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Provisional Peer-Reviewed Toxicity Values for

Lactonitrile
(CASRN 78-97-7)

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TABLE OF CONTENTS

COMMONLY USED ABBREVIATIONS AND ACRONYMS[1]

α2u-g	alpha 2u-globulin
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
ALD	approximate lethal dosage
ALT	alanine aminotransferase
AR	androgen receptor
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service registry number
CBI	covalent binding index
CHO	Chinese hamster ovary (cell line cells)
CL	confidence limit
CNS	central nervous system
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DEN	diethylnitrosamine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
ER	estrogen receptor
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GSH	glutathione
GST	glutathione-S-transferase
Hb/g-A	animal blood-gas partition coefficient
Hb/g-H	human blood-gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IVF	in vitro fertilization
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MOA	mode of action
MTD	maximum tolerated dose
NAG	*N*-acetyl-β-D-glucosaminidase
NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
OCT	ornithine carbamoyl transferase
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
PND	postnatal day
POD	point of departure
POD_{ADJ}	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RBC	red blood cell
RDS	replicative DNA synthesis
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase, also known as AST
SGPT	serum glutamic pyruvic transaminase, also known as ALT
SSD	systemic scleroderma
TCA	trichloroacetic acid
TCE	trichloroethylene
TWA	time-weighted average
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_C	composite uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
U.S.	United States of America
WBC	white blood cell

[1]Abbreviations and acronyms not listed on this page are defined upon first use in the PPRTV document.

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR LACTONITRILE (CASRN 78-97-7)

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 at least two National Center for Environment Assessment (NCEA) scientists and an independent external peer review by at least 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.

PPRTV assessments are eligible to be updated on a 5-year cycle to incorporate new data or methodologies that might impact the toxicity values or characterization of potential for adverse human-health effects and are revised as appropriate. Questions regarding nomination of chemicals for update can be sent to the appropriate U.S. Environmental Protection Agency (EPA) Superfund and Technology Liaison (https://www.epa.gov/research/fact-sheets-regional-science).

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. 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.

This document has been reviewed in accordance with U.S. EPA policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

QUESTIONS REGARDING PPRTVs

Questions regarding the content of this PPRTV assessment should be directed to the EPA Office of Research and Development's (ORD's) NCEA, Superfund Health Risk Technical Support Center (513-569-7300).

INTRODUCTION

Lactonitrile, CASRN 78-97-7, belongs to a class of compounds known as cyanohydrins. It is used mainly as a solvent and as an intermediate in the manufacture of lactic acid and its derivatives, such as ethyl lactate (Lewis and Hawley, 2007; Cholod, 2001). Lactonitrile is listed on EPA's Toxic Substances Control Act (TSCA) public inventory (U.S. EPA, 2015a), is registered with Europe's Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) program (ECHA, 2017), and was assessed under the EPA's High Production Volume (HPV)/Organisation for Economic Co-operation and Development (OECD) Screening Information Data Set (SIDS) Programme (OECD, 2004). Lactonitrile is listed as an EPA Extremely Hazardous Substance (S302) and has been assigned a Threshold Planning Quantity of 1,000 pounds (U.S. EPA, 2015b).

The empirical formula for lactonitrile is C_3H_5NO, and its structure is shown in Figure 1. Synonyms for lactonitrile include the following: laktonitrile, lactonitrite, 2-hydroxypropanenitrile, and 2-hydroxypropionitrile. Commercial production of lactonitrile occurs by the base-catalyzed reaction of equimolar amounts of acetaldehyde and hydrogen cyanide in the presence of 1.5 of 20% sodium hydroxide (Cholod, 2001). Table 1 summarizes the physicochemical properties of lactonitrile. Lactonitrile is a straw-yellow liquid at room temperature. It is unstable at high pH and will undergo acid hydrolysis to form propanoic acid (Cholod, 2001). The hydrolysis half-life for lactonitrile was reported as 15 days at pH 9 and 25°C; however, it was not hydrolyzed at pH 4 or 7 (ECHA, 2017). Lactonitrile's vapor pressure indicates that it will exist almost entirely as a vapor in the atmosphere. The estimated half-life of vapor-phase lactonitrile in air by reaction with photochemically produced hydroxyl radicals is 6.7 days. The moderate vapor pressure and Henry's law constant for lactonitrile indicate that it may volatilize from either dry or moist surfaces. The estimated high water solubility and low soil adsorption coefficient for lactonitrile indicate that the compound may leach to groundwater or undergo runoff after a rain event. Based on screening tests, lactonitrile may also undergo ready biodegradation in the environment (ECHA, 2017).

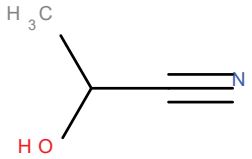

Figure 1. Lactonitrile Structure

Table 1. Physicochemical Properties of Lactonitrile (CASRN 78-97-7)

Property (unit)	Value
Physical state	Liquid
Boiling point (°C)	160.96[a]
Melting point (°C)	−40[a]
Density (g/cm^3 at 20°C)	0.9877[b]
Vapor pressure (mm Hg at 25°C)	0.119[a]
pH (unitless)	NA
pKa (unitless)	NA
Solubility in water (mg/L at 25°C)	4.66×10^5 [a]
Octanol-water partition coefficient (log K_{ow})	−0.94[a]
Henry's law constant (atm-m^3/mol at 25°C)	9.8×10^{-6} (estimated)[a]
Soil adsorption coefficient K_{oc} (L/kg)	1 (estimated)[a]
Atmospheric OH rate constant (cm^3/molecule-sec at 25°C)	1.6×10^{-12} (estimated)[a]
Atmospheric half-life (d)	6.7 (estimated)[a]
Relative vapor density (air = 1)	2.45[c]
Molecular weight (g/mol)	71[a]
Flash point (closed cup in °C)	77[c]

[a]U.S. EPA (2012c).
[b]Lide (2008).
[c]Lewis (2012).

NA = not applicable.

A summary of available toxicity values for lactonitrile from EPA and other agencies/organizations is provided in Table 2.

Table 2. Summary of Available Toxicity Values for Lactonitrile (CASRN 78-97-7)

Source (parameter)[a, b]	Value (applicability)	Notes	Reference(s)
Noncancer			
IRIS	NV	NA	U.S. EPA (2017)
HEAST	NV	NA	U.S. EPA (2011a)
DWSHA	NV	NA	U.S. EPA (2012a)
ATSDR	NV	NA	ATSDR (2017)
IPCS	NV	NA	IPCS (2017); WHO (2017)
Cal/EPA	NV	NA	Cal/EPA (2014); Cal/EPA (2017a); Cal/EPA (2017b)
OSHA	NV	NA	OSHA (2006); OSHA (2011)
NIOSH	NV	NA	NIOSH (2016)
ACGIH	NV	NA	ACGIH (2016)
DOE (PAC)	PAC-1: 0.24 mg/m^3; PAC-2: 2.6 mg/m^3; PAC-3: 16 mg/m^3	Based on TEEL values	DOE (2015)
USAPHC (air-MEG)	1-hr critical: 150 mg/m^3; 1-hr marginal: 18 mg/m^3; 1-hr negligible: 10 mg/m^3	Based on TEEL values	U.S. APHC (2013)
Cancer			
IRIS	NV	NA	U.S. EPA (2017)
HEAST	NV	NA	U.S. EPA (2011a)
DWSHA	NV	NA	U.S. EPA (2012a)
NTP	NV	NA	NTP (2014)
IARC	NV	NA	IARC (2015)
Cal/EPA	NV	NA	Cal/EPA (2011); Cal/EPA (2017a); Cal/EPA (2017b)
ACGIH	NV	NA	ACGIH (2016)

[a]Sources: ACGIH = American Conference of Governmental Industrial Hygienists; ATSDR = Agency for Toxic Substances and Disease Registry; Cal/EPA = California Environmental Protection Agency; DOE = Department of Energy; DWSHA = Drinking Water Standards and Health Advisories; HEAST = Health Effects Assessment Summary Tables; IARC = International Agency for Research on Cancer; IPCS = International Programme on Chemical Safety; IRIS = Integrated Risk Information System; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; USAPHC = U.S. Army Public Health Command.
[b]Parameters: MEG = military exposure guideline; PAC = protective action criteria; TEEL = temporary emergency exposure limit.

NA = not applicable; NV = not available.

Non-date-limited literature searches were conducted in June 2015 and updated in April 2017 for studies relevant to the derivation of provisional toxicity values for lactonitrile. Searches were conducted using the U.S. EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: PubMed, ToxLine (including TSCATS1), and Web of Science. The following databases were searched outside of HERO for health-related data: American Conference of Governmental Industrial Hygienists (ACGIH), Agency for Toxic Substances and Disease Registry (ATSDR), California Environmental Protection Agency (Cal/EPA), U.S. EPA Integrated Risk Information System (IRIS), U.S. EPA Health Effects Assessment Summary Tables (HEAST), U.S. EPA Office of Water (OW), U.S. EPA TSCATS2/TSCATS8e, U.S. HPV, European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), Japan Existing Chemical Data Base (JECDB), European Chemicals Agency (ECHA), OECD SIDS, OECD International Uniform Chemical Information Database (IUCLID), OECD HPV, National Institute for Occupational Safety and Health (NIOSH), National Toxicology Program (NTP), Occupational Safety and Health Administration (OSHA), and Defense Technical Information Center (DTIC).

