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Provisional Peer-Reviewed Toxicity Values for

Lewisite
(CASRN 541-25-3)

Superfund Health Risk Technical Support Center
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COMMONLY USED ABBREVIATIONS AND ACRONYMS

α2u-g	alpha 2u-globulin
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
ALD	approximate lethal dosage
ALT	alanine aminotransferase
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
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	glutamic oxaloacetic transaminase, also known as AST
SGPT	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_H	intraspecies uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UF_D	database uncertainty factor
U.S.	United States of America
WBC	white blood cell

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR LEWISITE (CASRN 541-25-3)

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.

This document has been reviewed in accordance with U.S. Environmental Protection Agency 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 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

Lewisite, CASRN 541-25-3, was manufactured as a poison gas and skin blistering agent (vesicant). It has an odor like geraniums (HSDB, 2010). This chemical was proclaimed a high risk chemical with little or no use for peaceful purposes and is listed in Schedule 1 of the Annex on Chemicals for the Chemical Weapons Convention. Lewisite may exist as the *trans* or *cis* isomer, but in aqueous solution, the *cis* isomer is photoconverted to the *trans* isomer (NRC, 2013). Lewisite has moderate vapor pressure, and if released into the air, it is expected to exist solely in the vapor phase. Once in the air, lewisite is expected to degrade slowly. Although lewisite has low water solubility, it rapidly hydrolyzes. As such, volatilization of lewisite from an aquatic source is expected to decrease over time when released in an aqueous environment (HSDB, 2010). The empirical formula for lewisite is $C_2H_2AsCl_3$ (see Figure 1). A table of physicochemical properties for lewisite is provided below (see Table 1).

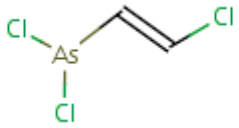

Figure 1. Lewisite Structure

Table 1. Physicochemical Properties of Lewisite (CASRN 541-25-3)[a]

Property (unit)	Value
Boiling point (°C)	Decomposes at 190
Melting point (°C)	0.1
Density (g/cm^3 at 20ºC)	1.888
Vapor pressure (mmHg at 25°C)	0.58
pH (unitless)	ND
Solubility in water (mg/L; temperature not reported)	500, rapid hydrolysis
Relative vapor density (air = 1)	7.1
Molecular weight (g/mol)	207.32

[a]HSDB (2010).

ND = no data.

Lewisite is an unstable compound; thus, environmental exposures may be to a mixture of lewisite with one or more of its degradation products and/or frequently occurring impurities. Reactions and reaction products of lewisite under various conditions have been reviewed by Munro et al. (1999). As noted by the authors, lewisite hydrolyzes readily in water, forming the water-soluble product 2-chlorovinyl arsonous acid (CVAA) and hydrochloric acid. The equilibrium between lewisite, lewisite oxide and CVAA is not a true equilibrium, because once

in solution, lewisite is completely converted to CVAA. Dehydration of CVAA forms the insoluble 2-chloroarsenous oxide (lewisite oxide). In basic solution, the *trans* isomer of lewisite is cleaved to yield acetylene and sodium arsenite. In addition, the *cis* isomer of lewisite may be photoconverted to the *trans* isomer, and the trivalent form of arsenic in lewisite oxide is generally oxidized to pentavalent arsenic under environmental conditions. Impurities found in the synthesized form of lewisite include bis(2-chlorovinyl)chloroarsine (also known as lewisite-2 or L-2), tris(2-chlorovinyl)arsine (also known as lewisite-3 or L-3), and arsenic trichloride [reviewed by NRC (2013); Munro et al. (1999)].

A summary of available toxicity values for lewisite from EPA and other agencies/organizations is provided in Table 2. The only organizations that have derived chronic toxicity values for lewisite are the U.S. Army and the National Research Council (NRC). In 1996, the U.S. Army derived an interim chronic oral reference dose (RfD) [documented in SERDP (1997)] of 1×10^{-4} mg/kg-day based on a no-observed-adverse-effect level (NOAEL) of 0.6 mg/kg-day (the highest dose tested) for forestomach lesions in male and female rats exposed via intragastric intubation 5 days/week for 23 weeks in a two-generation reproductive toxicity study (Sasser et al., 1999; Sasser et al., 1989b). Forestomach lesions were observed at higher doses in a subchronic-duration toxicity study in rats (Sasser et al., 1996; Sasser et al., 1989a). The U.S. Army authors converted the NOAEL to a continuous exposure dose of 0.44 mg/kg-day and divided this point-of-departure by a total uncertainty factor (UF) of 3,000 (including UFs of 10 each for interspecies extrapolation, variability in human sensitivity, and extrapolation from subchronic- to chronic-duration exposure; and a UF of 3 for database deficiencies) to obtain the interim RfD.

