg-day dose group survived (NTP, 2000). Male rats administered 60 mg/kg-day in the 32-day interim evaluation group showed statistically significantly lower mean body weights than the control group rats, but the decrease was less than 10%.

In the 32-day interim evaluation, clinical toxicity effects were observed within minutes of dosing and were dose dependent (NTP, 2000). The clinical toxicity effects observed were lethargy, lacrimation, tremors, convulsions, ataxia, and abnormal breathing. The study authors did not specify at which doses these effects were observed, or whether one sex was affected

more than the other, but stated that the effects disappeared within several hours after dosing. In addition, in this interim evaluation group, no effects were observed on hematology in males or females when measured on Day 4, while on Day 32, there was a statistically significant decrease in hemoglobin at the 60-mg/kg-day dose in males, and a statistically significant decrease in hematocrit, hemoglobin, and erythrocytes at doses ≥30 mg/kg-day in females. Clinical chemistry results (32-day interim evaluation group) on Day 4 showed statistically significant decreases in males in urea nitrogen at doses ≥15 mg/kg-day, ALT at doses of 60 mg/kg-day, and sorbitol dehydrogenase at doses ≥15 mg/kg-day. On Day 32, only ALT levels remained statistically significantly decreased (at 60 mg/kg-day). In females, there was a statistically significant decrease in ALT on Day 4 at doses ≥7.5 mg/kg-day. On Day 32 in females, urea nitrogen was statistically significantly decreased at doses ≥60 mg/kg-day, and ALT was statistically significantly decreased at doses ≥15 mg/kg-day.

In the 13-week evaluation (NTP, 2000), 2/10 male rats in the 60-mg/kg-day dose group died during the first week. Only one male rat survived as long as 32 days. The remainder of the male rats and one female rat in the 120-mg/kg-day dose group died before the end of the study. When compared with the controls, male rats in the 60-mg/kg-day dose group had a slightly lower (9.5%), but statistically significant, final mean body weight. Females in the 120-mg/kg-day dose group also had lower (7.6%), but statistically significant, final mean body weights. Clinical toxicity effects of the 13-week group were observed within minutes of dosing and included lethargy, lacrimation, tremors, convulsions, ataxia, and abnormal breathing. These effects disappeared within several hours of dosing. Minimal anemia, as indicated by decreased hematocrit values, hemoglobin concentrations, and erythrocyte counts, was observed on Day 32 in males administered 60 or 120 mg/kg-day and females administered 30 mg/kg-day or greater. At Week 13, the anemia had improved, as was revealed only by small (not statistically significant) decreases in hemoglobin observed in males in the 60-mg/kg-day dose group and females in the 120-mg/kg-day dose group. ALT was statistically significantly decreased in females only at the highest dose (120 mg/kg-day).

A number of changes were observed in the relative and absolute organ weights at both the 32-day interim evaluation and the 13-week evaluation (NTP, 2000). In the 32-day interim evaluation at sacrifice, the absolute and relative liver weights of the female rats in the 120-mg/kg-day dose group were significantly greater (>10% difference in both absolute and relative) than those of the controls. Female rats in the 60- and 120-mg/kg-day dose groups had significantly greater stomach weights than the stomach weights of the control group. The absolute weights of the right kidney and thymus of the male rats in the 60-mg/kg-day dose group, and the absolute and relative thymus weights of female rats in the 120-mg/kg-day dose group were significantly less than those of the control group. Relative heart, stomach, and right testis weights were significantly greater in male rats in the 60-mg/kg-day dose group than the control group.

In the 13-week evaluation, males in the 30- and 60-mg/kg-day dose groups had absolute and relative liver weights that were statistically significantly greater than controls (at 30 mg/kg-day, absolute and relative liver weights >10% difference; at 60 mg/kg-day, absolute liver weights <10% difference, relative liver weights >10% difference) (NTP, 2000). Males in the 30- and 60-mg/kg-day dose groups also had relative lung weights that were statistically significantly greater than controls (9.7% and 10.4%, respectively). Males in the 60-mg/kg-day

dose group also had absolute stomach weights that were statistically significantly greater than controls. The relative stomach weights of all dose groups of males were statistically significantly greater than those in the control group. However, absolute stomach weights of males were only statistically significantly increased in the 60-mg/kg-day dose group. Absolute and relative stomach weights of females in the 60- or 120-mg/kg-day dose groups were statistically significantly increased compared to the control group. Females that were administered 60 or 120 mg/kg-day had absolute thymus weights that were statistically significantly decreased compared to controls, and females in the 120-mg/kg-day dose group also had statistically significantly decreased relative thymus weight when compared to the control groups. Females in the 120-mg/kg-day dose group had relative heart, right kidney, and liver weights that were statistically significantly greater (right kidney weights showed <10% difference; liver weights >10% difference) than the control groups. No significant differences were observed in reproductive organ weights or sperm motility parameters between the males in any of the dose groups and the controls. In the 60- or 120-mg/kg-day dose groups, females had significantly longer estrous cycles than the control group. Females administered the 60-mg/kg-day dose group spent more time in diestrus than those in the control group.

The study authors stated that no gross lesions observed at necropsy were attributed to methacrylonitrile administration (NTP, 2000). The study authors identified the olfactory epithelium of the nasal cavity as the primary target of methacrylonitrile toxicity in rats examined microscopically. Treatment-related changes consisting of necrotic and metaplastic effects were observed in the olfactory mucosa of the 60- and 120-mg/kg-day dose groups in both the 32-day interim and 13-week evaluations in females. Necrosis of the olfactory epithelium was characterized by the observation of cells undergoing different stages of necrosis. Characterization of metaplasia was indicated by replacement of damaged olfactory epithelium with respiratory epithelium and/or an undifferentiated type of epithelium. Due to the higher survival rate of females in the 120-mg/kg-day dose group, olfactory toxicity was more apparent in females than males, with statistically significant dose-related increases in the occurrence and severity of olfactory lesions occurring in females at both the 32-day interim and 13-week evaluations. In males, statistically significant increases in olfactory metaplasia were observed only at 60 mg/kg-day, in both the 32-day interim and 13-week evaluations. The study authors identified a NOAEL of 30 mg/kg-day for necrotic and metaplastic effects of the olfactory epithelium in rats. However, based on the statistically significant increase in relative lung and liver weights in male rats at 30 mg/kg-day, a LOAEL is identified at 30 mg/kg-day (adjusted for dosing schedule), and a NOAEL is identified at 10.7 mg/kg-day (equivalent to 11 mg/kg-day).

NTP, 2000; mouse study

In the same peer-reviewed 13-week NTP gavage study in rats discussed above, groups of 20 male and 20 female $B6C3F_1$ mice were administered 0-, 0.75-, 1.5-, 3-, 6-, or 12-mg/kg-day methacrylonitrile (99.9% purity) (equivalent to 0, 0.54, 1.1, 2.1, 4.3, and 8.6 mg/kg-day) by gavage in deionized, purified water, for 5 days/week, for 13 weeks (NTP, 2000). Ten animals of each sex from each dose group were preselected for interim evaluations at 32 days then sacrificed and examined. The remaining mice were sacrificed and examined at 13 weeks. All animals were necropsied, and the weights of the heart, right kidney, liver, lung, stomach (without contents), right testis, and thymus were recorded. A complete histopathological examination was conducted on all control mice, male and female mice in the 12-mg/kg-day dose group, and all mice that died before scheduled evaluations. Statistical data analyses were performed using the

Fisher's Exact test, the parametric comparison procedures of Williams and Dunnett, the nonparametric multiple comparison methods of Shirley and Dunn, Jonckheere's test, and the Mann-Whitney U test.

In the 32-day interim evaluation, one male in the 12-mg/kg-day dose group died during Week 3, and no reason was provided (NTP, 2000). Two females administered 1.5 mg/kg-day died before the end of the study as a result of gavage dosing errors (as noted in the report). All dosed groups had final mean body weights and mean body-weight gains that were similar to the control groups. Clinical toxicity effects were dose dependent and included lethargy, tremors, ataxia, convulsions, and abnormal breathing. All clinical toxicity effects were observed within minutes of dosing and disappeared within 2 to 3 hours after dosing.

In the mice evaluated at the end of the 13-week evaluation, two females in the 12-mg/kg-day dose group died early; one death was the result of a gavage dosing error, and no reason was provided for the other (NTP, 2000). All dosed mice had similar final mean body weights and mean body-weight gains compared to the control groups. Clinical findings for the 13-week evaluation were similar to those observed at the 32-day interim evaluation: effects were dose dependent and included lethargy, tremors, ataxia, convulsions, and abnormal breathing. These toxic effects were observed within minutes of dosing and disappeared within 2 to 3 hours after dosing.

Minimal differences were observed in organ weights at the 32-day interim evaluation and the 13-week evaluation (NTP, 2000). In the 32-day interim evaluation, males in the 12-mg/kg-day dose group had absolute thymus weights that were significantly less than the control groups. In the 32-day interim evaluation, the stomach weights of male mice that received 3 mg/kg-day or greater were significantly increased compared to the control group. At Week 13, males in the two highest dose groups (6 and 12 mg/kg-day) had significantly increased relative stomach weights (14% and 21% respectively), with increased (18%) absolute mean stomach weight only at the highest dose. NTP stated that the toxicological significance of the stomach-weight changes (without corresponding gross or microscopic pathologic changes) was difficult to determine; EPA considers the increased stomach weights not to be of toxicological significance. No significant differences in reproductive organ weights or sperm motility patterns were observed between male dosed and control groups. In addition, there were no biologically-significant differences observed in estrous cycle length or in the relative length of time spent in estrous stages between female dosed and control groups.

No gross or microscopic treatment-related lesions were observed in any of the treated groups (NTP, 2000). There were two unexplained deaths in the mice, one male at the 32-day interim evaluation and one female at the end of the study, both in the 12-mg/kg-day dose group, but they were not attributed to chemical treatment by the study authors, who stated that no obvious chemical-related effects were observed in male or female mice administered doses up to 12 mg/kg-day in this study. After analyzing the study results and data EPA reached the same conclusion and a NOAEL of 8.6 mg/kg-day (adjusted for dosing schedule) is established for this study.

MHLW, 2001

A study performed by the Ministry of Health, Labour and Welfare of Japan (MHLW, 2001) examined the subchronic and reproductive effects of methacrylonitrile administration to

Sprague-Dawley rats (see reproductive section below for a summary of the reproductive effects from this study). The study report is available only in a secondary source (OECD, 2002) and is not published in the peer-reviewed literature. OECD (2002) summarizes that MHLW (2001) met the requirements of GLP, but peer-review status was not stated. Groups of 12 male and 12 female rats received doses of 0-, 7.5-, 15-, or 30-mg/kg-day methacrylonitrile (purity not provided) by gavage daily. Males were dosed for 46 days from 14 days prior to mating; females received treatment for 39−51 days from 14 days prior to mating, through mating and pregnancy, to Day 4 of lactation. No clinical signs of toxicity were noted in the rats. No significant changes were noted in body weight or feed consumption in males or females. Hematological analysis (presumed at study termination) reported decreases in erythrocyte counts and hemoglobin concentrations in males in the 30-mg/kg-day dose group. Analysis of blood chemistry showed a decrease in potassium in males at 15 and 30 mg/kg-day, an increase in creatinine in males at 30-mg/kg-day, and increases in total bilirubin and glucose in females at 30-mg/kg-day. Histopathological examination revealed slight extramedullary hematopoiesis in the spleen in 1/12, 0/12, 3/12, and 7/12 females at 0, 7.5, 15, and 30 mg/kg-day, respectively (Table B.2). Only the response at 30 mg/kg-day was statistically significant. The study authors of the summary (OECD, 2002) stated that, based on anemia at 30-mg/kg-day in males, the NOAEL for repeated dose toxicity was considered to be 15 mg/kg-day. EPA considers the slight splenic extramedullary hematopoiesis in females not to be of toxicological significance. A NOAEL of 15 mg/kg-day and a LOAEL of 30 mg/kg-day (adjusted for dosing schedule) are identified, based on anemia in males.