REVIEW OF POTENTIALLY RELEVANT DATA (NONCANCER AND CANCER)

Tables 3A and 3B provide overviews of the relevant noncancer and cancer databases, respectively, for lactonitrile and include all potentially relevant repeated-dose short-term-, subchronic-, and chronic-duration studies as well as reproductive and developmental toxicity studies. Principal studies are identified in bold. The phrase "statistical significance" and the term "significant(ly)," used throughout the document, indicate a p-value of < 0.05 unless otherwise specified.

Table 3A. Summary of Potentially Relevant Noncancer Data for Lactonitrile (CASRN 78-97-7)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration	Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Human							
1. Oral (mg/kg-d)							
ND							
2. Inhalation (mg/m^3)							
ND							
Animal							
1. Oral (mg/kg-d)							
Combined subchronic toxicity and R/D screening study	**10 M/10 F, Crj:CD(SD) rat; gavage (vehicle not specified); 7 d/wk, 6 wk (2 wk premating through 2 wk postmating [M] or PND 3 [F])**	**ADD: 0, 1.2, 6, 30**	**Systemic: Increased absolute and relative liver weights, and centrilobular hypertrophy in males; increased absolute liver weight in females; transient clinical signs of toxicity in both sexes (hypolocomotion, hypopnea, salivation)** R/D: No observed effects	**Systemic: 6** R/D: 30	**Systemic: 30** R/D: NDr	**UNEP (2003); Mitsubishi Chemical Safety Institute Ltd. (1992b)**	**NPR, PS** Based on UNEP summary of Japanese language publication with data tables in English
2. Inhalation (mg/m^3)							
ND							

[a]Duration categories are defined as follows: Acute = exposure for ≤24 hours; short term = repeated exposure for 24 hours to ≤30 days; long term (subchronic) = repeated exposure for >30 days ≤10% lifespan for humans (>30 days up to ~90 days in typically used laboratory animal species); and chronic = repeated exposure for >10% lifespan for humans (more than ~90 days to 2 years in typically used laboratory animal species) (U.S. EPA, 2002).
[b]Dosimetry: Values are presented as ADDs (mg/kg-day) for oral noncancer effects. In contrast to other repeated exposure studies, values from animal gestational exposure studies are not adjusted for exposure duration in calculation of the ADD.
[c]Notes: NPR = not peer reviewed; PS = principal study.

ADD = adjusted daily dose; F = female(s); LOAEL = lowest-observed-adverse-effect level; M = male(s); ND = no data; NDr = not determined; NOAEL = no-observed-adverse-effect level; PND = postnatal day; R/D = reproductive/developmental; UNEP = United Nations Environment Programme.

Table 3B. Summary of Potentially Relevant Cancer Data for Lactonitrile (CASRN 78-97-7)							
Category	**Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration**	**Dosimetry**	**Critical Effects**	**NOAEL**	**LOAEL**	**Reference (comments)**	**Notes**
Human							
1. Oral (mg/kg-d)							
ND							
2. Inhalation (mg/m^3)							
ND							
Animal							
1. Oral (mg/kg-d)							
ND							
2. Inhalation (mg/m^3)							
ND							

LOAEL = lowest-observed-adverse-effect level; ND = no data; NOAEL = no-observed-adverse-effect level.

HUMAN STUDIES

Human data are limited to a single case report. A fatal case of poisoning, attributed to lactonitrile, was reported in a worker exposed to lactonitrile while cleaning discharge pipes in a factory where acrylonitrile was produced (Nagata et al., 1968). The man experienced severe headache, nausea, palpitation, and abdominal pain while working. He was found unconscious after leaving work and admitted to a hospital under a preliminary diagnosis of cyanide poisoning. Despite medical intervention, the patient died. Autopsy observations included intense posterior lividity, petechial hemorrhages in conjunctiva and in mucosa of the renal pelvis, blood and fluid in pericardium, and marked congestion of all viscera. Gas chromatography was used to verify the presence of acrylonitrile, but showed that lactonitrile was present in much higher quantities in the waste products collected from the factory discharge pipes, the deceased patient's clothing (some items of which, most notably the undershirt and mask, had no acrylonitrile at all), and blood and urine collected postmortem. The authors hypothesized that in the heavy rain that was falling, lactonitrile has dissolved in water and saturated in the thick, tight clothing, from which it was absorbed into the body.

ANIMAL STUDIES

Oral Exposures

Repeated-dose oral toxicity data are limited to an unpublished OECD Test Guideline 422 combined repeated-dose and reproductive/developmental (R/D) screening toxicity study conducted by the Mitsubishi Chemical Safety Institute Ltd. (1992b) and written in Japanese. Data tables are presented in English, and an English summary is available in the OECD SIDS dossier (UNEP, 2003).

UNEP (2003); Mitsubishi Chemical Safety Institute Ltd. (1992b)

Groups of male and female Crj:CD(SD) rats (10/sex/group) were exposed to lactonitrile (purity 92.3%) at doses of 0, 1.2, 6, or 30 mg/kg-day via gavage, 7 days/week for 14 days before mating, and during a mating period of up to 2 weeks. Daily dosing of females continued through gestation until Postnatal Day (PND) 3; males continued to receive daily gavage doses for 2 weeks postmating (~6 weeks total exposure). It is unclear from the English summary what vehicle was used; however, the summary indicates that concurrent vehicle controls were used. Rats were evaluated daily for mortality and clinical signs of toxicity. Body weight and food consumption were measured weekly. Reproductive endpoints that were evaluated included the length of time between initial pairing and detection of coitus (precoital interval); mating, fertility, implantation, delivery, gestational and viability indices; numbers of corpora lutea, implantation sites, and live and dead offspring; gestation length; and F1 birth weight. On PND 4, all F0 and F1 animals were weighed and sacrificed. At terminal sacrifice, blood was collected from F0 males for clinical chemistry (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], γ-glutamyl transferase [GGT], total bilirubin, blood urea nitrogen [BUN], creatinine, glucose, total cholesterol, triglyceride, total protein, albumin, calcium, inorganic phosphate, sodium, potassium, chloride) and hematology (red blood cell [RBC] count, white blood cell [WBC] count, and differential, reticulocyte count, platelet count, hematocrit [Hct], hemoglobin [Hb] concentration, mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC]). Based on data presentation, it is unclear whether clinical chemistry or hematological evaluations were conducted in F0 females. The thymus, liver, kidneys, testes, and epididymides from F0 animals were removed and weighed. All F0 and F1 animals were examined grossly, and the liver, heart, spleen, kidneys, adrenals, testes, and epididymides were fixed for histopathological evaluation in

control and high-dose F0 animals. The livers were also examined microscopically in low- and mid-dose F0 males.

One control male died on Day 23 of the experiment due to "manipulation error" (not further defined) and one high-dose female died on Day 41 of the experiment (2 days postpartum) due to disseminated intravascular coagulation which could be treatment-related, but hematology was not evaluated in female rats. No other mortalities occurred. Transient clinical signs of toxicity (decreased locomotion, hypopnea, salivation) were observed in the majority of high-dose males and females; clinical signs of toxicity were not observed in low- or mid-dose rats. No exposure-related changes were observed in body weight or food consumption. All reproductive endpoints were comparable between the exposure and control groups. Slight, but statistically significant increases were observed in several clinical chemistry values in high-dose males, including increased total protein (8%), albumin (9%), and calcium (4%), but the clinical relevance of these small changes is unclear (see Table B-1). Similarly, the relevance of a statistically significant 17% decrease in serum AST is unknown while serum ALT levels did not differ from control (see Table B-1). There is an upward trend in serum bilirubin (75, 200, and 150%, at the low, middle, and high dose, respectively) in F0 males compared with control animals. Elevated bilirubin reached statistical significance at the mid-dose (200%) but not at the high and low dose (see Table B-1).

No significant hematological differences were observed between exposed and control F0 males. Absolute and relative liver weights were statistically and biologically significantly elevated by 23 and 21%, respectively, in F0 males exposed to 30 mg/kg-day lactonitrile, but not in the lower dose F0 male groups; absolute liver weights were biologically (>10%) but not statistically elevated at 30 mg/kg-day in exposed F0 females compared with control animals (see Table B-2). No exposure-related changes were observed in thymus, kidney, testes, or epididymides weights in F0 rats (see Table B-2). Elevated liver weights in high-dose males were accompanied by a statistically significantly increased incidence of grossly observed liver enlargement, and microscopically observed centrilobular hypertrophy; these findings were not observed in any other dose group (see Table B-3). A centrilobular fatty change was observed in a single male rat (1/10 animals) in the high-dose group, but this increase was not statistically significant. No other exposure-related lesions were observed in F0 animals. No abnormal gross findings were reported in exposed F1 pups.