In 1996, the Material/Chemical Risk Assessment (MCRA) Working Group of the Environmental Risk Assessment Program, a multiagency work group consisting of EPA, U.S. Department of Defense (DOD), and U.S. Department of Energy (DOE) representatives, reviewed the U.S. Army's interim RfD (SERDP, 1997). The working group concluded that the RfD was not verifiable due to deficiencies in the lewisite database. The group recommended that inorganic arsenic (RfD, 3×10^{-4} mg/kg-day) be used as a surrogate for lewisite based on its similarity to the interim RfD for lewisite, and the observation that lewisite would degrade to inorganic arsenic in the environment.

The NAS (1999) was asked to review the Army's interim RfD. Upon review of the available data, the NAS' NRC recommended against using the proposed RfD and suggested instead that the RfD should be based on a lowest-observed-adverse-effect level (LOAEL) of 0.07 mg/kg-day for mortality and gastric lesions in rabbits exposed during gestation (Hackett et al., 1987). The NRC reasoned that rabbits might be more susceptible to lewisite than rats, and that this increased susceptibility outweighed concerns raised in the U.S. Army's assessment regarding the small numbers of surviving rabbits in each dose group. The NRC suggested that the LOAEL of 0.07 mg/kg-day in rabbits be combined with a total UF of 9,000 (with UFs of 3 each for interspecies extrapolation and variability in human sensitivity and UFs of 10 each for extrapolation from a LOAEL to a NOAEL, extrapolation from a 14-day exposure to a chronic-duration exposure, and database deficiencies) to obtain an RfD that rounded to 1×10^{-5} mg/kg-day.

Table 2. Summary of Available Toxicity Values for Lewisite (CASRN 541-25-3)

Source/ Parameter[a,b]	Value (applicability)	Notes	Reference
Noncancer			
IRIS	NV	NA	U.S. EPA (2015)
HEAST	NV	NA	U.S. EPA (2011a)
DWSHA	NV	NA	U.S. EPA (2012a)
ATSDR	NV	NA	ATSDR (2015)
WHO	NV	NA	WHO (2015)
Cal/EPA	NV	NA	Cal/EPA (2014); Cal/EPA (2015a); Cal/EPA (2015b)
U.S. Army (RfD)	1×10^{-4} mg/kg-d	Route: oral, intragastric intubation Species: rat Duration: 23 wk in a 2-generation reproduction study	SERDP (1997)
NRC (RfD)	1×10^{-5} mg/kg-d	Route: oral, intragastric intubation Species: rabbit Duration: 14 d during gestation	NAS (1999)
OSHA	NV	NA	OSHA (2006)
NIOSH	NV	NA	NIOSH (2015)
ACGIH	NV	NA	ACGIH (2015)
Cancer			
IRIS	NV	NA	U.S. EPA (2015)
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 (2015a); Cal/EPA (2015b)
ACGIH	NV	NA	ACGIH (2015)

[a]Sources: ACGIH = American Conference of Governmental Industrial Hygienists; ATSDR = Agency for Toxic Substances and Disease Research; Cal/EPA = California Environmental Protection Agency; DWSHA = Drinking Water Standards and Health Advisories; HEAST = Health Effects Assessment Summary Tables; IARC = International Agency for Research on Cancer; IRIS = Integrated Risk Information System; NIOSH = National Institute for Occupational Safety and Health; NRC = National Resource Council; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; WHO = World Health Organization.
[b]Parameters: RfD = reference dose

NA = not applicable; NV = not available.