Gagnaire et al., 1998

In a peer-reviewed study, Gagnaire et al. (1998) administered doses of 0-, 50-, 70-, and 90-mg/kg-day (calculated to be equivalent to 0, 36, 50, and 64 mg/kg-day) methacrylonitrile (99% purity) by gavage to Sprague-Dawley rats, for 5 days/week, for 12 weeks. There were 10 rats in the control group, 12 rats in the 50−70-mg/kg-day dose groups, and 16 rats in the 90-mg/kg-day dose group. Two rats died in the 50-mg/kg-day dose group, and eight rats died in the 90-mg/kg-day dose group. A 13% body-weight decrease was observed in the 90-mg/kg-day dose group at the end of the 12th week of treatment. Electrophysiological parameters were evaluated, consisting of the measurement of motor conduction velocity and sensory conduction velocity of the tail nerve, and the amplitudes of the sensory action potential and of the muscular action potential. No changes were noted in these parameters in the dosed groups as compared to the control groups. No other effects were noted. Due to the high mortality in this study and specialized design, neither a LOAEL nor a NOAEL is assigned.

Chronic Studies

Based on the above 13-week NTP (2000) study, a chronic study was performed by NTP (2001) to study the effects of methacrylonitrile on F344/N rats. This peer-reviewed study was performed in accordance with GLP standards. Groups of 50 male and 50 female F344/N rats were administered methacrylonitrile (>99% purity) by gavage in deionized water. The animals were administered doses of 0, 3, 10, or 30 mg/kg-day (calculated to be equivalent to 0, 2.14, 7.14, and 21.4 mg/kg-day), for 5 days/week, for 104 to 105 weeks. All animals were observed twice daily and weighed at the beginning of the studies, every 4 weeks, and at necropsy. Clinical findings were recorded on Days 8 and 29, every 4 weeks thereafter, and at necropsy. A complete histopathological analysis was performed on all animals. In addition to gross lesions and tissue masses, the study authors examined the following tissues: adrenal gland, bone with marrow,

brain, clitoral gland, esophagus, heart and aorta, large intestine, small intestine, kidney, liver, lung, lymph nodes, mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach, testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. Statistical analyses were performed using the Kaplan and Meier method, Cox's method, the Poly-k test, Shirley and Dunn's tests, Jonckheere's test, the Dixon and Massey test, and the Mann-Whitney test.

The survival of all the dosed groups was similar to the survival of the control groups (NTP, 2001). In the 30-mg/kg-day dose group, mean body weights were less (not statistically significantly less and <10% difference) than those of the control groups after Weeks 21 and 37 for males and females, respectively. No clinical effects related to methacrylonitrile administration were observed.

Nonneoplastic effects were observed in the nose, liver, bone marrow, and pancreatic exocrine gland (NTP, 2001). In the males and females administered 30 mg/kg-day, the incidences of olfactory epithelial atrophy and metaplasia of the nose were significantly greater than in the control groups. Study authors ranked average severity of the olfactory epithelial atrophy as ranging from minimal to mild. Additionally, the study authors considered a diagnosis of metaplasia of the olfactory epithelium to consist of complete loss of sensory and sustentacular cells, which were replaced by thin pseudostratified, ciliate, nonciliated cuboidal, or columnar epithelial cells (NTP, 2000). Histopathological evaluation of organs other than the nose showed significantly greater incidences of diffuse cytoplasmic vacuolization in the liver in the group of 30-mg/kg-day males and in all dosed groups of females (≥3 mg/kg-day) compared to control groups. All male dosed groups experienced an increased incidence of pancreatic exocrine gland hyperplasia; however, this incidence was comparable with the historical control range (NTP, 2001), and, therefore, was not considered by the study authors to be the result of methacrylonitrile exposure. Bone marrow hyperplasia was increased in female rats receiving 30 mg/kg-day.

Regarding neoplastic effects, methacrylonitrile did not increase the incidence of pancreatic exocrine gland neoplasms (NTP, 2001). Male rats exhibited a negative trend in the incidence of mononuclear cell leukemia. In the 30-mg/kg-day dose group, male rats had significantly fewer incidences of mononuclear cell leukemia than vehicle controls and had incidences slightly lower than the historical range in controls (NTP, 2000). No increase in neoplasms was observed. The study authors concluded that there was no evidence of carcinogenic activity in male or female rats based on this study.

The study authors did not indicate a NOAEL or LOAEL for this study; however, they did state that methacrylonitrile administration caused significant increases in the incidences of nonneoplastic lesions of the nose and liver in rats (NTP, 2001). A LOAEL of 2.14 mg/kg-day (adjusted for dosing schedule) is identified based on cytoplasmic vacuolization in the liver of female rats. A NOAEL is not identified because this effect occurred at the lowest dose in female rats.

In the same chronic NTP gavage study, groups of 50 male and 50 female $B6C3F_1$ mice were administered 0-, 1.5-, 3-, or 6-mg/kg-day (calculated to be equivalent to 0, 1.07, 2.14, and 4.29 mg/kg-day) methacrylonitrile (>99% purity) by gavage in deionized water, for 5 days/week, for 2 years (NTP, 2001). All animals were observed twice daily and weighed at the beginning of

the studies, every 4 weeks, and at necropsy. Clinical findings were recorded on Days 8 and 29, every 4 weeks thereafter, and at necropsy. Following sacrifice, all animals were necropsied and inspected for grossly and microscopically visible lesions on all organs and tissues. A complete histopathological analysis was performed on all animals. In addition to gross lesions and tissues masses, the following tissues were examined adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder, heart and aorta, large intestine, small intestine, kidney, liver, lung, lymph nodes, mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach, testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. Statistical analyses were performed using the Kaplan and Meier method, Cox's method, the Poly-k test, Shirley and Dunn's tests, Jonckheere's test, the Dixon and Massey test, and the Mann-Whitney test.

All dosed groups had mean body weights that were not significantly different from the body weights of the control groups (NTP, 2001). No increase in nonneoplastic or neoplastic lesions was reported. The study authors concluded that there was no evidence of carcinogenic activity in male or female mice based on this study (NTP, 2001).

The study authors did not indicate a NOAEL or LOAEL for this study (NTP, 2001). Based on lack of increased incidences of nonneoplastic effects, a NOAEL of 4.29 mg/kg-day, the highest dose administered, is identified. A LOAEL is not identified.

Developmental Toxicity Studies

NTP, 1993a; George et al., 1996; rat study

NTP performed a peer-reviewed study (NTP, 1993a; George et al., 1996) that measured the effects of methacrylonitrile in distilled water administered by gavage to pregnant Sprague-Dawley rats. This study was performed in accordance with GLP standards. Groups of 26 female rats received daily doses of 0-, 5-, 25-, or 50-mg/kg-day methacrylonitrile on Gestational Days (GDs) 6 through 15. On GDs 0, 3, 6, 9, 12, 15, 18, and 20, the body weights of the sperm-positive females, along with the food and water consumption weights, were recorded. Clinical signs were recorded beginning on GD 6 through GD 20. On GD 20, approximately 25−26 females per group had confirmed pregnancies. At that time, all were sacrificed and examined for clinical status and gestational outcome. Maternal body, liver, and uterine weights were recorded, and external, visceral, and skeletal malformation inspections were carried out on all live fetuses. Additionally, the number of implantation sites, resorptions, late fetal deaths, live fetuses, and early resorptions were assessed. Statistical analyses were performed using analysis of variance (ANOVA), William's multiple comparison test, and Dunnett's test.

Both absolute and relative maternal feed consumption were significantly decreased between GDs 6 through 9 in the 50-mg/kg-day dose group, while relative maternal feed consumption was significantly increased between GDs 18 through 20 in the 50-mg/kg-day dose group (NTP, 1993a; George et al., 1996). Between GDs 9 through 12, maternal feed consumption was significantly greater than the controls at 25 mg/kg-day but significantly less than the controls in the 50-mg/kg-day dose group. Maternal death, morbidity, or distinguishing clinical signs were not detected. In addition, no significant adverse effect on maternal body weights or weight changes was observed. No effect was observed on gravid uterine weights at any dose level; however, maternal liver weight (absolute, relative, and adjusted) was statistically significantly increased (but the liver-weight differences based on absolute liver-weight changes

were <10%) in the 25- and 50-mg/kg-day dose groups. On GD 20, each pregnant animal had at least one live fetus. Average number of implantation sites per litter, postimplantation deaths per litter, live fetuses per litter, or mean fetal weight per litter did not differ between dosed and control groups. The incidence of fetal morphological abnormalities was not affected by the administration of methacrylonitrile. Therefore, a NOAEL of 50 mg/kg-day, the highest dose administered, is identified for both maternal and developmental toxicity. A LOAEL is not identified.

NTP, 1993b; George et al., 1996; rabbit study

The NTP performed another peer-reviewed experiment (NTP, 1993b; George et al., 1996) that measured the effects of methacrylonitrile in distilled water administered by gavage to pregnant New Zealand White rabbits. GLP standards were followed in this study. Animals (17−22/dose group) received daily doses of 0-, 1-, 3-, or 5-mg/kg-day methacrylonitrile on GDs 6−19. On GDs 0, 3, 6−19, 25, and 30, the maternal weights were recorded. Daily observation for clinical signs occurred before, during, and after dosing. Approximately 17−22 females per group had confirmed pregnancies on GD 30; all animals were sacrificed and examined for clinical status and gestational outcome. Maternal body, liver, and uterine weights were recorded following sacrifice. External, visceral, and skeletal malformation inspections were performed on all live fetuses. Additionally, the number of implantation sites, resorptions, late fetal deaths, live fetuses, and early resorptions were measured. Statistical analyses were performed using ANOVA, William's multiple comparison test, and Dunnett's test.

One animal in the 3-mg/kg-day dose group and another animal in the 5-mg/kg-day dose group died on GD 20 (NTP, 1993b; George et al., 1996). The cause of death was not provided in the study. Alopecia and resistance to dosing were observed more frequently in the dosed groups than in the control groups; however, the study authors stated that these clinical signs were not dose related and remained fairly constant throughout the study. Body weights and body-weight changes were not affected by exposure to methacrylonitrile at any dose. Gravid uterine and liver weights were unaffected by methacrylonitrile administration. Resorptions occurred in the controls and in every dose group but were not dose related; five litters were resorbed in the controls, four litters were resorbed in the 1-mg/kg-day dose group, four were resorbed in the 3-mg/kg-day dose group, and three were resorbed in the 5-mg/kg-day dose group. The number of implantation sites per litter, percentage postimplantation loss per litter, live litter size, or mean fetal body weight per litter were all unaffected by treatment. However, the percentage of male pups per litter in the 5-mg/kg-day dose group was significantly decreased compared to the number of male pups in the control group. Nevertheless, there was no effect on total live litter size, and the study authors suggested that "…the reduction in the ratio of live male fetuses in the high-dose group was not biologically significant," and EPA agrees with this conclusion. Administration of methacrylonitrile did not affect the occurrence of external, visceral, or skeletal malformations or variations in fetuses at any dose level. A NOAEL at the highest dose tested of 5 mg/kg-day for developmental toxicity in rabbits is established in this study. A LOAEL is not identified. Presuming that the two maternal deaths were unrelated to treatment, a NOAEL of 5 mg/kg-day is identified for maternal toxicity also.

Farooqui and Villarreal, 1992

Farooqui and Villarreal (1992) examined the maternal toxicity of methacrylonitrile in Sprague-Dawley rats. This was a peer-reviewed study, but it was not stated whether the study was conducted according to GLP standards. Animals were divided into three dose groups of six

pregnant animals each, and each group was administered methacrylonitrile (purity not provided) in safflower oil via gavage. Group I received 50 mg/kg-day during the first week of gestation, Group II received 50 mg/kg-day during the second week of gestation, and Group III received 100 mg/kg-day, also during the second week of gestation. Physical signs of toxicity and their severity were observed daily. All animals were weighed throughout the study. Litter size was measured and recorded. At the end of the study, all animals were sacrificed and examined for the occurrence and severity of morphological or physiological abnormalities.