A systemic no-observed-adverse-effect level (NOAEL) of 6 mg/kg-day and a lowest-observed-adverse-effect level (LOAEL) of 30 mg/kg-day are identified based on a >10% increase in absolute and relative liver weights and centrilobular hypertrophy in males, a >10% increase in absolute liver weight in females, and transient clinical signs of toxicity in both sexes (hypolocomotion, hypopnea, salivation).

Inhalation Exposures

No data regarding the chronic- or subchronic-duration toxicity of lactonitrile to animals following repeated-dose inhalation exposure have been identified.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Acute Toxicity

Oral median lethal dose (LD_{50}) values of 21−87 mg/kg have been reported in rats, with deaths occurring as low as 10 mg/kg (UNEP, 2003; Hartung, 1993; Mitsubishi Chemical Safety Institute Ltd., 1992a; Smyth et al., 1969). Clinical signs observed in male and female rats (SD/Crj:CD) at acute exposures (≥21 mg/kg) included hypopnea, decreased activity, ataxia, pronation, and convulsions; focal hemorrhaging in the lung, and dilation of the atrium were observed in animals that died (Mitsubishi Chemical Safety Institute Ltd., 1992a). An inhalation study reported no deaths among six rats (sex and strain not reported) exposed to 62.5 ppm of lactonitrile for 4 hours and 6/6 deaths after exposure to 125 ppm for 4 hours (Smyth et al., 1969). A single application of lactonitrile to the skin of rabbits (sex and strain not reported) resulted in an LD_{50} value of 20 mg/kg (UNEP, 2003; Smyth et al., 1969). For intraperitoneal (i.p.) administration, an LD_{50} value of 15 mg/kg was reported in male CD-1 mice (Kaplita and Smith, 1986). Clinical signs of toxicity exhibited were dyspnea, ataxia, hypothermia, and convulsions. No other details regarding toxic effects were provided. Furthermore, the acute-duration data came from either secondary sources, are published in a foreign language or lack details on experimental methods, thus making interpretation questionable.

Genotoxicity

A limited number of genotoxicity studies indicate that lactonitrile is not mutagenic to bacteria but has the potential to cause clastogenic effects in yeast and mammalian cells (see Table 4 and below for details).

Lactonitrile was not mutagenic to *Salmonella typhimurium* or *Escherichia coli*, with or without metabolic activation (FDSC HRI, 2016a; UNEP, 2003; Zeiger et al., 1992). Chromosomal aberrations (CAs) were induced in Chinese hamster lung (CHL) cells, with and without metabolic activation (FDSC HRI, 2016b; UNEP, 2003). Additionally, lactonitrile induced chromosomal malsegregation and respiratory deficiency in *Saccharomyces cerevisiae* strain D61.M (Zimmermann and Mohr, 1992).

Table 4. Summary of Lactonitrile (CASRN 78-97-7) Genotoxicity

Endpoint	Test System	Doses/Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	Reference(s)
Genotoxicity studies in prokaryotic organisms						
Mutation	*Salmonella typhimurium* TA97, TA98, TA100, TA1535, and TA1537	0, 0.1, 0.3, 1.0, 3.0, 10, 33, 100, 333, 500 µg/plate	−	−	Cytotoxicity was observed >100 µg/plate.	Zeiger et al. (1992)
Mutation	*S. typhimurium* TA100, TA1535, TA98, and TA1537	0, 4.69, 9.38, 18.75, 37.5, 75, 150 µg/plate	−	−	Cytotoxicity was observed at 150 µg/plate.	FDSC HRI (2016a); UNEP (2003)
Mutation	*Escherichia coli* WP2 uvrA	0, 75, 150, 300, 600, 1,200, 2,400 µg/plate	−	−	Cytotoxicity was observed at 2,400 µg/plate.	FDSC HRI (2016a); UNEP (2003)
Genotoxicity studies in nonmammalian eukaryotic organisms						
Mitotic malsegregation	*Saccharomyces cerevisiae* strain D61.M; continuous assay (28°C for 16 hr) and cold-shock assay (4 hr at 28°C, 16-hr ice bath, 4 hr at 28°C)	0, 1.96, 2.45, 2.94, 3.43, 4.41, 5.86 mg/mL (continuous assay) 0, 2.45, 2.94, 4.41, 5.87, 7.81, 9.47 mg/mL (cold-shock assay)	+	ND	Increased incidence of malsegregation was observed at ≥2.94 mg/mL in continuous incubation and ≥4.41 mg/mL in cold-shock assay. An increased incidence of respiratory-deficient cells was noted at ≥4.41 mg/mL in continuous incubation and ≥5.87 mg/mL in cold-shock assay.	Zimmermann and Mohr (1992)
Genotoxicity studies in mammalian cells in vitro						
CAs	CHL cells	0, 0.10, 0.19, 0.38 mg/mL (−S9; 24- or 48-hr incubation) 0, 0.18, 0.36, 0.71 mg/mL (±S9; 6-hr incubation)	+	+	CAs were induced at ≥0.38 mg/mL after 24−48 hr without metabolic activation and at 0.71 mg/mL after 6 hr with or without metabolic activation. In a separate assay, cytotoxicity was observed at 1.00 mg/mL without metabolic activation and ≥0.38 mg/mL with metabolic activation.	UNEP (2003); FDSC HRI (2016b)

[a]+ = positive; − = negative

CA = chromosomal aberration; CHL = Chinese hamster lung; ND = no data.

DERIVATION OF PROVISIONAL VALUES

Tables 5 and 6 present summaries of noncancer and cancer reference values, respectively.

Table 5. Summary of Noncancer Reference Values for Lactonitrile (CASRN 78-97-7)

Toxicity Type (units)	Species/Sex	Critical Effect	p-Reference Value	POD Method	POD (HED)	UF_C	Principal Study
Screening Subchronic p-RfD (mg/kg-d)	Rat/F	Elevated absolute liver weight	2×10^{-3}	$BMDL_{10}$	0.5	300	Mitsubishi Chemical Safety Institute Ltd. (1992b)
Screening Chronic p-RfD (mg/kg-d)	Rat/F	Elevated absolute liver weight	2×10^{-4}	$BMDL_{10}$	0.5	3,000	Mitsubishi Chemical Safety Institute Ltd. (1992b)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

$BMDL_{10}$ = 10% benchmark dose lower confidence limit; F = female(s); HED = human equivalent dose; NDr = not determined; POD = point of departure; p-RfC = provisional reference concentration; p-RfD = provisional reference dose; UF_C = composite uncertainty factor.

Table 6. Summary of Cancer Reference Values for Lactonitrile (CASRN 78-97-7)

Toxicity Type (units)	Species/Sex	Tumor Type	Cancer Value	Principal Study
p-OSF $(mg/kg\text{-}d)^{-1}$	NDr			
p-IUR $(mg/m^3)^{-1}$	NDr			

NDr = not determined; p-IUR = provisional inhalation unit risk; p-OSF = provisional oral slope factor.

DERIVATION OF ORAL REFERENCE DOSES

The available human and animal data are not considered sufficiently reliable to use in deriving subchronic or chronic provisional reference doses (p-RfDs) for lactonitrile. Repeated-dose oral toxicity data for lactonitrile are limited to an unpublished OECD combined repeated-dose and R/D screening toxicity test conducted by the Mitsubishi Chemical Safety Institute Ltd. (1992b) and written in Japanese. Because the study is not available in English, it cannot be independently reviewed; therefore, it is not appropriate to use as the basis of a provisional toxicity value. However, study data tables are provided in English, and there is an English language summary of the study in the OECD SIDS dossier (UNEP, 2003); therefore, this study is suitable for the derivation of "screening-level" values for subchronic and chronic oral exposure to lactonitrile (see Appendix A). Further, the NOAEL for systemic toxicity (6 mg/kg-day), identified in the Mitsubishi Chemical Safety Institute Ltd. (1992b) summary is close to LD_{50} values (21−87 mg-kg-day) derived from several acute toxicity studies (UNEP, 2003; Hartung, 1993; Mitsubishi Chemical Safety Institute Ltd., 1992a; Smyth et al., 1969). The

close proximity of LD_{50} values to NOAELs found in the principal study supports the derivation of screening values rather than provisional values.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No subchronic- or chronic-duration, repeated-exposure studies were located regarding toxicity of lactonitrile to humans or animals by inhalation; therefore, neither subchronic nor chronic provisional reference concentrations (p-RfCs) are derived.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

No relevant cancer data are available. Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), there is *"Inadequate Information to Assess Carcinogenic Potential"* for lactonitrile by either oral or inhalation exposure (see Table 7).

Table 7. Cancer Weight-of-Evidence Descriptor for Lactonitrile (CASRN 78-97-7)

Possible WOE Descriptor	Designation	Route of Entry (oral, inhalation, or both)	Comments
"Carcinogenic to Humans"	NS	NA	There are no human data to support this.
"Likely to Be Carcinogenic to Humans"	NS	NA	There are no suitable human or animal studies to support this.
"Suggestive Evidence of Carcinogenic Potential"	NS	NA	There are no suitable human or animal studies to support this.
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	**Both**	**Available studies are insufficient to assess carcinogenic potential.**
"Not Likely to Be Carcinogenic to Humans"	NS	NA	There are no suitable human or animal studies to support this.