After reviewing the NAS (1999) recommendations, the U.S. Army (2000) concluded that the interim RfD of 1×10^{-4} mg/kg-day is a more appropriate estimate of the toxicity from chronic-duration oral exposure to lewisite and should be used when lewisite or its degradation products (CVAA or lewisite oxide) are present in the environment. The Army indicated that these products are unlikely to be present in the environment and that risk evaluations of other lewisite degradation products (arsenicals) in the environment should employ the EPA RfD for inorganic arsenic (0.003 mg/kg-day).

Literature searches were conducted in July 2013, June 2014 and updated in September 2015 for studies relevant to the derivation of provisional toxicity values for lewisite (CASRN 541-25-3). Searches were conducted using EPA's Health and Environmental Research Online (HERO) database of scientific literature. The following databases were searched: PubMed, ToxLine (including TSCATS1), and Web of Science. The following databases were searched outside of HERO for health-related values: ACGIH, ATSDR, Cal/EPA, U.S. EPA IRIS, U.S. EPA HEAST, U.S. EPA OW, U.S. EPA TSCATS2/TSCATS8e, NIOSH, NTP, OSHA, and RTECS.

REVIEW OF POTENTIALLY RELEVANT DATA (NONCANCER AND CANCER)

Tables 3A and 3B provide an overview of the relevant databases for lewisite and include all potentially relevant and repeated short term-, subchronic-, and chronic-duration studies. Principal studies are identified in bold. The phrase "statistical significance," used throughout the document, indicates a *p*-value of <0.05 unless otherwise noted.

Table 3A. Summary of Potentially Relevant Noncancer Data for Lewisite (CASRN 541-25-3)

Category	Number of Male/Female, Strain, 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]					
ND								
			2. Inhalation (mg/m^3)					
ND								
Animal								
			1. Oral (mg/kg-d)[a]					
Subchronic	**10 M/10 F, S-D rat, lewisite in sesame oil via intragastric intubation, 5 d/wk, 13 wk**	**0, 0.01, 0.10, 0.50, 1.0, or 2.0 mg/kg-d** **ADD: 0, 0.0071, 0.071, 0.36, 0.71, 1.4**	**Mortality due to inflammatory lesions of the respiratory tract at ≥0.36 mg/kg-d.**	**0.071**	**0.0049 (F)**	**0.36 (FEL)**	**Sasser et al. (1996); Sasser et al. (1989a)**	**PR PS**
Subchronic/ chronic	10 rats, sex and strain unspecified, 98 d at 10 ppm, 133 d at 16 ppm, in drinking water	0, 10[c], 16[d] ppm	Mortality	NDr	NDr	ND	U.S. Army, 1941	NPR
Reproductive	20 M/25 F, S-D rat, lewisite in sesame oil via intragastric intubation, 5 d/wk, 23 wk each generation (13 wk premating, and during mating, gestation, and lactation)	0, 0.10, 0.25, or 0.60 mg/kg-d ADD: M: 0.071, 0.18, 0.43 F: 0.076, 0.19, 0.46	Mortality due to inflammatory lesions of the respiratory tract at ≥0.071 mg/kg-d.	NDr	0.0052 (F)	0.071 (FEL) for mortality in F1 male rats	Sasser et al. (1999); Sasser et al. (1989b)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for Lewisite (CASRN 541-25-3)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (comments)	Notes[b]
Developmental	0 M/10 F, CD rat, dose-range-finding study, lewisite in sesame oil via intragastric intubation on GDs 6−15	0, 0.5, 1.0, 2.0, or 2.5 mg/kg-d ADD: 0, 0.5, 1.0, 2.0, 2.5	Mortality and gross gastrointestinal lesions at ≥2.0 mg/kg-d. Due to uncertainty regarding the possible contribution of toxicity towards one death attributed to dosing trauma at 1.0 mg/kg-d, the next lower dose of 0.5 mg/kg-d, at which no effects were reported, was designated the NOAEL.	0.5	NDr	2.0 (FEL)	Hackett et al. (1992); Hackett et al. (1987)	NPR
	0 M/25 F, CD rat, main developmental study, lewisite in sesame oil via intragastric intubation on GDs 6−15	0, 0.5, 1.0, and 1.5 mg/kg-d ADD: 0, 0.5, 1.0, 1.5	No effects observed.	1.5	NDr	NDr	Hackett et al. (1992); Hackett et al. (1987)	NPR
Developmental	0 M/8 F, New Zealand rabbit, dose-range-finding study, lewisite in sesame oil via intragastric intubation on GDs 6−19	0, 0.5, 1.0, 1.5, or 2.0 mg/kg-d ADD: 0, 0.5, 1.0, 1.5, 2.0	Hemorrhage of the gastric mucosa at ≥0.5 mg/kg-d. The study authors attributed high mortality at this dose entirely to dosing trauma, but there is some uncertainty regarding the possible contribution of lewisite toxicity to these deaths. Deaths attributed to lewisite toxicity (sans gavage error) were reported at ≥1.0 mg/kg-d.	NDr	NDr	0.5 (FEL)	Hackett et al. (1992); Hackett et al. (1987)	NPR
Developmental	0 M/18 F, New Zealand rabbit, main developmental study, lewisite in sesame oil via intragastric intubation on GDs 6−19	0, 0.07, 0.20, and 0.60 mg/kg-d ADD: 0, 0.07, 0.20, 0.60	Mortality due to gastric lesions at ≥0.07 mg/kg-d.	NDr	0.002	0.07 (FEL)	Hackett et al. (1992); Hackett et al. (1987)	NPR