Clinical signs of toxicity were observed within 1 hour following methacrylonitrile administration. These signs were mild to severe and included ataxia, trembling, convulsions, salivation, and irregular breathing; however, these signs disappeared at various times depending on the dose of methacrylonitrile. Animals in Group I gained weight progressively more over the first 15 days of gestation, yet never gained weight to the same extent as the controls. Following this weight gain, the animals then started to lose weight, which was not observed in the control group. Weight gain was observed in Group II, but at a much slower rate and to a lesser extent than both the controls and Group I animals. Likewise, animals in Group III gained weight, but at a much slower rate and to a lesser extent than the control group. Body-weight gain in all groups was significantly less ($p < 0.01$) than the weight gain in the control group. Animals in Groups I and III did not deliver litters, and only one animal in Group II delivered a litter. These results differed significantly from the control group, which delivered normal size litters on GD 20. In a number of the Group I animals, mild-to-severe edema in the fallopian tubes was observed, while severe edema in the fallopian tubes was observed in the majority of rats in both Groups II and III. One of the animals in Group III also presented with a globular structure in one of the fallopian tubes. The control groups experienced edema; however, it was not to the extent of the edema observed in Groups I through III. In fact, the treated groups experienced edema that was significantly (statistical method not provided) greater than the controls. A LOAEL of 50 mg/kg-day is identified for this study based on reproductive effects, including effects on fertility and edema in the fallopian tubes. A NOAEL is not identified because these effects were noted at the lowest dose (50 mg/kg-day).

Villarreal et al. 1988

Villarreal et al. (1988) administered 50-mg/kg-day methacrylonitrile (purity not provided) to pregnant Sprague-Dawley rats (number not provided) during the first week of gestation and administered 100 mg/kg-day to pregnant Sprague-Dawley rats during the second week of gestation. A dose-dependent significant (not stated whether statistically significant) reduction in maternal body-weight gain was observed. Control rats delivered litters of a normal size, and the rats administered methacrylonitrile failed to maintain their pregnancies and aborted the fetuses. At the end of gestation, dose-dependent, mild-to-severe edema was observed in the fallopian tubes of treated rats. No further details were provided on this study, including the number of rats, whether there was a control group, and whether the route of administration was via gavage, feed, or drinking water. GLP and peer-review status were not discussed, and the study was published as part of conference proceedings results. A LOAEL and a NOAEL cannot be identified from this study because litters were aborted at 50 mg/kg-day.

Reproductive Toxicity Studies

NTP, 1997; range-finding study (Task 1)

NTP (1997) exposed Sprague-Dawley rats to methacrylonitrile by gavage according to Reproductive Assessment by Continuous Breeding (RACB) protocols. This study was performed according to GLP standards. During the dose range-finding phase (Task 1) of this study, doses of 0-, 15-, 30-, 50-, and 70-mg/kg-day methacrylonitrile (99.97% purity) were administered by gavage to groups of eight male and eight female animals for 28 days. The animals were observed for clinical signs, and body weights and feed and water consumption were recorded. The doses used in this task were used to determine the doses in the continuous breeding phase (Task 2), which was the main part of this study. Throughout Task 1, the mean body weights of males in the dosed groups decreased. Compared to controls, terminal body weights were reduced by 1%, 9%, 13%, and 16% in males in the 15-, 30-, 50-, and 70-mg/kg-day dose groups, respectively. Female terminal body weights were reduced by 1.5%, 3%, 7%, and 8% in the 15-, 30-, 50-, and 70-mg/kg-day dose groups, respectively. Total body-weight gain, compared to controls, was decreased in males by 11%, 34%, 63%, and 71% in the 15-, 30-, 50-, and 70-mg/kg-day dose groups, respectively; all body-weight-gain decreases, except at 15 mg/kg-day, were statistically significant at the 0.05-level of significance; individual *p*-values were not reported. Female total body-weight gains decreased by 7%, 27%, 42%, and 51% in the 15-, 30-, 50-, and 70-mg/kg-day dose groups, respectively, reaching statistical significance ($p < 0.05$) only at 50 mg/kg-day and above. Daily feed consumption was decreased in males by 3%, 15%, 17%, and 23%, in the 15-, 30-, 50-, and 70-mg/kg-day dose groups, respectively. Female feed consumption was reduced by 5%, 7%, 9%, and 12%, in the 15-, 30-, 50-, and 70-mg/kg-day dose groups, respectively. Water consumption was increased for males only at the highest doses. Water consumption was increased for females as well, but only by 7% at the two lowest dose levels. Two males in the 70-mg/kg-day dose group were found dead on Day 3, one on Day 4, and one on Day 29. Gross necropsy on these animals revealed lesions in the liver and gastrointestinal tract. Other lesions were observed, but the locations were not specified. A mottled liver and pale kidneys, testes, and stomach, as well as foci on the liver and foci and dark areas on the kidneys, were also reported in these male rats. Very few clinical signs were observed during this task in either male or female rats, specified by the study authors only as diarrhea and hunched and thin body presentation. A LOAEL of 30 mg/kg-day for decreased body weight and body-weight gain in males and females is established for this study. The NOAEL is 15 mg/kg-day.

NTP, 1997; continuous-breeding phase (Task 2)

Task 2 involved four groups of animals (F0) containing 20 pairs per group. One group served as the control while the remaining three groups were administered doses of 0, 2, 7, and 20 mg/kg-day. Animals in the F0 generation were dosed via gavage every day from day 1 until the day before necropsy. After 7 days of dosing, animals were paired and housed for 16 weeks. Following this period, the pairs were separated, but dosing continued. Any litters produced during the 16-week mating period were counted and weighed by sex on PND 1 and then sacrificed. Litters born following the 16-week cohabitation period were assigned as the F1 generation and remained with the dams until PND 21. Until approximately 80 days of age, selected weanlings from the control and high-dose groups were reared in the same sex groups. Animals from the F1 generation were dosed via gavage and were used for the second-generation fertility assessment (Task 4 described below). Following weaning of the F1 generation, hematology and clinical chemistry determinations were carried out on selected F0 animals that

were then necropsied. In addition, terminal body weights and organ weights were recorded, and sperm analysis was performed.

In Task 2, one control male, one control female, one male at 20 mg/kg-day, and two females at 20 mg/kg-day died during Task 2 (NTP, 1997). For one control male and one 20 mg/kg-day female, the cause of death could not be determined. The remaining three animals that died before scheduled evaluations died as a result of a gavage error. Clinical signs of toxicity included abrasions, alopecia, swellings, discharge, thinness, lacrimation, tissue masses, vaginal discharge, paleness, missing tip of tail, opacity, squinting, ulcers, rales, and salivation. Less than 30% of animals per group (characterized by the study authors as a low-to-moderate incidence) experienced these symptoms, and dose-related variation was not observed.

Exposure to methacrylonitrile during Task 2 did not affect fertility, as was indicated by similar pregnancy indices (number delivering/number cohabitated) in the control and dosed groups. As the number of litters increased for each pair, the pregnancy index decreased. When compared to the controls, there were no differences in the mean average litters per pair, number of live pups per litter, proportion of pups born alive, sex ratio, live pup weight, or adjusted live pup weight in the dosed groups. The mean cumulative days to litter were similar among dose groups. F0 male body weights were recorded on the day of their mate's delivery. The body weights of males in the 20-mg/kg-day dose group were 3−6% less than the control group. This observation continued throughout the study, as evidenced by reduced body weights on Weeks 6, 12, and 18. On Weeks 6, 12, and 18, mean body weights of females were similar among dose groups. Average feed consumption of each dosed group, both male and female, was similar to the average feed consumption of the control group.

No treatment-related hematology or clinical chemistry variations were observed in Task 2, other than a decreased (4%) hemoglobin value in females in the 7-mg/kg-day dose group and an increase (7%) in total protein in males in the 2-mg/kg-day dose group (NTP, 1997). The study authors considered these changes not treatment related, because they were small and not dose related. Body weights at the end of the study for the 20-mg/kg-day F0 males were 5% less (not statistically significant) than the controls. Average body weights for females at the end of the study were similar among all dose groups. In the 2-mg/kg-day F0 males, the mean absolute adrenal weight was statistically significantly increased; however, no other absolute organ weight differences were observed at the other doses. Many of the relative organ weights were increased for males as compared to controls; the study authors stated that this was due to the slight decrease in body weight. Both males and females in the 20-mg/kg-day dose group experienced an increase in relative liver weights (13% increase in males, 12% increase in females) as compared to the controls. There were statistically-significant increases in abnormal sperm morphology observed in males dosed at 2 and 20 mg/kg-day, but only about 1% of sperm in treated animals was reported as abnormal (Table B3). Additionally, the relative weights of the right cauda epididymis (14% increase), right epididymis (12% increase), and stomach (19% increase) in males in the 20-mg/kg-day dose group were greater than those of the controls. The number of spermatids per milligram testis, and the total number of spermatids per testis were similar between all dose groups and the controls. The mean epididymal sperm density was slightly increased (5.5%) in the high-dose group but not statistically significant. Males and females of the F0 generation were free of treatment-related gross lesions. Cysts on the epididymis and spleen, liver foci, small testis, and epididymis; torsion of the abdominal fat;

nodule on the kidneys; and abscess on the skin were observed but considered incidental because no dose-related response was observed. One F0 male in the 20-mg/kg-day dose group had cell necrosis in the hepatocytes; however, the study authors did not consider this to be treatment related.

Adult body weights of the F1 males in the 20-mg/kg-day dose group were decreased by 9−10% during Weeks 2−4 when compared to controls. Adult body weights of the F1 females in the 20-mg/kg-day dose group were decreased by 6% on Week 2 but were comparable to controls on Week 4. Mean feed consumption in the 20 mg/kg-day males in the F1 generation was decreased by 8−11% during Weeks 2 and 4 compared to controls, while mean feed consumption for the F1 females was similar between the control and the 20-mg/kg-day dose groups.

A LOAEL of 20 mg/kg-day is established for the continuous-breeding phase (Task 2) for this study (NTP, 1997) based on increased epididymal weights (12−14%) and stomach weights (19%) in F0 males and increased relative liver weights (12−13%) in F0 males and females. The NOAEL is 7 mg/kg-day. Although increased mean adrenal weights were noted in the F0 males at 2 mg/kg-day, adrenal organ weights were not increased at any other dose, so this effect does not appear to be dose related. There were also unexplained deaths including one control male, one male at 20 mg/kg-day, and two females at 20 mg/kg-day during Task 2. As these deaths occurred in the controls as well as in the dosed animals, the deaths are judged not to be treatment related.

NTP, 1997; fertility assessment (Task 4)

Due to lack of evidence of reproductive toxicity in the continuous breeding phase, the crossover-mating task (Task 3) was omitted, and a fertility assessment (Task 4) was performed. Twenty male and 20 female F1 weanlings were randomly selected from the control and 20-mg/kg-day dose groups for rearing to adulthood (only animals from the control and 20-mg/kg-day dose groups were used, due to the absence of reproductive toxicity in Task 2). Oral gavage dosing began on PND 22 and continued until 81 ± 11 days of age. Approximately 1 week before PND 81 ± 11, at least one male and female from each litter (avoiding sibling mating) were selected to obtain 20 breeding pairs. All litters produced were evaluated on PND 1. Selected F1 females were subjected to vaginal cytology sampling. Following sampling, predetermined animals, both males and females, from the F1 generation were analyzed for hematology and clinical chemistry determinations. Terminal body and organ weights were obtained, and sperm analyses were performed. The data for number of pups from each litter, number of live and dead pups, number of male and female pups, and total pup weight of each sex were obtained. Statistical data analyses (for the entire study) were performed using ANOVA, Dunnett's test, Dunn's test, Shirley's test, Jonckheere's test, Wilcoxon's test, and the Cochran-Armitage test.

In Task 4, it was observed that the proportion of pups born alive in the final litter (to the F1 generation) was similar between the control and 20-mg/kg-day dose group. Male pups born to the 20-mg/kg-day dose group experienced a slight decrease in mean pup survival on PNDs 4, 7, 14, and 21; however, pup survival was similar to controls when both sexes were considered. Average pup weights were similar among groups during the lactation phase of the last litter. On PND 21, average body weights of the male and female pups born to the 20-mg/kg-day dose group were similar to controls. On PND 81 ± 11, the mean body weights of the F1 females born

to the 20-mg/kg-day dose group were 7% lower than controls, while the mean body weight of the F1 males born to the 20-mg/kg-day dose group were not statistically significantly different from the controls. On PND 1, no alteration in anogenital distance for the final litter was observed.