NA = not applicable; NS = not selected; WOE = weight of evidence.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

The absence of suitable data precludes development of cancer potency values for lactonitrile.

APPENDIX A. SCREENING PROVISIONAL VALUES

For reasons noted in the main Provisional Peer-Reviewed Toxicity Value (PPRTV) document, it is inappropriate to derive provisional toxicity values for lactonitrile. However, information is available for this chemical, which although insufficient to support derivation of a provisional toxicity value under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in an appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the main documents to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there is considerably more uncertainty associated with the derivation of an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center.

Derivation of a Screening Subchronic Provisional Reference Dose

The unpublished Japanese-language Organisation for Economic Co-operation and Development (OECD) combined repeated-dose and reproductive/developmental (R/D) screening toxicity test conducted by the Mitsubishi Chemical Safety Institute Ltd. (1992b) and summarized in UNEP (2003). The UNEP (2003) described the report as "well controlled" and performed according to Good Laboratory Practices (GLP), and is considered the principal study for use in deriving the screening provisional reference doses (p-RfDs). The critical effect was increased absolute liver weight in female rats observed at 30 mg/kg-day.

The subchronic-duration study by Mitsubishi Chemical Safety Institute Ltd. (1992b) conducted daily administration of lactonitrile by gavage to Crj:CD(SD) rats (10/sex/group) for 6 weeks, including prior to mating and during a mating period. The report included a comprehensive assessment of body weight, hematology (males only), serum chemistry (males only), organ weights, and gross pathology of various organs. The authors also performed a microbiological exam of several organs at the control (0 mg/kg-day) and high dose (30 mg/kg-day), and male livers at all doses.

The study, however, is available as a secondary summary source and foreign language publication. A lowest-observed-adverse-effect level (LOAEL) of 30 mg/kg-day with a corresponding no-observed-adverse-effect level (NOAEL) of 6 mg/kg-day is identified based on >10% increase in absolute and relative liver weights in male F0 rats, >10% increase in absolute liver weight in female rats, and clinical signs of toxicity in male and female rats.

Potential points of departure (PODs) (see Table A-1) were modeled using the EPA's Benchmark Dose Software (BMDS, Version 2.6). The incidence data for centrilobular hypertrophy and clinical signs of toxicity were not suitable for benchmark dose (BMD) modeling because effects were only observed in high-dose animals (zero incidence in control and lower-dose animals). Details of the BMD modeling can be found in Appendix C. PODs considered for derivation of the screening subchronic p-RfD are presented in Table A-1. Bilirubin was elevated (75, 200, and 150% respectively) at 1.2, 6, and 30 mg lactonitrile/kg-day in male rats, and the effect is consistent with liver toxicity. But the effect was not considered as a POD because only the mid-dose (6 mg/kg-day) was statistically significant. Further, because

the reference range of total bilirubin varies by up to 1,200% in humans (Chernecky and Berger, 2013) and a comparable amount in CD rats (Giknis and Clifford, 2006), the toxicological significance of a 200% elevation in bilirubin is uncertain.

Table A-1. PODs Considered for Derivation of the Screening Subchronic p-RfD from the 6-Week Gavage Study in Crj:CD(SD) F0 Rats (2 Weeks Premating through 2 Weeks Postmating [Males] or PND 3 [Females])[a]

Species/Sex	Critical Effect	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	POD Method	POD (mg/kg-d)
Rat/M	Increased absolute liver weight	6	30	$BMDL_{10}$	10
Rat/M	Increased relative liver weight	6	30	$BMDL_{10}$	10
Rat/F	Increased absolute liver weight	6	30	$BMDL_{10}$	2
Rat/M	Centrilobular hypertrophy	6	30	NOAEL	6
Rat/M & F	Clinical signs of toxicity	6	30	NOAEL	6

[a]Mitsubishi Chemical Safety Institute Ltd. (1992b).

$BMDL_{10}$ = 10% benchmark dose lower confidence limit; F = female(s); LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; PND = postnatal day; POD = point of departure; p-RfD = provisional reference dose.

The lowest available POD is a 10% benchmark dose lower confidence limit ($BMDL_{10}$) of 2 mg/kg-day for female rats with elevated absolute liver weight that is biologically significant (>10% change). This POD is protective of other effects observed following lactonitrile exposure, including liver-weight changes in male and female rats. These liver-weight increases are consistent with the centrilobular hypertrophy, gross liver enlargement, and centrilobular fatty changes reported in male rats. Based on the consistency in these effects within the study, **the $BMDL_{10}$ for elevated absolute liver weight in female rats (2 mg/kg-day) is selected as the POD for derivation of the screening subchronic p-RfD.**

The $BMDL_{10}$ of 2 mg/kg-day was converted to a human equivalent dose (HED) according to current U.S. EPA (2011b) guidance. In *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), the EPA endorses body-weight scaling to the 3/4 power (i.e., $BW^{3/4}$) as a default to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for the purpose of deriving a p-RfD from effects that are not portal-of-entry effects.

Following U.S. EPA (2011b) guidance, the POD for systemic toxicity is converted to an HED through the application of a dosimetric adjustment factor (DAF)[2] derived as follows:

$$DAF = (BW_a^{1/4} \div BW_h^{1/4})$$

where

DAF = dosimetric adjustment factor
BW_a = animal body weight
BW_h = human body weight

Using a reference BW_a of 0.25 kg for rats and a reference BW_h of 70 kg for humans, the resulting DAF is 0.24 (U.S. EPA, 2011b). Applying this DAF to the $BMDL_{10}$ of 2 mg/kg-day yields a POD (HED) as follows:

$$\begin{aligned} \text{POD (HED)} &= BMDL_{10}\text{ (mg/kg-day)} \times DAF \\ &= 2\text{ mg/kg-day} \times 0.24 \\ &= 0.5\text{ mg/kg-day} \end{aligned}$$

The screening subchronic p-RfD for lactonitrile was derived using the POD (HED) and a composite uncertainty factor (UF_C) of 300 (reflecting an interspecies uncertainty factor [UF_A] of 3, an intraspecies uncertainty factor [UF_H] of 10, and a database uncertainty factor [UF_D] of 10):

$$\begin{aligned} \textbf{Screening Subchronic p-RfD} &= \text{POD (HED)} \div UF_C \\ &= 0.5\text{ mg/kg-day} \div 300 \\ &= \mathbf{2 \times 10^{-3}\text{ mg/kg-day}} \end{aligned}$$

Table A-2 summarizes the uncertainty factors for the screening subchronic p-RfD for lactonitrile.

[2]As described in detail in *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), rate-related processes scale across species in a manner related to both the direct ($BW^{1/1}$) and allometric scaling ($BW^{3/4}$) aspects such that $BW^{3/4} \div BW^{1/1} = BW^{-1/4}$, converted to a $DAF = BW_a^{1/4} \div BW_h^{1/4}$.

Table A-2. Uncertainty Factors for the Screening Subchronic p-RfD for Lactonitrile (CASRN 78-97-7)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty in characterizing the toxicokinetic or toxicodynamic differences between rats and humans following oral lactonitrile treatment. The toxicokinetic uncertainty has been accounted for by calculating an HED through application of a DAF as outlined in the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_D	10	A UF_D of 10 is applied to account for the limited toxicity database for lactonitrile, specifically the lack of a repeated-dose systemic toxicity study longer than 6 wk; lack of a repeated-dose toxicity study in a second species; lack of a comprehensive evaluation in females; reproductive and developmental toxicity in a second species; and the lack of evaluation of CNS toxicity based on behavioral and clinical signs of toxicity.
UF_H	10	A UF_H of 10 is applied for intraspecies variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of lactonitrile in humans.
UF_L	1	A UF_L of 1 is applied because the POD is a $BMDL_{10}$.
UF_S	1	A UF_S of 1 is applied because a subchronic-duration study was selected as the principal study.
UF_C	300	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

$BMDL_{10}$ = 10% benchmark dose lower confidence limit; CNS = central nervous system; DAF = dosimetric adjustment factor; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies variability uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

Derivation of a Screening Chronic Provisional Reference Dose

The unpublished Japanese-language OECD combined repeated-dose and R/D screening toxicity test conducted by the Mitsubishi Chemical Safety Institute Ltd. (1992b) is considered the principal study for use in deriving the screening chronic p-RfD. No studies evaluating chronic-duration exposure to lactonitrile have been identified. Therefore, the $BMDL_{10}$ of 2 mg/kg-day for elevated absolute liver weight in female rats is used as the POD for the screening chronic p-RfD. An additional subchronic-to-chronic uncertainty factor (UF_S) of 10 is applied to account for extrapolation from a subchronic-duration study.

The screening chronic p-RfD for lactonitrile is derived using the POD (HED) and a UF_C of 3,000 (reflecting a UF_A of 3, a UF_H of 10, a UF_D of 10, and a UF_S of 10):

$$\textbf{Screening Chronic p-RfD} = \text{POD (HED)} \div UF_C$$
$$= 0.5 \text{ mg/kg-day} \div 3{,}000$$
$$= \mathbf{2 \times 10^{-4} \text{ mg/kg-day}}$$

Table A-3 summarizes the uncertainty factors for the screening chronic p-RfD for lactonitrile.