No differences were observed between the control and the 20-mg/kg-day dose group with regard to reproductive performance in Task 4. This observation was supported by similar pregnancy indices, number of pups per litter, pup weight, proportion of pups born alive, sex ratio of pups, and gestation length between the 20-mg/kg-day and control groups. Immediately following delivery, the mean dam weights were similar between the 20-mg/kg-day and control groups. After dams delivered, their male partners were weighed, and the body weights of the males in the 20-mg/kg-day dose group were 8% less (not statistically significant) than the controls. The data for the amount of time spent in different estrous stages, cycle length, number of cycles, number of cycling females, or number of females with regular cycles were not different between the 20-mg/kg-day dose group and the control group.

During Weeks 2 and 4 of Task 4, a decrease in adult F1 male body weights at the 20-mg/kg-day dose group was observed when compared to controls. The females in the same group experienced a mean body-weight decrease in Week 2 but not in Week 4. Feed consumption of the 20-mg/kg-day dose group was unaffected by methacrylonitrile administration when compared to controls, with the exception of males receiving 20 mg/kg-day during Weeks 2 and 4, when the mean feed consumption was decreased in males by 8−11%. Alopecia and swelling at a low-to-moderate level of severity were the only clinical observations noted during Task 4, and dose-related differences were not observed. Following unintentional removal from the animal room, two animals were removed from the study. No animals died before scheduled evaluation during Task 4.

In Task 4, changes noted in hematology or clinical chemistry parameters were an increase (3%) in mean corpuscular hemoglobin in females in the 20-mg/kg-day dose group and a decrease (33%) in mean serum ALT in females in this same dose group. These were not considered biologically significant by the study authors because they were small and not associated with other changes in liver or kidney parameters. Terminal body weights of the males (F1) in the 20-mg/kg-day dose group were decreased by 9% compared to controls. No differences were observed in body weights of F1 females. No differences were noted in the absolute organ weights of either males or females. In F1 males at the 20-mg/kg-day dose group, increases were observed in relative weights of the liver (13%), right epididymis (7%), ventral prostate (16%), and stomach (11%) compared to controls. In F1 females in the 20-mg/kg-day dose group, the relative liver weight was increased (13%) compared to controls (Table B.4). No differences were noted in sperm analysis parameters, epididymal sperm morphology, or testicular head counts. Epididymal sperm density was decreased by 19% in males at the 20-mg/kg-day dose group. Cell necrosis of the hepatocytes, cytoplasmic vacuolization of the hepatocytes, renal tubule degeneration, and renal tubule regeneration were observed by microscopic examination in F1 males. In F1 females, microscopic examination revealed cell necrosis of the hepatocytes and cytoplasmic vacuolization of the hepatocytes. The study authors (NTP, 1997) did not consider any of the microscopic lesions to be treatment related because the incidence was similar between the control and treated group; EPA agrees with the study authors' assessment.

A LOAEL of 20 mg/kg-day for the fertility assessment (Task 4) of the NTP (1997) study is identified based on decreased epididymal sperm density and increased organ weights (liver, stomach, and ventral prostate) in F1 males. A NOAEL was not established in this phase of the study, but a NOAEL of 7 mg/kg-day was determined in Task 2 for similar effects in F0 rats (see discussion in subchronic p-RfD derivation section below).

MHLW, 2001

A study performed by the Ministry of Health, Labour and Welfare of Japan (MHLW, 2001) examined the reproductive effects of methacrylonitrile administration to Sprague-Dawley rats. This study was summarized previously in the subchronic study section (page 19) and only the reproductive toxicity outcomes are summarized here. Reproductive performance such as mating, fertility, delivery, and lactation suffered no effects from methacrylonitrile administration. Estrous cycles during the premating period were also not affected. A NOAEL for reproductive toxicity for both males and females of 30 mg/kg-day, the highest dose tested, is identified for this study. A LOAEL is not identified.

Other Studies

No additional studies could be located regarding the effects of oral exposure of animals to methacrylonitrile.

Inhalation Exposures

The effects of inhalation exposure of animals to methacrylonitrile have been evaluated in one short-term study on several species (Pozzani et al., 1968), in two subchronic studies in the same study report (Pozzani et al., 1968), and in one developmental (Saillenfait et al., 1993) study.

Short-term Studies

Pozzani et al. (1968) carried out a series of short-term studies on methacrylonitrile inhalation in rats, mice, guinea pigs, rabbits, and dogs. In the first study, groups of 6 male and female Harlan-Wistar rats were exposed to 85,500-ppm (calculated to be equivalent to 234,270 mg/m^3) methacrylonitrile for 0.47, 0.93, 1.88, 3.75, 7.5, and 14 minutes. This exposure resulted in mortality ratios of 0/6, 0/6, 1/6, 6/6, 6/6, and 6/6, respectively. In those animals that died, prostration and loss of consciousness preceded death. The surviving rats gained weight normally during a subsequent 14-day observation period. No other details were included in this study (Pozzani et al., 1968).

In another short-term experiment by Pozzani et al. (1968), groups of six Harlan-Wistar rats (male and female), six A/J strain mice (male), six albino guinea pigs (male), and four albino rabbits (male) were exposed to various concentrations (not provided in study) for single 4-hour periods in order to determine LC_{50} values. LC_{50} values were determined at 328 ppm (calculated to be equivalent to 899 mg/m^3) for male rats, 496 ppm (1359 mg/m^3) and 700 ppm (1918 mg/m^3) for female rats, 36 ppm (99 mg/m^3) for mice, 88 ppm (241 mg/m^3) for guinea pigs, and 37 ppm (100 mg/m^3) for rabbits. Three female dogs (two mongrels and one cocker spaniel) were also exposed to methacrylonitrile, but an LC_{50} was not determined. The study authors stated that the dogs were considerably less resistant to methacrylonitrile vapor than the rat. The responses of all the species were dose related and consisted of vomiting (dogs only), loss of consciousness, tonic-clonic convulsions, and death. Most of the survivors gained weight normally during the 14-day observation period. No gross lesions attributable to exposure were found in any of the

animals at autopsy. A NOAEL and LOAEL could not be identified because death—a frank effect—was seen in all animal species tested.

Subchronic Studies

Pozzani et al. (1968); rat study

The rat study by Pozzani et al. (1968) is selected as the principal study for deriving the subchronic and chronic p-RfCs. Pozzani et al. (1968) analyzed the effects of methacrylonitrile inhalation exposure on Harlan-Wistar rats. Groups of 12 male and 12 female rats were exposed to methacrylonitrile vapor (purity not provided) for an average of 7 hours/day, 5 days/week, for 91 days, at doses of 0, 19.3, 52.6, and 109.3 ppm (median measured concentrations) (calculated to be equivalent to 0, 11.0, 30.1, and 62.5 mg/m^3). This is a published, peer-reviewed study; however, it was not stated whether GLP standards were followed. Body-weight changes and liver and kidney weights were measured, and gross and microscopic pathological evaluations were performed. Nineteen tissues (unspecified, but excluding the brain) were sampled from each rat for microscopic examination.

Throughout the first day of exposure, seven male rats from the 109.3-ppm methacrylonitrile group died, and another male from the 52.6-ppm group died on the second day. No explanations were given for the deaths. Prior to death, loss of consciousness was observed; however, convulsions were not reported. One male rat in the 109.3-ppm group appeared prostrate on the 11th exposure day but recovered the next day. Upon autopsy, no other effects were observed. No gross or microscopic lesions were observed in either the rats that died or the survivors. Comparisons of body-weight gains of the dosed groups against the control groups were performed on the 5th, 29th, 59th, and 91st days of exposure (absolute body weights were not provided). Both males and females in the 109.3-ppm group and the females in the 52.6-ppm group had weight gains that were significantly lower than the control groups after 5 days of exposure. Significant body-weight losses were not observed in the treated rats compared to the controls. Relative liver weights were increased for males by 9.7% ($p < 0.05$) at 52.6 ppm and 28% ($p < 0.01$) at 109.3 ppm relative to controls. Relative liver weights were increased for females exposed to 52.6 ppm by 5.4% (NS) and 109.3 ppm by 27% ($p < 0.001$) relative to controls. A NOAEL of 52.6 ppm (30.1 mg/m^3) and a LOAEL of 109.3 ppm (62.5 mg/m^3) are identified from this study based on increased (>10%) relative liver weights in males and females.

Pozzani et al. (1968); dog study

In the same study report, Pozzani et al. (1968) performed a separate experiment in which doses of 0-, 3.2-, 8.8-, and 13.5-ppm methacrylonitrile (equivalent to 0, 9, 24, and 37 mg/m^3, as calculated in IRIS (U.S. EPA, 1987a) were administered to groups of male beagles (3 dogs per group). Pozzani et al. (1968) was selected as the principal study in IRIS (U.S. EPA, 1987a) to calculate the oral chronic RfD. The values were converted from inhalation exposure to oral exposure using an older method prior to current guidance on route-to-route conversion methodology (U.S. EPA, 1994). The corresponding HEC exposure levels are 0, 1.8, 4.9, and 7.6 mg/m^3, using the current inhalation dosimetry methods (U.S. EPA, 1994). There is no RfC derived in IRIS, but this study was also used to calculate the subchronic and chronic inhalation RfCs in HEAST (U.S. EPA, 2010). The animals were exposed for an average of 7 hours/day, 5 days/week, for 90 days. Body-weight changes and liver and kidney weights were measured. Additionally, hematocrit, total white blood cell count, differential count, and blood urea nitrogen

(BUN), serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and serum alkaline phosphatase (SAP) levels were measured.

In the 13.5-ppm dose group, one dog experienced tonic convulsions, trouble standing, tachycardia, severely blocked optic discs, and loss of control over his hindquarters after 49 days of exposure. However, all of these symptoms subsided within a day. Gross or microscopic lesions were not observed. Another dog at the 13.5-ppm level also experienced loss of control over his hindquarters, tonic convulsions, tachycardia, hurried and deep respiration, nystagmus, and blocked optic discs after 39 days of exposure. These symptoms also subsided within a day. This was the only animal in which microscopic lesions were observed, consisting of a marked malacia of the floor of the third ventricle of the brain with massive accumulation of gitter cells and some demyelinization of the corpus callosum. In one dog administered 8.8 ppm, noticeable increases of SGOT and SGPT levels were observed, but these values decreased considerably 3 days later and were normal after the 41st, 61st, and 89th exposure days. A dog receiving the 3.2-ppm dose experienced a slight-but-transitory reversal in its neutrophil-lymphocyte ratio. No other symptoms were observed. The study authors provided a "no-ill effect" range, or NOAEL, of 3.2 to 8.8 ppm (9−24 mg/m^3). The EPA (1987a) established a LOAEL of 24 mg/m^3 and a NOAEL of 9 mg/m^3 based on transient increased SGOT and SGPT levels from this study.

Chronic Studies

No studies could be located regarding the effects of chronic inhalation exposure of animals to methacrylonitrile.

Developmental Studies

In a developmental study, Saillenfait et al. (1993) exposed groups of 20−23 pregnant Sprague-Dawley rats to methacrylonitrile for 6 hours/day on GDs 6−20. The study authors did not state whether the study was performed in accordance with GLP standards. The concentrations of methacrylonitrile (99% purity) were 0, 12, 25, 50, and 100 ppm (calculated to be equivalent to 0, 32.9, 68.5, 137, and 274 mg/m^3). Daily observations of rats were performed throughout pregnancy. On GDs 0, 6, and 21, maternal body weights were recorded. Following sacrifice, the uterus of each female was removed, weighed, dissected, and inspected for implantation and resorption sites and live and dead fetuses. Weight, external irregularities, and the sex of live fetuses were recorded. Live fetuses were then sacrificed and examined microscopically for skeletal abnormalities. Statistical analyses using the Wilcoxon's and Fisher's Exact tests were performed for the number of implantation sites, live fetuses, and body weights.

During this study, maternal deaths were not observed (Saillenfait et al., 1993). Weight gains among all dose groups did not differ from the control group after correction for uterus weight. During GDs 6 through 21, a slight decrease in weight gain was observed; however, this was a reflection of lower fetal body weights. Methacrylonitrile exposure did not affect occurrence of pregnancy, average number of implantation sites, or average number of live fetuses. In the 100-ppm dose group, one litter was completely resorbed. Additionally, the 100-ppm dose group exhibited an increase in the incidences of nonsurviving implants and resorptions; however, this was not a statistically significant increase. The male-to-female sex ratio was similar among all dose groups, with the exception of the 50-ppm dose group, where a significant decrease in the number of males was observed. The study authors stated that this

decrease appeared to be a random occurrence; however, no justification for this conclusion was provided. Body-weight gain of both male and female pups was significantly ($p < 0.01$ male, $p < 0.05$ female) reduced (>10%) at 100-ppm methacrylonitrile exposure. Gross malformations in fetuses were not observed in any dose group. Occurrence of external, visceral, and skeletal variants was similar between dose and control groups. The study authors concluded that a NOAEL of 50 ppm (137 mg/m^3) and a LOAEL of 100 ppm (274 mg/m^3) could be established, based on significantly reduced fetal body weights.