Table A-3. Uncertainty Factors for the Screening Chronic p-RfD for Lactonitrile (CASRN 78-97-7)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty in characterizing the toxicokinetic or toxicodynamic differences between rats and humans following oral lactonitrile treatment. The toxicokinetic uncertainty has been accounted for by calculating an HED through application of a DAF as outlined in the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_D	10	A UF_D of 10 is applied to account for the limited toxicity database for lactonitrile, specifically the lack of a repeated-dose systemic toxicity study longer than 6 wk; lack of a repeated-dose toxicity study in a second species; lack of a comprehensive evaluation in females; reproductive and developmental toxicity in a second species; and the lack of evaluation of CNS toxicity based on behavioral and clinical signs of toxicity.
UF_H	10	A UF_H of 10 is applied for intraspecies variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of lactonitrile in humans.
UF_L	1	A UF_L of 1 is applied because the POD is a $BMDL_{10}$.
UF_S	10	A UF_S of 10 is applied because a subchronic-duration study was selected as the principal study.
UF_C	3,000	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

$BMDL_{10}$ = 10% benchmark dose lower confidence limit; CNS = central nervous system; DAF = dosimetric adjustment factor; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies variability uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

APPENDIX B. DATA TABLES

Table B-1. Selected Clinical Chemistry Endpoints in Male Crj:CD(SD) F0 Rats Exposed to Lactonitrile by Gavage up to 6 Weeks (2 Weeks Premating through 2 Weeks Postmating)[a]

Endpoint[b]	Exposure Group, mg/kg-d			
	0	1.2	6	30
GOT (AST) (IU/L)	81 ± 12.6	77 ± 10.8 (−4.9%)	77 ± 10.5 (−4.9%)	67 ± 5.9* (−17.3%)
GPT (ALT) (IU/L)	27 ± 6.4	27 ± 7.3 (0%)	25 ± 4.0 (−7.4%)	24 ± 6.4 (−11.1%)
ALP (IU/L)	251 ± 65.8	236 ± 37.3 (−6%)	219 ± 38.7 (−12.7%)	225 ± 47.7 (−10.4%)
Total bilirubin (mg/dL)	0.04 ± 0.05	0.07 ± 0.05 (+75%)	0.12 ± 0.04* (+200%)	0.10 ± 0.07 (+150%)
Total protein (g/dL)	6.43 ± 0.276	6.42 ± 0.167 (−0.2%)	6.53 ± 0.233 (+1.6%)	6.93 ± 0.276** (+7.8%)
Albumin (g/dL)	3.85 ± 0.122	3.79 ± 0.094 (−1.6%)	3.88 ± 0.134 (+0.8%)	4.2 ± 0.169** (+9.1%)
Calcium (mg/dL)	9.1 ± 0.25	9.1 ± 0.19 (0%)	9.2 ± 0.3 (+1.1%)	9.5 ± 0.2* (+4.4%)

[a]Mitsubishi Chemical Safety Institute Ltd. (1992b).
[b]Values are reported as mean ± SD for 9 control rats and 10 rats/exposure group.
*Statistically significantly different from control value at $p < 0.05$, as reported by the study authors.
**Statistically significantly different from control value at $p < 0.01$, as reported by the study authors.

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate amino transferase; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; SD = standard deviation.

Table B-2. Body and Organ Weights for Male and Female Crj:CD(SD) F0 Rats Exposed to Lactonitrile by Gavage for 6 Weeks (2 Weeks Premating through 2 Weeks Postmating [Males] or PND 3 [Females])[a]

Endpoint[b]	Exposure Group, mg/kg-d			
	0	1.2	6	30
Male				
Sample size	**9**	**10**	**10**	**10**
Final body weight (g)	474 ± 31.0	472 ± 33.7 (−0.4%)	479 ± 36.7 (+1.1%)	480 ± 28.7 (+1.3%)
Liver				
Absolute (g)	12.94 ± 1.675	12.92 ± 1.712 (−0.2%)	13.23 ± 1.798 (+2.2%)	15.87 ± 1.661** (+22.6%)
Relative (%)	2.72 ± 0.201	2.73 ± 0.203 (+0.4%)	2.75 ± 0.176 (+1.1%)	3.30 ± 0.261** (+21.3%)
Kidney				
Absolute (g)	3.04 ± 0.271	2.97 ± 0.204 (−2.3%)	3.10 ± 0.278 (+2%)	3.12 ± 0.334 (+2.6%)
Relative (%)	0.64 ± 0.041	0.63 ± 0.016 (−1.6%)	0.65 ± 0.020 (+1.6%)	0.65 ± 0.062 (+1.6%)
Thymus				
Absolute (mg)	340 ± 62.7	382 ± 67.4 (+12.4%)	360 ± 95.2 (+5.9%)	381 ± 87.1 (+12.1%)
Relative (%)	0.072 ± 0.016	0.081 ± 0.016 (+12.5%)	0.075 ± 0.017 (+4.2%)	0.079 ± 0.016 (+9.7%)
Testes				
Absolute (g)	3.17 ± 0.168	3.17 ± 0.272 (0%)	3.33 ± 0.187 (+5%)	3.22 ± 0.389 (+1.6%)
Relative (%)	0.67 ± 0.070	0.67 ± 0.062 (0%)	0.70 ± 0.062 (+4.5%)	0.67 ± 0.086 (0%)
Epididymis				
Absolute (g)	1.26 ± 0.068	1.25 ± 0.089 (−0.8%)	1.27 ± 0.109 (+0.8%)	1.30 ± 0.144 (+3.2%)
Relative (%)	0.27 ± 0.025	0.26 ± 0.027 (−3.7%)	0.27 ± 0.026 (0%)	0.27 ± 0.034 (0%)

Table B-2. Body and Organ Weights for Male and Female Crj:CD(SD) F0 Rats Exposed to Lactonitrile by Gavage for 6 Weeks (2 Weeks Premating through 2 Weeks Postmating [Males] or PND 3 [Females])[a]

Endpoint[b]	Exposure Group, mg/kg-d			
	0	1.2	6	30
Female				
Sample size	**9**	**10**	**9**	**9**
Final body weight (g)	303 ± 15.6	307 ± 13.4 (+1.3%)	312 ± 20.5 (+3%)	315 ± 16.6 (+4%)
Liver				
Absolute (g)	12.61 ± 0.946	12.59 ± 1.135 (−0.2%)	13.62 ± 0.941 (+8%)	13.9 ± 1.33 (+10.2%)
Relative (%)	4.17 ± 0.347	4.10 ± 0.252 (−1.7%)	4.37 ± 0.206 (+4.8%)	4.41 ± 0.353 (+5.8%)
Kidney				
Absolute (g)	1.96 ± 0.174	1.98 ± 0.188 (+1%)	2.00 ± 0.105 (+2%)	1.98 ± 0.174 (+1%)
Relative (%)	0.65 ± 0.067	0.65 ± 0.056 (0%)	0.64 ± 0.031 (−1.5%)	0.63 ± 0.051 (−3.1%)
Thymus				
Absolute (mg)	239 ± 53.6	253 ± 54.6 (+5.9%)	224 ± 51.7 (−6.3%)	231 ± 73.6 (−3.3%)
Relative (%)	0.079 ± 0.019	0.082 ± 0.015 (+3.8%)	0.071 ± 0.015 (−10.1%)	0.073 ± 0.020 (−7.6%)

[a]Mitsubishi Chemical Safety Institute Ltd. (1992b).
[b]Values are reported as mean ± SD for 9−10 rats/group.
*Statistically significantly different from control value at $p < 0.05$, as reported by the study authors.
**Statistically significantly different from control value at $p < 0.01$, as reported by the study authors.

PND = postnatal day; SD = standard deviation.

Table B-3. Gross and Microscopic Liver Findings for Male and Female Crj:CD(SD) F0 Rats Exposed to Lactonitrile by Gavage for 6 Weeks (2 Weeks Premating Through 2 Weeks Postmating [Males] or PND 3 [Females])[a]

Endpoint	Exposure Group, mg/kg-d							
	Male				Female			
	0	1.2	6	30	0	1.2	6	30
Gross necropsy findings								
Liver enlargement	0/9[b]	0/10	0/10	9/10*	0/10	0/10	0/10	0/9[c]
Microscopic findings								
Centrilobular hypertrophy	0/9	0/10	0/10	7/10*	0/10	NDr	NDr	0/9
Centrilobular fatty change	0/9	0/10	0/10	1/10	0/10	NDr	NDr	0/9
Glycogen accumulation in hepatocytes	0/9	0/10	0/10	0/10	1/10	NDr	NDr	1/9
Microgranuloma	4/9	4/10	6/10	2/10	0/10	NDr	NDr	0/9

[a]Mitsubishi Chemical Safety Institute Ltd. (1992b).
[b]One male died due to manipulation error on the 23rd day.
[c]One female died due to disseminated intravascular coagulation on PND 2.
*Statistically significantly different from control value at $p < 0.05$, Fisher's exact test calculated for this review.