Reproductive Studies

No studies could be located regarding the reproductive effects of inhalation exposure of animals to methacrylonitrile.

Other Studies

No additional studies could be located regarding the effects of inhalation exposure of animals to methacrylonitrile.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Tests Evaluating Carcinogenicity, Genotoxicity, and/or Mutagenicity

The genotoxicity and mutagenicity of methacrylonitrile has been tested in several in vitro (Zeiger et al., 1987; MHLW, 2001; Zimmering et al., 1989; Knaap et al., 1985; Wu et al., 2009; Vasanthakumari et al., 1997) and in vivo (Shelby et al., 1993; MacGregor et al., 1990) studies. These genotoxicity tests are summarized in Table 3.

In several Ames mutagenicity tests, methacrylonitrile demonstrated negative results using *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 in the presence and absence of metabolic activation (Zeiger et al., 1987; MHLW, 2001; Knaap et al., 1985; Wu et al., 2009). Another study by MHLW (2001) produced negative results in the Ames mutagenicity test using *Escherichia coli* WP2 uvr A in the presence and absence of metabolic activation. In male *Drosophila melanogaster* administered 6000-ppm methacrylonitrile, no sex-linked recessive lethal mutations were observed in germ cells (Zimmering et al., 1989). In a brief abstract, Knaap et al. (1985) reported that there was no evidence for mutagenicity in an Ames assay in *Salmonella typhimurium* TA98 and TA100 (both with and without activation), in L5178 mouse lymphoma cells, or in a sex-linked recessive lethal test in *Drosophila melanogaster*. The same abstract reported positive results observed in a fluctuation test in *Klebsiella pneumoniae* (Knaap et al., 1985). In an assay for unscheduled DNA synthesis, results were inconclusive for concentrations tested up to 40 ηM/plate in human HepG2 cells (Vasanthakumari et al., 1997). A chromosomal aberration test in Chinese hamster lung cells found positive results with metabolic activation but negative results without activation (MHLW, 2001). Wu et al. (2009) observed DNA damage in an in vitro comet assay in human lymphocytes and Hep G2 cells.

Table 3. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Genotoxicity	Reverse mutation: *Salmonella typhimurium* strains TA97,TA98, TA100, TA1535, TA1537 with and without activation (in vitro)	Negative	Doses tested up to 10,000 µg/plate	Zeiger et al. (1987)
	Reverse mutation: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 with and without activation (in vitro)	Negative	Doses tested up to 5000 µg/plate	MHLW (2001)
	Reverse mutation: *Salmonella typhimurium* strains TA98, TA100 with and without activation (in vitro)	Negative	No details reported in brief abstract	Knaap et al. (1985)
	Reverse mutation: *Salmonella typhimurium* strains TA98, TA100	Negative	Doses tested up to 3000 µg/plate	Wu et al. (2009)
	Reverse mutation: *Escherichia coli* WP2 uvr with and without activation (in vitro)	Negative	Doses tested up to 5000 µg/plate	MHLW (2001)
	Sex-linked recessive lethal mutations: *Drosophila melanogaster*	Negative	Doses tested up to 6000 ppm	Zimmering et al. (1989)
	Sex-linked recessive lethal mutations: *Drosophila melanogaster*	Negative	No details reported in brief abstract	Knaap et al. (1985)
	Gene mutation test: mouse lymphoma L5178Y cells (HPRT and TK loci) (in vitro)	Negative	No details reported in brief abstract	Knaap et al. (1985)
	Fluctuation test: *Klebsiella pneumoniae* (in vitro)	Positive	No details reported in brief abstract	Knaap et al. (1985)
	Unscheduled DNA synthesis: human HepG2 cells (in vitro)	Inconclusive	Doses tested up to 40 ηm/plate	Vasanthakumari et al. (1997)
	Comet assay: DNA damage in human lymphocytes and Hep G2 cells	Positive	Positive doses tested at 250 µM or greater	Wu et al. (2009)
	Micronucleus test: rat bone marrow (in vivo)	Negative	Doses tested up to 200 mg/kg	Shelby et al. (1993)
	Chromosome aberration test: Chinese hamster lung cells with and without activation (in vitro)	Equivocal	Negative (without activation), Positive (with activation)	MHLW (2001)
	Micronucleus test: mouse bone marrow (in vivo)	Negative	Doses tested up to 25 mg/kg	Shelby et al. (1993)
	Micronucleus test: $B6C3F_1$ mouse peripheral blood erythrocytes (in vivo)	Negative	Doses tested up to 12 mg/kg	MacGregor et al. (1990)

Table 3. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Other toxicity studies (exposures other than oral or inhalation)	Dermal LD_{50} in male albino New Zealand rabbits	LD_{50} value determined to be 0.32 mL/kg (0.19−0.51 mL/kg)	None	Pozzani et al. (1968)
	Intragastric LD_{50} in rats	LD_{50} value determined to be 0.24 g/kg (0.16−0.36 g/kg)	None	Pozzani et al. (1968)

Following administration of methacrylonitrile to male rats in vivo, significant induction of micronuclei occurred in the 200 mg/kg dose group. However, no induction of micronucleated polychromatic erythrocytes was observed in a second trial, and the study authors concluded that the test was negative overall (Shelby et al., 1993). In male mice, no induction of micronucleated polychromatic erythrocytes was observed following administration of methacrylonitrile (Shelby et al., 1993). A third in vivo study found no induction of polychromatic erythrocytes in the blood of male and female mice (MacGregor et al., 1990). Overall, in vitro and in vivo genotoxicity assays suggest that methacrylonitrile is not mutagenic.

Other Toxicity Studies (Exposures Other Than Oral or Inhalation)

The effects of dermal and intragastric exposure of animals to methacrylonitrile have been evaluated in two acute studies from the same study report (Pozzani et al., 1968). Table 3 summarizes these effects.

Pozzani et al. (1968) applied a single dose of methacrylonitrile to the shaved skin of male albino New Zealand rabbits for 24 hours. Four rabbits received a dose of 0.5 mL/kg, while another group of four rabbits received a dose of 0.25 mL/kg. Within 3 hours and 45 minutes, all four rabbits receiving the highest dose experienced gasping and convulsions and then died. Only one of the four rabbits at the lower dose (0.25 ml/kg) experienced gasping and convulsions before death, which occurred within 2 hours and 40 minutes of methacrylonitrile application. The study authors reported that the remaining three rabbits displayed no symptoms and gained weight normally during the following 14-day recovery period. Based on this experiment, the study authors determined a dermal LD_{50} value of 0.32 mL/kg (0.19−0.51 mL/kg).

In a separate experiment in the same study, Pozzani et al. (1968) determined an intragastric LD_{50} value of 0.24 g/kg (0.16−0.36 g/kg) following a single intragastric dose administered in rats. Groups of five rats (sex not provided) were administered 0.1, 0.2, or 0.4 g/kg. Four rats in the highest dose group died on the day of dosing; the surviving rat died on the night of dosing. At both the 0.1- and 0.2-g/kg doses, one rat in each dose group died. All animals that died following exposure experienced prostration and convulsion within 90 minutes of administration. The same symptoms were experienced by some of the survivors, but to a lesser extent. All survivors gained weight normally during the 14-day recovery period.

Metabolism/Toxicokinetic Studies

Absorption of methacrylonitrile occurs through the skin, respiratory tract, and gastrointestinal tract and is distributed to all major tissues (Smyth et al, 1962; Pozzani et al., 1968; Tanii and Hashimoto, 1984; Farooqui and Mumtaz, 1991; Ghanayem et al., 1992). Metabolism studies show that methacrylonitrile is metabolized via the cytochrome P-450 mixed function oxidase system and through conjugation with reduced glutathione. This results in the production of several metabolites possibly via an epoxide intermediate including cyanide and acetone (Farooqui and Mumtaz, 1991; Ghanayem et al., 1992; Ghanayem and Burka, 1996).

DERIVATION OF PROVISIONAL VALUES

Table 4 presents a summary of noncancer reference values. Table 5 presents a summary of cancer values for methacrylonitrile. IRIS data are indicated in the table, if available.

Table 4. Summary of Noncancer Reference Values for Methacrylonitrile (CASRN 126-98-7)

Toxicity type (units)	Species/Sex	Critical Effect	p-Reference Dose	POD Method	POD	UF_C	Principal Study
Subchronic p-RfD (mg/kg-d)	Rat/M	Increased relative lung weight in males	5×10^{-2}	BMDL	5.06 mg/kg-d	100	NTP (2000)
Chronic RfD (mg/kg-d) (IRIS; U.S. EPA, 1987a)	Dog/M	Increase in SGOT, SGPT levels	1×10^{-4}	NOAEL/LOAEL (converted from inhalation exposure)	0.34 mg/kg-d	3000	Pozzani et al. (1968)
Subchronic p-RfC (mg/m^3)	Rat/M, F	liver weight	3×10^{-1}	NOAEL/LOAEL	30.1 mg/m^3	100	Pozzani et al. (1968)
Chronic p-RfC (mg/m^3)	Rat/M, F	liver weight	3×10^{-2}	NOAEL/LOAEL	30.1 mg/m^3	1000	Pozzani et al. (1968)

Table 5. Summary of Cancer Reference Values for Methacrylonitrile

Toxicity Value	Reference Value	Tumor Type or Precursor Effect	Species/Sex	Principal Study
p-OSF	N/A	N/A	N/A	N/A
p-IUR	N/A	N/A	N/A	N/A

N/A = not available.

A study by Farooqui et al. (1990) demonstrated the metabolism of methacrylonitrile to cyanide by the cytochrome P-450 mixed function oxidase system. The study authors observed localization of metabolic activity in the microsomal (rather than the nuclear, mitochondrial, or cytosolic) fraction of the liver and noted the requirement for NADPH and oxygen during metabolism. The study authors also reported that the rate of methacrylonitrile metabolism was increased by treatments that enhanced hepatic cytochrome P-450 content, and similarly, decreased when the hepatic cytochrome P-450 content was reduced. Acetone has also been identified as a metabolite formed from methacrylonitrile metabolism, and it was suggested that metabolism to acetone may further induce metabolism of methacrylonitrile and other cytochrome P-450 substrates (Ghanayem et al., 1992). The elimination of methacrylonitrile (as unchanged methacrylonitrile, acetone, and CO_2) is primarily via expired air and urine (Ghanayem et al., 1992; Ghanayem and Burka, 1996).

DERIVATION OF ORAL REFERENCE DOSES

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

There are no human studies examining the health effects from subchronic oral exposure to methacrylonitrile. However, available animal subchronic and developmental/reproductive studies could be used to derive a subchronic p-RfD (see Table 6). There are three available subchronic oral animal studies for methacrylonitrile (NTP, 2000; MHLW, 2001; Gagnaire et al., 1998). Of these three, NTP (2000) is selected for the purposes of deriving a subchronic p-RfD. This is a peer-reviewed study, was performed according to GLP standards, and meets the standards of study design and performance, with numbers of animals, examination of potential toxicity information, and presentation of data included in the study report. MHLW (2001) was summarized in OECD (2002), and the original study was not available for review. A NOAEL of 15 mg/kg-day and a LOAEL of 30 mg/kg-day are identified from this study, based on anemia in males and extramedullary hematopoiesis in the spleen in females. Gagnaire et al. (1998) exposed rats for 12 weeks to doses ranging up to 90 mg/kg-day; however, a NOAEL and a LOAEL cannot be identified because of the high level of mortality in this study (2/12 rats died in the lowest dose group). NTP (2000) exposed both rats and mice to methacrylonitrile for 13 weeks, with increased relative liver weights and relative lung weights in male rats at 21.4 mg/kg-day and increased relative stomach weight in male mice at 4.3 mg/kg-day. Increased relative stomach weights at 6 and 9 mg/kg-day were reported for male mice in NTP (2000), but no stomach lesions were observed. NTP stated that the toxicological significance of the organ-weight changes (without corresponding gross or microscopic pathologic changes) was difficult to determine. EPA considers the increased stomach weights not to be of toxicological significance. A NOAEL of 10.7 mg/kg-day and a LOAEL of 21.4 mg/kg-day are identified for rats, and a NOAEL of 2.1 mg/kg-day and a LOAEL of 4.3 mg/kg-day are identified for mice.

There are six reproductive/developmental studies for methacrylonitrile (NTP, 1993a,b, 1997; MHLW, 2001; Farooqui and Villarreal, 1992; Villarreal et al., 1988). In the NTP (1993a) study, no developmental effects were noted at any dose in rats, resulting in a NOAEL of 50 mg/kg-day for developmental effects (with no LOAEL). A NOAEL of 50 mg/kg-day was also noted by NTP (1993a) for maternal effects, because the only maternal effects noted were increased liver weights, but the difference was less than 10%. In NTP (1993b), the only reproductive effect noted in rabbits was a statistically-significant decrease in the percentage of male fetuses, which was not considered biologically significant by the study authors. A NOAEL at the highest dose of 5 mg/kg-day was established. Farooqui and Villarreal (1992) and

Villarreal et al. (1988) only used two doses (50 and 100 mg/kg-day), with effects on fertility, edema in fallopian tubes, and other effects noted at 50 mg/kg-day in Farooqui and Villarreal (1992), and fetal deaths occurring at both doses in Villarreal et al. (1988). No reproductive effects were noted in MHLW (2001) at doses up to 30 mg/kg-day. NTP (1997) reported a decrease in epididymal sperm density in F1 males and organ-weight changes at 20 mg/kg-day. Similar effects were reported for F0 animals at the same exposure level. Therefore, a LOAEL of 20 mg/kg-day is established for NTP (1997) based on increased epididymal weights (12−14%) and stomach weights (19%) in F0 males, increased relative liver weights (12−13%) in F0 males and females (Task 2), and decreased (19%) epididymal sperm density and increased organ weights (liver, 13%; stomach, 11%; ventral prostate, 16%) in F1 males (Task 4). A NOAEL for the F1 animals is not established.

Table 6. Summary of Relevant Oral Subchronic or Developmental/Reproductive Studies for Methacrylonitrile

Reference	#/Sex (M/F)	Exposure (mg/kg-d)	Frequency/ duration	NOAEL[a] (mg/kg-d)	LOAEL[b] (mg/kg-d)	BMDL[b] (mg/kg-d)	Critical Endpoint
NTP (2000)	10/10 rat	0, 5, 11, 21, 43, 85*	5 d/wk for 13 wk, gavage	10.7	21.4	5.06	Increase in absolute and relative male lung weights.
MHLW (2001)	12/12 rat	0, 5.4, 11, 21	39−51 d, gavage (frequency not reported)	11	21	Not run	Anemia in males.
NTP (1993a)	0/26 rat	0, 5, 25, 50	GDs 6−15	50 (dev.) 50 (maternal)	None (dev.) None (maternal)	Not run	No adverse developmental or maternal effects
NTP (1997)	20/20 rat	0, 2, 7, 20 (F0) 0, 20 (F1)	15 wk (F0) Weaning to 80 d old (F1)	7	20	9.7	Decrease in epididymal sperm density in F1 males; organ-weight changes.
MHLW (2001)	12/12 rat	0, 5.4, 11, 21	39−51 d, gavage (frequency not reported)	21	None	Not run	No reproductive effects.
Farooqui and Villarreal (1992)	0/6 rat	50 (1st wk of gestation), 50, 100 (2nd wk of gestation)	1st or 2nd wk of gestation	None	50	Not run	Ataxia, decreased body weights, edema in fallopian tubes, effects on fertility.
NTP (1993b)	0/17−22 rabbit	0, 1, 3, 5	GD 6−19	5 (dev.) 5 (maternal)	None (dev.) None (maternal)	Not run	None

Principal study bolded.
*Doses adjusted for 5 d/wk dosing schedule.
[a]$NOAEL_{ADJ}$ = NOAEL × (gavage schedule).
[b]$LOAEL_{ADJ}$ = LOAEL × (gavage schedule).

Benchmark dose (BMD) modeling was conducted for the most sensitive toxicologically-significant endpoints reported by NTP (2000) and NTP (1997, F0 and F1 animals); Tables B.1 through B.3 present the data from the studies that were used in the BMD modeling results. Tables C.1 through C.4 present the corresponding results of the BMD modeling. The focus of the BMD modeling was on endpoints that were defined as critical effects in each of these studies, with a few other effects (manifesting at higher doses) included that could inform the determination of the POD. A BMR of 10% relative deviation was used for modeling liver weights, and a BMR of 1 standard deviation (1 SD) from the control mean was used for all other continuous effects. Table C.1 shows the BMD modeling results for the NTP (2000) 13-week gavage study in mice and rats (data in Table B.1). Tables C.2 and C.3 show the

results for the F0 and F1 generations for the NTP (1997) two-generation reproduction study, respectively (with corresponding data in Tables B.2 and B.3). Of all the endpoints modeled, the lowest BMDL was obtained for increased relative lung weight in male rats in the subchronic gavage study (NTP, 2000; Table C.1). Therefore, the $BMDL_{1SD}$ of 5.06 mg/kg-day for increased relative lung weight in male rats in the NTP (2000) subchronic study is selected as the POD.

NTP (2000) is selected as the principal study to determine the subchronic p-RfD because this study identified the lowest BMDL for potentially significant subchronic effects. The $BMDL_{1SD}$ of 5.06 mg/kg-day for increased relative lung weight in male mice (NTP, 2000) is used to establish the POD for the subchronic p-RfD, which is derived as follows:

$$\textbf{Subchronic p-RfD} = BMDL_{1SD} \div UF_C$$
$$= 5.06 \text{ mg/kg-day} \div 100$$
$$= \mathbf{5 \times 10^{-2} \text{ mg/kg-day}}$$

Tables 7 and 8, respectively, summarize the uncertainty factors and the confidence descriptor for the subchronic p-RfD for methacrylonitrile.

Derivation of Provisional Chronic RfD (Chronic RfD)

No chronic p-RfD is developed. IRIS (U.S. EPA, 1987a) provides an RfD of 1×10^{-4} mg/kg-day, based on the Pozzani et al. (1968) subchronic dog inhalation study. The critical effect (LOAEL) was increased SGOT and SGPT levels at 24 mg/m^3. A NOAEL of 9 mg/m^3 was used as the POD and converted to an oral exposure (0.34 mg/kg-day) based on a dog inhalation rate of 4.3 m^3/day, an absorption factor of 0.5, and an assumed dog body weight of 12.7 kg. A total UF of 3000 was used, including a 10-fold factor for interspecies extrapolation, a 10-fold factor for sensitive individuals, a 10-fold factor for the use of a subchronic NOAEL, and a 3-fold factor because only inhalation studies were available, neurotoxicity was not examined, and reproductive and chronic toxicity data were lacking (U.S. EPA, 1987a). However, additional studies published after the IRIS assessment was finalized were identified that may be relevant—particularly the chronic study by the National Toxicology Program (NTP, 2001).

Table 7. Uncertainty Factors for Subchronic p-RfD of Methacrylonitrile

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied for interspecies extrapolation to account for potential toxicokinetic and toxicodynamic differences between rats and humans. There are no data to determine whether humans are more or less sensitive than rats to the systemic toxicity of methacrylonitrile.
UF_D	1	A UF_D of 1 is applied because the database contains at least one acceptable two-generation reproduction study in rats (NTP, 1997); at least one acceptable developmental study (NTP, 1993a,b).
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
UF_L	1	A UF_L of 1 is applied because the POD is a BMDL.
UF_S	1	A UF_S of 1 is applied because a subchronic-duration study (NTP, 2000) was utilized as the principal study.
UF_C	100	

Table 8. Confidence Descriptor for Subchronic p-RfD for Methacrylonitrile

Confidence Categories	Designation[a]	Discussion
Confidence in Study	**H**	Confidence in the principal study (NTP, 2000) is high because the study was conducted according to GLP standards and meets the standards of study design and performance, with numbers of animals, examination of potential toxicity information, and presentation of data.
Confidence in Database	**H**	Confidence in the database is high because data are available for a variety of subchronic endpoints in rats and mice, and reproductive/developmental studies are available for rats, mice, and rabbits.
Confidence in Subchronic p-RfD	**H**	The overall confidence in the subchronic p-RfD is high.

[a]L = Low, M = Medium, H = High.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

Derivation of Subchronic Provisional RfC (Subchronic p-RfC)

There are no human subchronic studies available for methacrylonitrile. There is one subchronic inhalation study available in rats and dogs (Pozzani et al., 1968) and one developmental study in rats (Saillenfait et al., 1993). The Pozzani et al. (1968) study in dogs was used as the principal study in the derivation of the IRIS RfD (the dose was converted from inhalation to oral exposure in U.S. EPA, 1987a) and as the basis for the subchronic and chronic inhalation RfC in HEAST (U.S. EPA, 2010). However, only three dogs per dose group were used in Pozzani et al. (1968), and the critical endpoint used as the basis for the RfD and RfCs (increases in SGOT and SGTP in one dog) was transitory. The Pozzani et al. (1968) study in rats used 12 rats per group; higher relative liver weights were noted in males and females at 109.3 mg/m^3 (equivalent to 62.5 mg/m^3). Saillenfait et al. (1993) reported significantly reduced fetal body weights at 274 mg/m^3, resulting in the identification of a NOAEL of 137 mg/m^3 and a LOAEL of 274 mg/m^3.

The Pozzani et al. (1968) study in rats is used as the principal study to determine the subchronic p-RfC. This is a published, peer-reviewed study, but it was conducted prior to the establishment of GLP standards. The critical effect (increased liver weight) is supported in the oral-administration studies, having been observed in several rodent studies. A LOAEL of 62.5 mg/m^3 for increased relative liver weight in males and females is identified in this study, with a NOAEL of 30.1 mg/m^3. BMD modeling cannot be conducted because variance measures were not given for the reported endpoint means. The $NOAEL_{HEC}$ for extra-respiratory effects is used to establish the POD as follows.

$NOAEL_{HEC}$ = ppm × (MW ÷ 24.45) × (hours exposed ÷ 24) × (days exposed ÷ total days of study) × (blood:air partition coefficient for extra-respiratory effects). The standard value of 1 is used for the blood:air partition coefficient because a measured value is not available.

$NOAEL_{HEC}$ = 52.6 ppm × (67.09 ÷ 24.45) × (7 hr ÷ 24 hr) × (65 ÷ 91) × (1)
= 30.1 mg/m^3

The subchronic p-RfC is developed as follows:

Subchronic p-RfC = $NOAEL_{HEC, EXRESP} \div UF_C$
= 30.1 $mg/m^3 \div 100$
= **3×10^{-1} mg/m^3**

Tables 9 and 10, respectively, summarize the uncertainty factors and the confidence descriptor for the subchronic p-RfC for methacrylonitrile.

Derivation of Chronic Provisional RfC (Chronic p-RfC)

There are no chronic studies available for inhalation exposure to methacrylonitrile. Therefore, the subchronic rat study by Pozzani et al. (1968) will be used to derive the chronic p-RfC, for the same reasons as discussed above for the subchronic p-RfC.

The chronic p-RfC is developed as follows:

Chronic p-RfC = $NOAEL_{HEC, EXRESP} \div UF_C$
= 30.1 $mg/m^3 \div 1000$
= 3×10^{-2} mg/m^3

Tables 11 and 12, respectively, summarize the uncertainty factors and the confidence descriptor for the chronic p-RfC for methacrylonitrile.