NDr = not determined; PND = postnatal day.

APPENDIX C. BENCHMARK DOSE MODELING RESULTS

MODELING PROCEDURE FOR CONTINUOUS DATA

Benchmark dose (BMD) modeling of continuous data is conducted with EPA's Benchmark Dose Software (BMDS, Version 2.6). All continuous models available within the software are fit using a default benchmark response (BMR) of 1 standard deviation (SD) relative risk unless a biologically determined BMR is available (e.g., BMR 10% for body weight based on a biologically significant weight loss of 10%), as outlined in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). An adequate fit is judged based on the χ^2 goodness-of-fit *p*-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination is made as to whether the variance across dose groups is homogeneous. If a homogeneous variance model is deemed appropriate based on the statistical test provided by BMDS (i.e., Test 2), the final BMD results are estimated from a homogeneous variance model. If the test for homogeneity of variance is rejected ($p < 0.1$), the model is run again while modeling the variance as a power function of the mean to account for this nonhomogeneous variance. If this nonhomogeneous variance model does not adequately fit the data (i.e., Test 3; *p*-value < 0.1), the data set is considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest benchmark dose lower confidence limit/benchmark concentration lower confidence limit (BMDL/BMCL) is selected if the BMDL/BMCL estimates from different models vary >threefold; otherwise, the BMDL/BMCL from the model with the lowest Akaike's information criterion (AIC) is selected as a potential point of departure (POD) from which to derive the provisional reference dose/provisional reference concentration (p-RfD/p-RfC).

BMD MODELING TO IDENTIFY POTENTIAL PODs FOR THE DERIVATION OF A SCREENING p-RfD

The following data sets were selected for BMD modeling:

- Absolute liver-weight data from male rats administered lactonitrile for 6 weeks (2 weeks premating through 2 weeks postmating) (Mitsubishi Chemical Safety Institute Ltd., 1992b)
- Relative liver-weight data from male rats administered lactonitrile for 6 weeks (2 weeks premating through 2 weeks postmating) (Mitsubishi Chemical Safety Institute Ltd., 1992b)
- Absolute liver-weight data from female rats administered lactonitrile for 6 weeks (2 weeks premating through gestation and Postnatal Day [PND] 3) (Mitsubishi Chemical Safety Institute Ltd., 1992b).

Absolute Liver Weight Data from Male Rats Administered Lactonitrile for 6 Weeks (2 Weeks Premating through 2 Weeks Postmating)

The procedure outlined above was applied to the data for increased absolute liver weight in F0 male Crj:CD(SD) rats administered lactonitrile via gavage 7 days/week for 2 weeks prior to mating through 2 weeks postmating (6 weeks total exposure) (see Table C-1). Table C-2 summarizes the BMD modeling results. A BMR of 10% was selected, as this is generally considered a biologically significant change in liver weight for laboratory rodents. With the constant variance model applied, all models, except for the Exponential model 5 and the

Hill model, provided adequate fit to the data. BMDLs for models providing adequate fit were sufficiently close (differed by <threefold), so the model with the lowest AIC was selected (Exponential model 2). Thus, the 10% benchmark dose lower confidence limit ($BMDL_{10}$) of 10 mg/kg-day from this model is selected for this endpoint (see Figure C-1 and the BMD text output for details).

Table C-1. Absolute Liver Weights in Male and Female Rats Administered Lactonitrile via Gavage for 6 Weeks (2 Weeks Premating through 2 Weeks Postmating)[a]

	Dose, mg/kg-d			
	0	**1.2**	**6**	**30**
Male				
Sample size	9	10	10	10
Mean (g) (% change)	12.94 (0%)	12.92 (−0.2%)	13.23 (+2.2%)	15.87 (+22.6%)
SD (g)	1.675	1.712	1.798	1.661
Female				
Sample size	9	10	9	9
Mean (g) (% change)	12.61 (0%)	12.59 (−0.2%)	13.62 (+8.0%)	13.90 (+10.2%)
SD (g)	0.946	1.135	0.941	1.330

[a]Mitsubishi Chemical Safety Institute Ltd. (1992b).

SD = standard deviation.

Table C-2. BMD Modeling Results for Increased Absolute Liver Weight in Male Rats Exposed to Lactonitrile by Gavage for 6 Weeks (2 Weeks Premating through 2 Weeks Postmating)

Model	Variance *p*-Value[a]	Means *p*-Value[a]	Scaled Residual: Dose Nearest BMD[b]	AIC	BMD_{10} mg/kg-d	$BMDL_{10}$ mg/kg-d
Constant variance						
Exponential model 2[c, d]	**0.99**	**0.94**	**−0.2682**	**82.91**	**13.45**	**10.06**
Exponential model 3[c]	0.99	0.94	0.01288	84.79	16.91	10.13
Exponential model 4[c]	0.99	0.66	−0.3336	84.97	12.61	4.52
Exponential model 5[c]	0.99	NA	1.87×10^{-6}	86.78	7.39	4.70
Hill[c]	0.99	NA	3.33×10^{-7}	86.78	7.65	4.58
Linear[e]	0.99	0.91	−0.334	82.97	12.61	9.06
Polynomial (2-degree)[e]	0.99	0.92	0.0181	84.79	17.37	9.16
Polynomial (3-degree)[e]	0.99	0.92	0.0181	84.79	17.37	9.16
Power[c]	0.99	0.94	0.0113	84.79	16.63	9.16

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residual at dose nearest to the BMD.
[c]Power restricted to ≥1.
[d]Selected model.
[e]Coefficients restricted to be positive.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); BMR = benchmark response; NA = not applicable (computation failed).

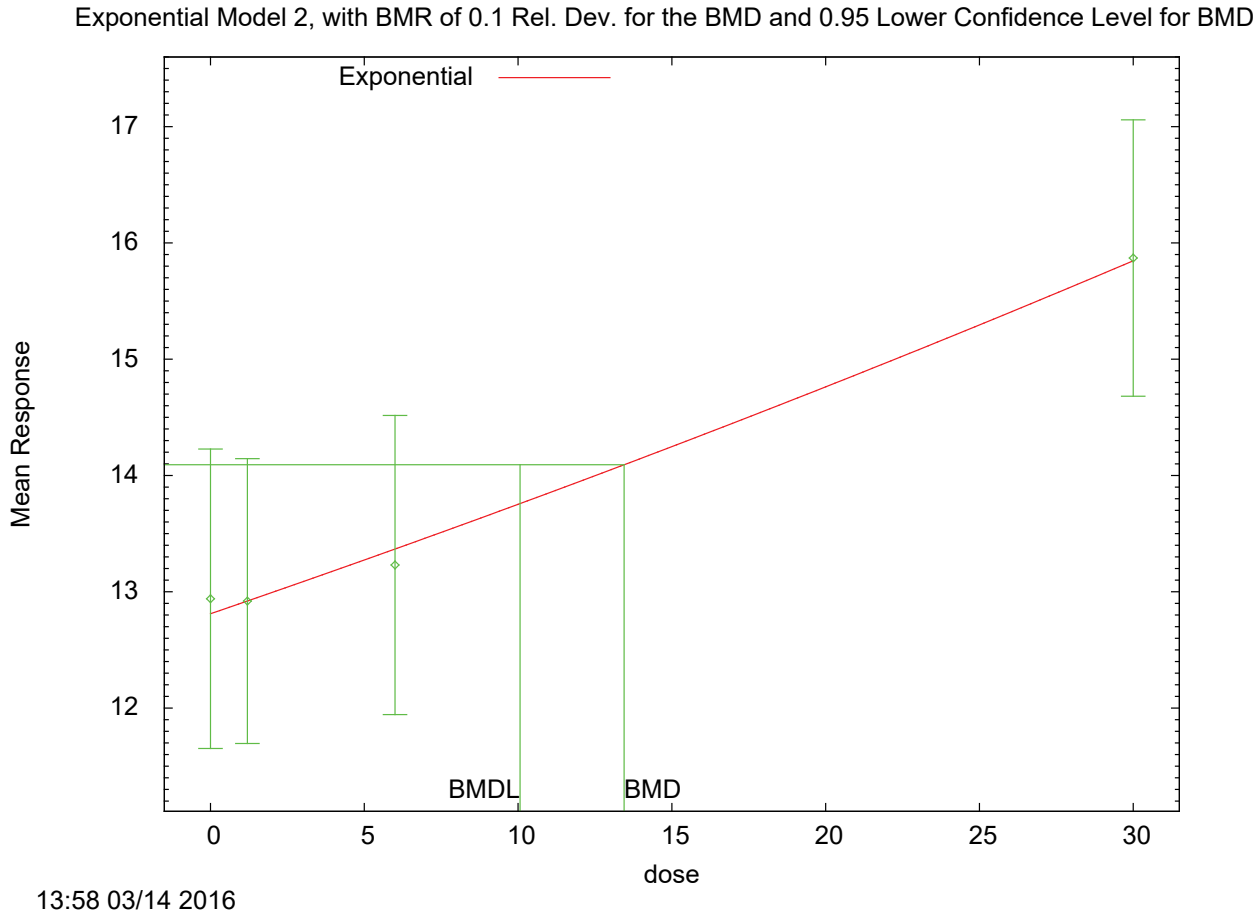

Figure C-1. Exponential (Model 2) for Increased Absolute Liver Weight in Male Rats Exposed to Lactonitrile by Gavage for 6 Weeks (2 Weeks Premating through 2 Weeks Postmating) (Mitsubishi Chemical Safety Institute Ltd., 1992b)

Text Output for Figure C-1:

```
=================================================================
        Exponential Model. (Version: 1.9;  Date: 01/29/2013)
        Input Data File:
C:/BMDS250_2014/Data/PTV-Lactonitrile/exp_AbsLvrWtmale_Exp-ConstantVariance-BMR10_RelD
ev-Up.(d)
        Gnuplot Plotting File:
                                                Mon Mar 14 14:58:33 2016
 =================================================================
 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 = Mean
Independent variable = Dose
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 = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
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
              --------          --------
                lnalpha          0.968728
                rho(S)                 0
                  a              12.8153
                  b           0.00705588
                  c                    0
                  d                    1

  (S) = Specified

                Parameter Estimates

               Variable          Model 2
               --------          -------
                lnalpha          0.972076
                    rho                 0
                      a           12.8113
                      b        0.00708778
                      c                 0
                      d                 1

        Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
      0      9        12.94        1.675
    1.2     10        12.92        1.712
      6     10        13.23        1.798
     30     10        15.87        1.661

             Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
      0         12.81        1.626           0.2375
    1.2         12.92        1.626        -0.001442
      6         13.37        1.626          -0.2682
     30         15.85        1.626          0.04529


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	-38.39019	5	86.78038
A2	-38.34946	8	92.69892
A3	-38.39019	5	86.78038
R	-47.41099	2	98.82198
2	-38.45548	3	82.91096

Additive constant for all log-likelihoods = -35.84. 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)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	18.12	6	0.005932
Test 2	0.08146	3	0.994
Test 3	0.08146	3	0.994
Test 4	0.1306	2	0.9368

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

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.

Benchmark Dose Computations:

Specified Effect = 0.100000

Risk Type = Relative deviation

Confidence Level = 0.950000

```
 BMD =      13.4471
BMDL =      10.0646
```

Relative Liver Weight Data from Male Rats Administered Lactonitrile for 6 Weeks (2 Weeks Premating through 2 Weeks Postmating)

The procedure outlined above was applied to the data for increased relative liver weight in F0 male Crj:CD(SD) rats administered lactonitrile via gavage, 7 days/week for 2 weeks prior to mating through 2 weeks postmating (6 weeks total exposure) (see Table C-3). Table C-4 summarizes the BMD modeling results. With the constant variance model applied, all models, except for the Exponential model 5 and the Hill model, provided adequate fit to the data. BMDLs for models providing adequate fit were sufficiently close (differed by <threefold), so the model with the lowest AIC was selected (Exponential model 2). Thus, the $BMDL_{10}$ of 10 mg/kg-day from this model is selected for this endpoint (see Figure C-2 and the BMD text output for details).

Table C-3. Relative Liver Weights in Male Rats Administered Lactonitrile via Gavage for 6 Weeks (2 Weeks Premating through 2 Weeks Postmating)[a]

	Dose, mg/kg-d			
	0	**1.2**	**6**	**30**
Sample size	9	10	10	10
Mean (%)	2.72	2.73	2.75	3.30
SD (%)	0.201	0.203	0.176	0.261

[a]Mitsubishi Chemical Safety Institute Ltd. (1992b).

SD = standard deviation.

Table C-4. BMD Modeling Results for Increased Relative Liver Weight in Male Rats Exposed to Lactonitrile by Gavage for 6 Weeks (2 Weeks Premating through 2 Weeks Postmating)

Model	Variance *p*-Value[a]	Means *p*-Value[a]	Scaled Residual: Dose Nearest BMD[b]	AIC	BMD_{10} mg/kg-d	$BMDL_{10}$ mg/kg-d
Constant variance						
Exponential model 2[c, d]	**0.63**	**0.63**	**−0.7994**	**−78.97**	**14.19**	**11.68**
Exponential model 3[e]	0.63	0.92	0.000139	−77.88	20.63	12.09
Exponential model 4[e]	0.63	0.28	−0.897	−76.74	13.41	10.77
Exponential model 5[e]	0.63	NA	0.0001451	−75.88	20.33	6.23
Hill[e]	0.63	NA	0.000147	−75.88	20.28	6.28
Linear[e]	0.63	0.56	−0.897	−78.74	13.41	10.77
Polynomial (2-degree)[e]	0.63	0.93	0.000545	−77.88	20.40	11.33
Polynomial (3-degree)[e]	0.63	0.96	6.82×10^{-5}	−77.89	22.10	11.33
Power[c]	0.63	0.92	0.00014	−77.88	20.34	11.32

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residual at dose nearest to the BMD.
[c]Power restricted to ≥1.
[d]Selected model.
[e]Coefficients restricted to be positive.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); BMR = benchmark response; NA = not applicable (computation failed).

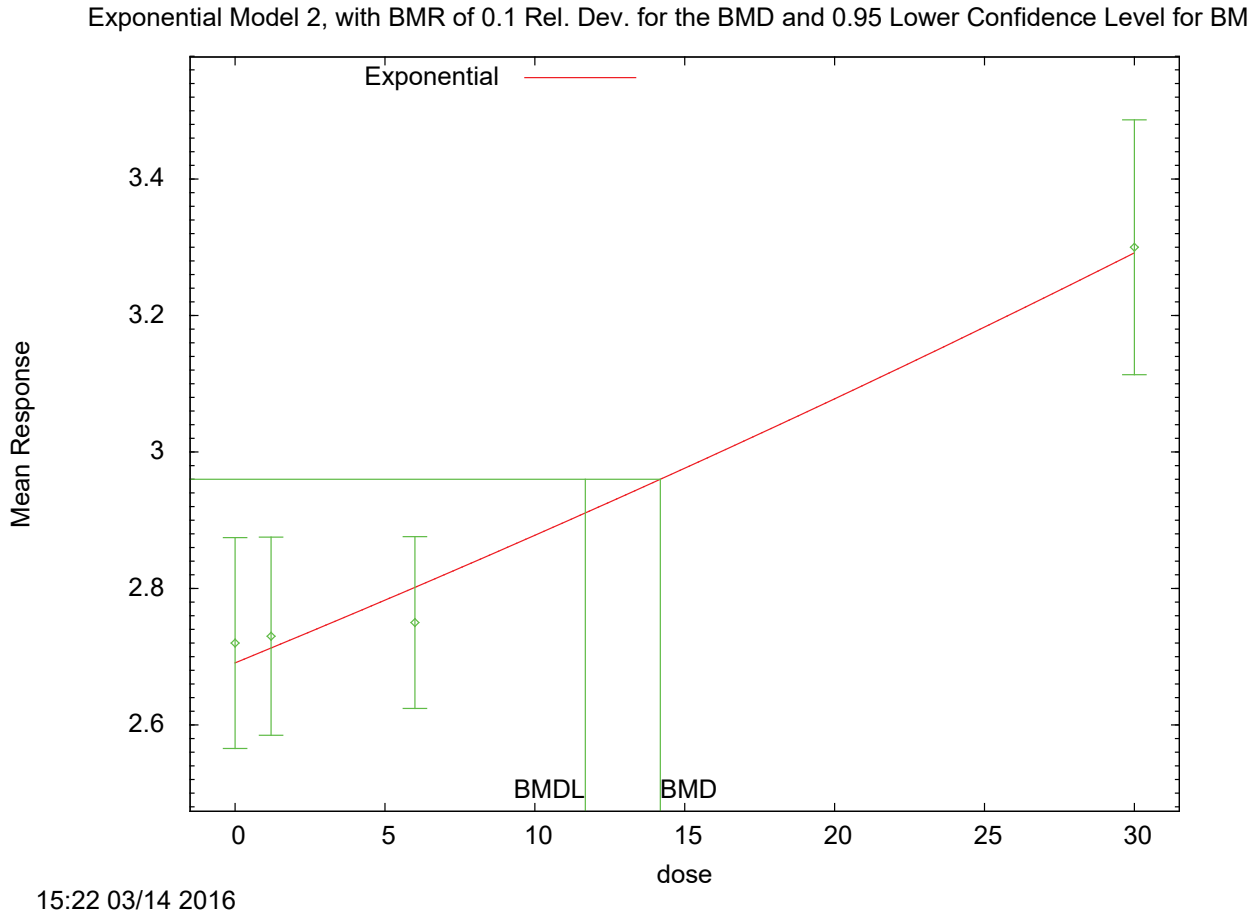

Figure C-2. Exponential (Model 2) for Increased Relative Liver Weight in Male Rats Exposed to Lactonitrile by Gavage for 6 Weeks (2 Weeks Premating through 2 Weeks Postmating) (Mitsubishi Chemical Safety Institute Ltd., 1992b)

Text Output for Figure C-2:

```
================================================================
        Exponential Model. (Version: 1.9;  Date: 01/29/2013)
        Input Data File:
C:/BMDS250_2014/Data/PTV-Lactonitrile/exp_RelLvrWtmale_Exp-ConstantVariance-BMR10_RelD
ev-Up.(d)
        Gnuplot Plotting File:
                                                Mon Mar 14 16:22:11 2016
 ================================================================
 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 = Mean
Independent variable = Dose
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 = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
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
               --------          --------
                 lnalpha            -3.20237
                     rho(S)                0
                       a              2.69213
                       b           0.00666932
                       c                    0
                       d                    1

  (S) = Specified

                 Parameter Estimates

                Variable          Model 2
                --------          -------
                 lnalpha          -3.17875
                     rho                 0
                       a           2.69089
                       b        0.00671863
                       c                 0
                       d                 1

        Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
      0      9         2.72        0.201
    1.2     10         2.73        0.203
      6     10         2.75        0.176
     30     10          3.3        0.261

             Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
      0         2.691       0.2041           0.4279
    1.2         2.713       0.2041           0.2685
      6         2.802       0.2041          -0.7994
     30         3.292       0.2041           0.1272
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	42.94622	5	-75.89245
A2	43.80104	8	-71.60208
A3	42.94622	5	-75.89245
R	25.0323	2	-46.0646
2	42.4856	3	-78.97121

Additive constant for all log-likelihoods = -35.84. 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)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	37.54	6	< 0.0001
Test 2	1.71	3	0.6348
Test 3	1.71	3	0.6348
Test 4	0.9212	2	0.6309

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

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.

Benchmark Dose Computations:

Specified Effect = 0.100000

Risk Type = Relative deviation

Confidence Level = 0.950000
BMD = 14.186
BMDL = 11.6832

Absolute Liver Weight Data from Female Rats Administered Lactonitrile for 6 Weeks (2 Weeks Premating through PND 3)

The procedure outlined above was applied to the data for increased (10.2%) absolute liver weight in F0 female Crj:CD(SD) rats administered lactonitrile via gavage 7 days/week for 2 weeks prior to mating through PND 3 (6 weeks total exposure) (see Table C-1). Table C-5 summarizes the BMD modeling results. With the constant variance model applied, all models, except for the Exponential model 5 and the Hill model, provided adequate fit to the data. The BMDLs for models providing adequate fit differed by >threefold, so the model with the lowest $BMDL_{10}$ was selected (Exponential model 4). Thus, the $BMDL_{10}$ of 2 mg/kg-day from this model is selected for this endpoint (see Figure C-3 and the BMD text output for details).

Table C-5. BMD Modeling Results for Increased Absolute Liver Weight in Female Rats Exposed to Lactonitrile by Gavage for 6 Weeks (2 Weeks Premating through PND 3)

Model	Variance *p*-Value[a]	Means *p*-Value[a]	Scaled Residual: Dose Nearest BMD[c]	AIC	BMD_{10} mg/kg-d	$BMDL_{10}$ mg/kg-d
Constant variance						
Exponential model 2[c]	0.6741	0.1585	−0.27	49.55785	32.1179	19.991
Exponential model 3[c]	0.6741	0.1585	0.01	49.55785	32.1179	19.991
Exponential model 4[c, d]	**0.6741**	**0.4235**	**−0.33**	**48.51447**	**9.40352**	**2.17676**
Exponential model 5[c]	0.6741	NA	1.87×10^{-6}	49.8756	6.57339	1.25823
Hill[c]	0.6741	NA	3.33×10^{-7}	49.8756	7.30683	NA
Linear[e]	0.6741	0.1638	−0.33	49.491986	31.8573	19.3007
Polynomial (2-degree)[e]	0.6741	0.1638	0.02	49.491986	31.8573	19.3007
Polynomial (3-degree)[e]	0.6741	0.1638	0.02	49.491986	31.8573	19.3007
Power[c]	0.6741	0.1638	0.01	49.491986	31.8573	19.3007

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residual at dose nearest to the BMD.
[c]Power restricted to ≥1.
[d]Selected model.
[e]Coefficients restricted to be positive.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); BMR = benchmark response; NA = not applicable (computation failed); PND = postnatal day.

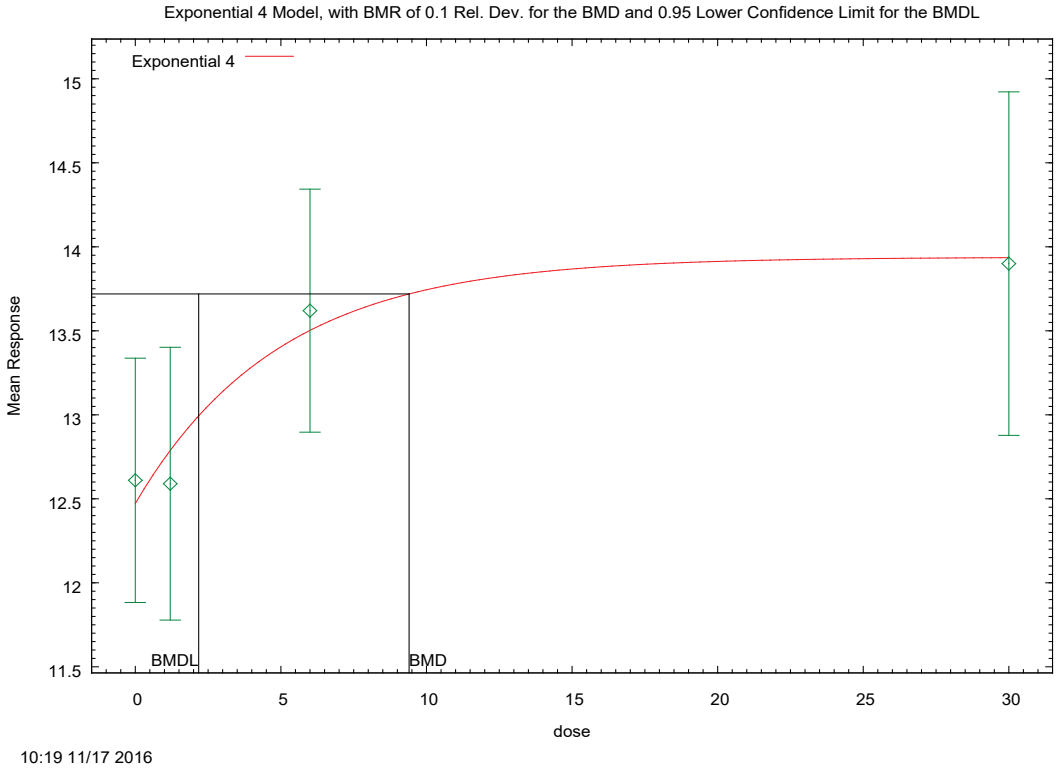

Figure C-3. Exponential (Model 4) for Increased Absolute Liver Weight in Female Rats Exposed to Lactonitrile by Gavage for 6 Weeks (2 Weeks Premating through PND 3) (Mitsubishi Chemical Safety Institute Ltd., 1992b)

Text Output for Figure C-3:

```
 ================================================================
        Exponential Model. (Version: 1.10;  Date: 01/12/2015)
        Input Data File: C:/Users/CCUBBIS/Desktop/LAC Female
Rat/exp_LACFemaleABSliver_Exp-ConstantVariance-BMR10-Up.(d)
        Gnuplot Plotting File:
                                              Wed Nov 23 10:44:58 2016
 ================================================================

 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 = Mean
Independent variable = Dose
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 = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
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 4	
lnalpha	0.0776715	
rho	0	Specified
a	11.9605	
b	0.0493555	
c	1.22027	
d	1	Specified

Parameter Estimates

Variable	Model 4	Std. Err.
lnalpha	0.0949857	0.255662
a	12.4722	0.296315
b	0.20211	0.144696
c	1.11758	0.0376269

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	9	12.61	0.946
1.2	10	12.59	1.135
6	9	13.62	0.941
30	9	13.9	1.33

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	12.47	1.049	0.3942
1.2	12.79	1.049	-0.5972
6	13.5	1.049	0.3361
30	13.94	1.049	-0.1008

```
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	-19.93692	5	49.87385
A2	-19.16914	8	54.33828
A3	-19.93692	5	49.87385
R	-25.0757	2	54.15141
4	-20.25724	4	48.51447

Additive constant for all log-likelihoods = -34. 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 6a: Does Model 4 fit the data? (A3 vs 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	11.81	6	0.06627
Test 2	1.536	3	0.6741
Test 3	1.536	3	0.6741
Test 6a	0.6406	1	0.4235

The p-value for Test 1 is greater than .05. There may not be a difference 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 6a is greater than .1. Model 4 seems to adequately describe the data.

Benchmark Dose Computations:

Specified Effect = 0.100000

```
       Risk Type = Relative deviation

Confidence Level = 0.950000

             BMD =      9.40352

            BMDL =      2.17676
```

APPENDIX D. REFERENCES

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