Table 9. Uncertainty Factors for Subchronic p-RfC of Methacrylonitrile

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied for animal-to-human extrapolation to account for the toxicodynamic portion of the UF_A because the toxicokinetic portion ($10^{0.5}$) has been addressed in dosimetric conversions. There are no data to determine whether humans are more or less sensitive than rats to the systemic toxicity of methacrylonitrile.
UF_D	3	A UF_D of 3 ($10^{0.5}$) is applied because the database contains at least one acceptable developmental inhalation study in rats (Saillenfait et al., 1993) but no two-generation inhalation reproduction study. The oral two-generation reproduction study (NTP, 1997) does not satisfy the requirement because of the indication that there could be significant metabolism in the liver, possibly leading to first-pass effects. In addition, the sperm effects reported in that study occurred at exposure levels similar to the critical effect for oral exposure and have the potential to be sensitive effects by the inhalation route.
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
UF_L	1	A UF_L of 1 is applied because the POD is a NOAEL.
UF_S	1	A UF_S of 1 is applied because a subchronic-duration study (Pozzani et al., 1968) was utilized as the principal study.
UF_C	100	

Table 10. Confidence Descriptors for Subchronic p-RfC for Methacrylonitrile

Confidence Categories	Designation[a]	Discussion
Confidence in study	**L**	Confidence in the principal study (Pozzani et al., 1968) is low because details of the study, including the tissues examined, were not provided. In addition, unexplained mortality occurred at the higher exposure levels.
Confidence in database	**L**	Confidence in the database is low because data are not available for a variety of subchronic endpoints, and data are not available on reproductive effects.
Confidence in subchronic p-RfC	**L**	The overall confidence in the subchronic p-RfC is low.

[a]L = Low, M = Medium, H = High.

Table 11. Uncertainty Factors for Chronic p-RfC of Methacrylonitrile

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied for animal-to-human extrapolation to account for the toxicodynamic portion of the UF_A because the toxicokinetic portion ($10^{0.5}$) has been addressed in dosimetric conversions. There are no data to determine whether humans are more or less sensitive than rats to the systemic toxicity of methacrylonitrile.
UF_D	3	A UF_D of 3 ($10^{0.5}$) is applied because the database contains at least one acceptable developmental inhalation study in rats (Saillenfait et al., 1993) but no acceptable inhalation two-generation reproduction study. The oral two-generation reproduction study (NTP, 1997) does not satisfy the requirement because of the indication that there could be significant metabolism in the liver, possibly leading to first-pass effects. In addition, the sperm effects reported in that study occurred at exposure levels similar to the critical effect for oral exposure and have the potential to be sensitive effects by the inhalation route.
UF_H	10	A UF_H of 10 for is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response to humans.
UF_L	1	A UF_L of 1 is applied because the POD is a NOAEL.
UF_S	10	A UF_S of 10 is applied because a subchronic-duration study (Pozzani et al., 1968) was utilized as the principal study.
UF_C	1000	

Table 12. Confidence Descriptors for Chronic p-RfC for Methacrylonitrile		
Confidence Categories	**Designation[a]**	**Discussion**
Confidence in study	**L**	Confidence in the principal study (Pozzani et al., 1968) is low because details of the study, including the tissues examined, were not provided. In addition, unexplained mortality occurred at the higher exposure levels.
Confidence in database	**L**	Confidence in the database is low because data are not available for a variety of subchronic endpoints, and data are not available on reproductive effects.
Confidence in subchronic p-RfC	**L**	The overall confidence in the subchronic p-RfC is low.

[a]L = Low, M = Medium, H = High.

CANCER WEIGHT-OF-EVIDENCE (WOE) DESCRIPTOR

No human data are available on the carcinogenicity of methacrylonitrile. Methacrylonitrile has not been classified previously for carcinogenicity based on EPA's 1986 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986) or EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005). The NTP (2001) conducted a 2-year cancer study by gavage in rats and mice and concluded that "…there was no evidence of carcinogenic activity of methacrylonitrile in male or female F344/N rats administered 3, 10, or 30 mg/kg-day and there was no evidence of carcinogenic activity of methacrylonitrile in male or female mice administered 1.5, 3, or 6 mg/kg." Genotoxicity and mutagenicity studies have shown primarily negative results. In in vitro studies, methacrylonitrile was negative for mutations in *Salmonella typhimurium* (Zeiger et al., 1987; MHLW, 2001; Knaap et al., 1985; Wu et al., 2009) and did not cause an increase in sex-linked recessive lethal mutations in *Drosophila melanogaster* (Zimmering et al., 1989). Methacrylonitrile was positive in the fluctuation test in *Klebsiella pneumoniae* (Knapp et al., 1985) and in a chromosome aberration test in Chinese hamster lung cells with metabolic activation (but negative without metabolic activation) (MHLW, 2001). In vivo, methacrylonitrile was negative for the induction of micronucleated polychromatic erythrocytes in rats and mice (Shelby et al., 1993; MacGregor et al., 1990). A mutagenic mode of carcinogenic action for methacrylonitrile is unlikely.

Therefore, given the negative mutagenicity data and negative data for carcinogenicity in two species, the cancer WOE descriptor for methacrylonitrile is judged as "*Not Likely to Be Carcinogenic to Humans*" by the oral route of exposure (Table 13).

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Provisional Oral Slope Factor (p-OSF)

No p-OFS can be derived due to inadequate carcinogenicity data.

Derivation of Provisional Inhalation Unit Risk (p-IUR)

No p-IUR can be derived due to inadequate carcinogenicity data.

Table 13. Cancer WOE Descriptor for Methacrylonitrile			
Possible WOE Descriptor	**Designation**	**Route of Entry (Oral, Inhalation, or Both)**	**Comments**
"Carcinogenic to Humans"	N/A	N/A	None
"Likely to Be Carcinogenic to Humans"	N/A	N/A	None
"Suggestive Evidence of Carcinogenic Potential"	N/A	N/A	None
"Inadequate Information to Assess Carcinogenic Potential"	N/A	Inhalation	Negative oral carcinogenicity data are strong enough for a descriptor of "*Not Likely to Be Carcinogenic to Humans*". There are inadequate data to assess methacrylonitrile carcinogenicity via the inhalation route of exposure.
"Not Likely to Be Carcinogenic to Humans"	**Selected**	**Oral**	**A 2-year gavage study in rats and mice showed no increase in cancer incidence (NTP, 2001). Mutagenicity studies have been primarily negative.**

APPENDIX A. PROVISIONAL SCREENING VALUES

No provisional screening values were derived.

APPENDIX B. DATA TABLES

Table B.1. Relative Organ Weights in Mice and Rats in 13-Week Gavage Study of Methacrylonitrile[a,b]

Rats	Vehicle Control	5.4 mg/kg-d	11 mg/kg-d	21 mg/kg-d	43 mg/kg-d	85.7 mg/kg-d
Male rats						
Animals examined	10	10	10	10	8	0
Liver	33.45 ± 0.60	35.04 ± 0.50	35.28 ± 0.38	39.04 ± 0.97**	40.09 ± 1.39**	-
Lung	4.43 ± 0.09	4.54 ± 0.11	4.71 ± 0.13	4.86 ± 0.14*	4.89 ± 0.09**	-
Female rats						
Animals examined	10	10	10	10	10	9
Liver	32.89 ± 0.75	32.43 ± 0.54	33.22 ± 0.53	34.06 ± 0.60	33.25 ± 108	38.34 ± 113**
Thymus	1.26 ± 0.06	1.29 ± 0.08	1.24 ± 0.05	1.24 ± 0.05	1.10 ± 0.09	0.87 ± 0.03**

[a]NTP (2000).
[b]Values Given as Mean ± Std. Error (100 × Organ Weight [mg]/Body Weight [g]).
*Significantly different ($p \leq 0.05$) from the vehicle control group by William's or Dunnett's test.
** $p \leq 0.01$.

Table B.2. Sperm Morphology and Relative Organ Weights in F0 Rats in Two-Generation Gavage Study of Methacrylonitrile[a,b]

	Vehicle Control	2 mg/kg-d	7 mg/kg-d	20 mg/kg-d
Animals Examined	19	10	10	10
Males				
Epididymal sperm morphology (% abnormal)	0.29 ± 0.08	0.95 ± 0.22*	0.55 ± 0.12	1.20 ± 0.28*
Relative liver weight	36.52 ± 0.86	36.42 ± 1.03	38.60 ± 0.98	41.40 ± 0.79*
Relative cauda epididymus weight	0.43 ± 0.02	0.44 ± 0.02	0.45 ± 0.02	0.49 ± 0.01*
Females				
Relative liver weight	36.87 ± 0.86	38.53 ± 0.82	36.33 ± 1.44	41.29 ± 0.68*

[a]NTP (1997, Task 2).
[b]Values Given as Mean ± Std. Error (Organ Weight [mg]/Body Weight [g]).
*Significantly different ($p \leq 0.05$) from the vehicle control group by Shirley's or Dunn's test.

Table B.3. Sperm Endpoints and Relative Organ Weights in F1 Rats in Two-Generation Gavage Study of Methacrylonitrile[a]

	Vehicle Control	20 mg/kg-d
Animals Examined	20	20[b]
Males		
Cauda epididymal sperm density (no. of sperm per mg cauda × 10^3)	357.31 ± 11.83	288.53 ± 9.80
Epididymal sperm morphology (% abnormal)	0.25 ± 0.09	0.37 ± 0.15
Relative liver weight (% BW)	3.772 ± 0.3710	4.279 ± 0.4076
Relative ventral prostate weight (% BW)	0.198 ± 0.0437	0.230 ± 0.0380
Females		
Relative liver weight (%)	3.374 ± 0.3370	3.799 ± 0.4092

[a]NTP (1997, Task 4).
[b]19 for sperm endpoints.

APPENDIX C. BMD OUTPUTS

Table C.1. Benchmark Dose Modeling Results for 13-Week Gavage Study in Rats[a]

Model	BMR[b]	*p*-Value	AIC	BMD	BMDL	Notes
Male rats, relative lung weight						
Exponential (M4)	1 SD	0.945	−47.12	14.2	5.06	Selected model. M4 not a better fit than M2, statistically, but fits much better in the BMR range
Exponential (M2)	1 SD	0.395	−46.47	33.4	21.9	BMDL > LOAEL
Linear (poly 1º)	1 SD	0.418	−44.61	32.4	20.8	4-degree polynomial allowed; BMDL > LOAEL
Hill, unrestricted	1 SD	0.919	−45.44	13.4	4.85	Hill coefficient = 2.4
Power, unrestricted	1 SD	0.592	−46.40	18.3	2.61	Supralinear (power = 0.59)
Male rats, relative liver weight						
Linear (poly 1º)	10% RD	0.123	129.41	17.8	13.9	Slightly better fit than exponential
Exponential (M4)	10% RD	0.102	130.19	14.9	9.76	
Hill, unrestricted	10% RD	0.060	131.16	17.4	10.7	Relatively poor fit; Hill coefficient = 3.6
Power, unrestricted	10% RD	0.073	130.86	15.9	9.97	Relatively poor fit; supralinear (power = 0.84)
Female rats, relative liver weight						
Polynomial (2º)	10% RD	0.585	169.50	49.1	25.6	4-degree polynomial allowed
Exponential (M4)	10% RD	0.413	170.43	63.3	42.7	
Hill, unrestricted	10% RD	0.377	171.52	66.5	45.4	Hill coefficient = 1.5
Power, unrestricted	10% RD	0.582	169.52	66.5	45.4	Power = 1.5
Female rats, relative thymus weight						*Modeled variance; variance model did not fit (p = 0.076)*
Polynomial (2º)	1 SD	0.871	−132.68	63.9	55.9	4-degree polynomial allowed
Exponential (M2)	1 SD	0.319	−129.22	39.8	28.0	
Hill, unrestricted	1 SD	0.547	−128.72	66.5	failed	Hill coefficient = 2.3
Power, unrestricted	1 SD	0.751	−130.71	66.5	39.6	Power = 2.3

[a]NTP (2000).
[b]RD = relative deviation; SD = standard deviation.

Table C.2. Benchmark Dose Modeling Results for F0 Generation in a Two-Generation Rat Study[a]

Model	BMR[b]	*p*-Value	AIC	BMD	BMDL	Notes
F0 Males, relative liver weight						
Linear (poly 1º)	**10% RD**	**0.800**	**168.42**	**14.5**	**10.1**	3-degree polynomial allowed
Exponential (M2)	10% RD	0.789	168.46	14.7	10.6	
Hill	10% RD	NA	171.98	7.78	5.73	Saturated model[c]; Hill coefficient = 12.7
Hill, intercept specified	10% RD	0.933	169.98	7.74	failed	Intercept = control mean; Hill coefficient = 13.5
Power, unrestricted	10% RD	0.508	170.42	14.1	6.80	Slightly supralinear (power = 0.96)
F0 Males, relative cauda epididymus weight						
Linear (poly 1º)	1 SD	0.579	−220.07	27.5	17.9	3-degree polynomial allowed
Exponential (M2)	1 SD	0.570	−220.03	26.9	18.2	
Hill, restricted	1SD	0.304	−218.10	13.7	failed	Unrestricted model supralinear
Hill, unrestricted	1 SD	NA	−216.24	29.6	failed	Supralinear (power = 0.798); saturated model
Power, unrestricted	1SD	0.339	−218.24	29.6	16.4	Supralinear (power = 0.792)
F0 Males, percent sperm abnormalities						*No good model fits*
Linear (poly 1º)	1 SD	0.0006	5.95	12.14	6.592	Best fit, but inadequate
F0 females, relative liver weight						*No response near BMR, all fits suboptimal*
Polynomial (2º)	10% RD	0.446	172.38	17.1	13.7	4-degree polynomial allowed
Linear (poly 1º)	10% RD	0.069	176.10	14.1	10.2	Relatively poor fit
Exponential (M3)	10% RD	0.466	175.79	19.7	14.7	
Hill	10% RD	0.466	173.30	19.6	7.79	Hill coefficient at upper bound
Power	10% RD	0.466	173.30	19.7	14.6	power = 17.9

[a]NTP (1997, Task 2).
[b]RD = relative deviation; SD = standard deviation.
[c]Residual degrees of freedom = 0.

Table C.3. Benchmark Dose Modeling Results for F1 Generation in a Two-Generation Rat Study[a]

Model	BMR[b]	*p*-Value	AIC	BMD	BMDL	Notes
F1 Males, cauda epididymus sperm density						
Linear (poly 1º)	1 SD	-[c]	-	13.7	9.71	Saturated model[d]
F1 Males, relative liver weight						
Linear (poly 1º)	10% RD	-	-	14.9	10.4	Saturated model
F1 Males, relative ventral prostate weight						
Linear (poly 1º)	1 SD	-	-	25.0	14.9	Saturated model
F1 Males, relative stomach weight						
Linear (poly 1º)	1 SD	-	-	17.7	11.8	Saturated model
F1 females, relative liver weight						
Linear (poly 1º)	10% RD	-	-	15.9	10.6	Saturated model

[a]NTP (1997, Task 2).
[b]SD = standard deviation.
[c]Saturated models; all *p*-values = NA; AIC values not relevant.
[d]Residual degrees of freedom = 0.

BMDS Output for Critical Effect: Increased Relative and Absolute Lung Weight in Male Rats in 13-Week Gavage Study (NTP, 2000)

```
================================================================
        Exponential Model. (Version: 1.61;  Date: 7/24/2009)
        Input Data File:
C:\Usepa\BMDS21\Data\exp_Methacrylonitrile_NTP_2000_male_Setting.(d)
        Gnuplot Plotting File:
                                                Wed Jun 01 13:23:27 2011
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = m_lung_rel
   Independent variable = d
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
   rho is set to 0.
   A constant variance model is fit.

   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   MLE solution provided: Exact


                                Initial Parameter Values

     Variable          Model 2           Model 3           Model 4           Model 5
     --------          -------           -------           -------           -------
      lnalpha          -2.15512          -2.15512            -2.15512          -
2.15512
          rho(S)              0                 0                   0
0
            a           4.51545           4.51545              4.2085
4.2085
            b        0.00230953        0.00230953           0.0384462
0.0384462
            c                --                --               1.22003
1.22003
            d                --                 1                 --
1

     (S) = Specified
```

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-2.09311	-2.09311	-2.14831	-2.15503
rho	0	0	0	0
a	4.52092	4.52092	4.41104	4.4289
b	0.00224199	0.00224199	0.0751941	0.0897299
c	--	--	1.11822	1.1033
d	--	1	--	1.70506

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	10	4.43	0.285
5.36	10	4.54	0.348
10.7	10	4.71	0.411
21.4	10	4.86	0.443
42.9	8	4.89	0.255

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	4.521	0.3511	-0.8188
	5.36	4.576	0.3511	-0.3204
	10.7	4.631	0.3511	0.7143
	21.4	4.743	0.3511	1.053
	42.9	4.977	0.3511	-0.7036
3	0	4.521	0.3511	-0.8188
	5.36	4.576	0.3511	-0.3204
	10.7	4.631	0.3511	0.7143
	21.4	4.743	0.3511	1.053
	42.9	4.977	0.3511	-0.7036
4	0	4.411	0.3416	0.1755
	5.36	4.584	0.3416	-0.4076
	10.7	4.699	0.3416	0.09925
	21.4	4.828	0.3416	0.2944
	42.9	4.912	0.3416	-0.1806
5	0	4.429	0.3404	0.01021
	5.36	4.543	0.3404	-0.02839
	10.7	4.706	0.3404	0.03332
	21.4	4.865	0.3404	-0.04217
	42.9	4.886	0.3404	0.03022

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)

```
           Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	27.72296	6	-43.44593
A2	29.71493	10	-39.42987
A3	27.72296	6	-43.44593
R	21.971	2	-39.94201
2	26.23462	3	-46.46924
3	26.23462	3	-46.46924
4	27.55938	4	-47.11876
5	27.72061	5	-45.44122

Additive constant for all log-likelihoods = -44.11. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

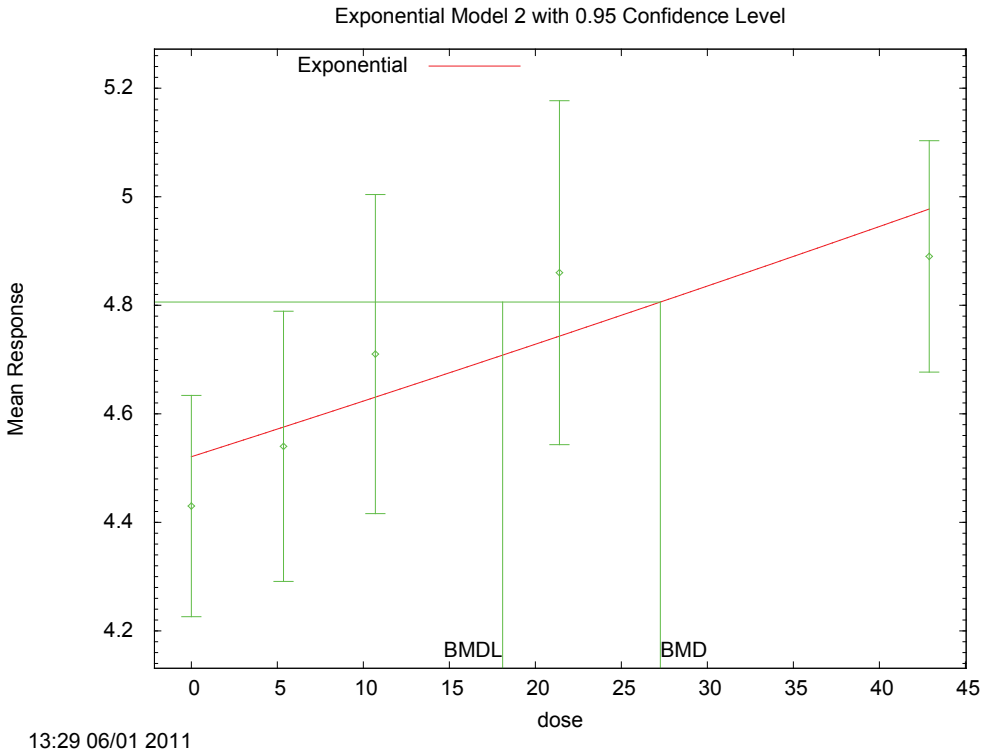
Test 7a: Does Model 5 fit the data? (A3 vs 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

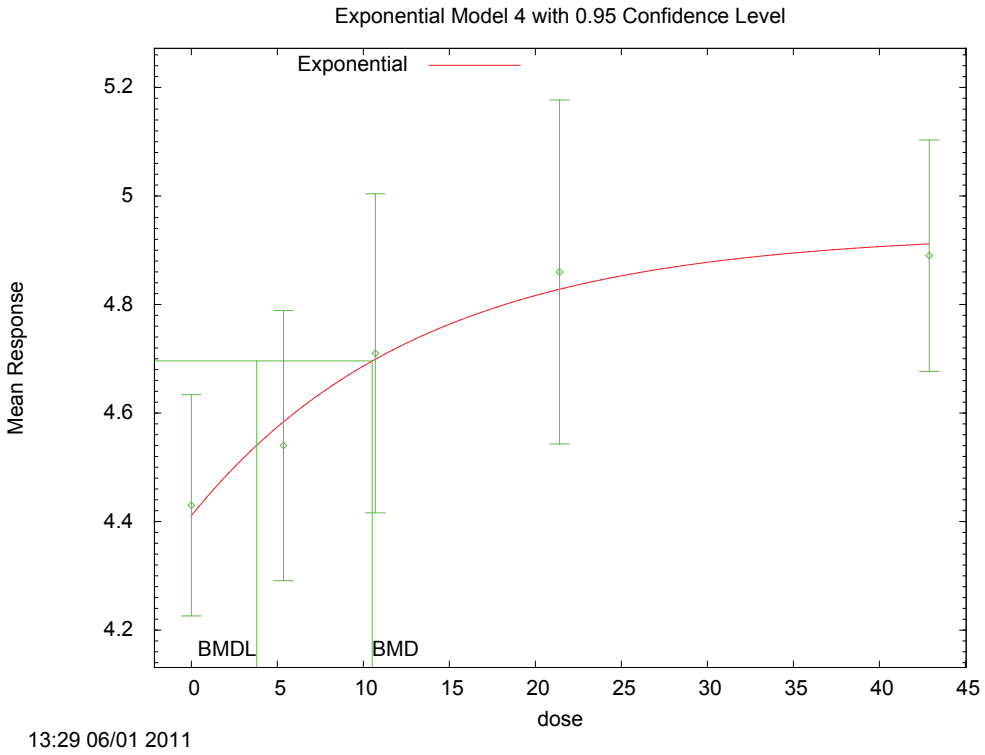
Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	15.49	8	0.05033
Test 2	3.984	4	0.4082
Test 3	3.984	4	0.4082
Test 4	2.977	3	0.3952
Test 5a	2.977	3	0.3952
Test 5b	-2.842e-013	0	N/A
Test 6a	0.3272	2	0.8491
Test 6b	2.65	1	0.1036
Test 7a	0.004712	1	0.9453
Test 7b	2.972	2	0.2263
Test 7c	0.3225	1	0.5701

The p-value for Test 1 is greater than .05. There may not be a

```
  diffence between responses and/or variances among the dose levels
  Modelling the data with a dose/response curve may not be appropriate.

  The p-value for Test 2 is greater than .1.  A homogeneous
  variance model appears to be appropriate here.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 4 is greater than .1.  Model 2 seems
  to adequately describe the data.

  The p-value for Test 5a is greater than .1.  Model 3 seems
  to adequately describe the data.

  Degrees of freedom for Test 5b are less than or equal to 0.
  The Chi-Square test for fit is not valid.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.

  The p-value for Test 6b is greater than .05.  Model 4 does
  not seem to fit the data better than Model 2.

  The p-value for Test 7a is greater than .1.  Model 5 seems
  to adequately describe the data.

  The p-value for Test 7b is greater than .05.  Model 5 does
  not seem to fit the data better than Model 3.

  The p-value for Test 7c is greater than .05.  Model 5 does
  not seem to fit the data better than Model 4.


Benchmark Dose Computations:

  Specified Effect = 1.000000

         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000


            BMD and BMDL by Model

   Model             BMD               BMDL
  -------         ------------        ----------
     2              33.3643            21.8638
     3              33.3643            21.8638
     4              14.1537            5.06031
     5              13.3654            5.36922
```


Exponential Model 2 with 0.95 Confidence Level
Exponential
Mean Response
5.2
5
4.8
4.6
4.4
4.2
BMDL
BMD
0
5
10
15
20
25
30
35
40
45
dose
13:29 06/01 2011


Exponential Model 4 with 0.95 Confidence Level
Exponential
Mean Response
5.2
5
4.8
4.6
4.4
4.2
BMDL
BMD
0
5
10
15
20
25
30
35
40
45
dose
13:29 06/01 2011

APPENDIX D. REFERENCES

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