Table 3A. Summary of Potentially Relevant Noncancer Data for Lewisite (CASRN 541-25-3)								
Category	**Number of Male/Female, Strain, 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)								
ND								

[a]Dosimetry: Oral doses are expressed as adjusted daily dose (ADD in mg/kg-day).
[b]Notes: PS = principal study; PR = peer reviewed; NPR = not peer reviewed.
[c]Exposed to 10 ppm for 98 days.
[d]Exposed to 16 ppm for 133 days.
Bold text indicates the principal study.

Treatment/exposure duration (unless otherwise noted): Short-term = repeated exposure for >24 hours ≤30 days (U.S. EPA, 2002); long-term (subchronic) = repeated exposure for >30 days ≤10% lifespan for humans (more than 30 days up to approximately 90 days in typically used laboratory animal species) (U.S. EPA, 2002); chronic = repeated exposure for >10% lifespan for humans (more than approximately 90 days to 2 years in typically used laboratory animal species) (U.S. EPA, 2002).

ADD = adjusted daily dose; F = female(s); FEL = frank effect level; GD = Gestation Day; M = male(s); ND = no data; NDr = not determined.

Table 3B. Summary of Potentially Relevant Cancer Data for Lewisite (CASRN 541-25-3)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry	Critical Effects	BMDL/ BMCL	Reference (comments)	Notes
Human						
1. Oral (mg/kg-d)						
ND						
2. Inhalation (mg/m^3)						
Carcinogenicity	55 male workers engaged in manufacture of lewisite at a poison gas factory in Japan were followed for lung cancer incidence for 50 yr. 969 workers at the same factory but not engaged in poison gas manufacture served as controls.	Based on job description only; no quantitative exposure data.	2 cases of lung cancer were observed in the lewisite-exposed group (3.6%); 38 lung cancers were observed in the control group (3.9%). Statistical analysis was not performed due to the small number of lewisite-exposed workers.	NDr	Doi et al. (2011)	PR
Animal						
1. Oral (mg/kg-d)						
ND						
2. Inhalation (mg/m^3)						
ND						

PR = peer reviewed; ND = no data; NDr = not determined.

HUMAN STUDIES

Oral Exposures

No studies have been identified.

Inhalation Exposures

Doi et al. (2011) evaluated the incidence of lung cancers in former workers in a lewisite production facility in Okunojima Island in Hiroshima Prefecture, Japan (see Table 3B). The factory primarily produced mustard gas, but lewisite and other poison gases were also produced. Former workers were recruited through a variety of means including house-to-house canvassing, television advertisement, and inquiry upon admittance to hospitals in the area. Workers directly engaged in the manufacture of poison gases were selected for participation; selection criteria included male gender, living continuously in the Hiroshima Prefecture after retirement from the factory, and follow-up data available for more than 2 years. Lung cancer diagnosis (confirmed by pathology) was obtained from clinical records, postmortem examinations, or notification from hospitals or public health authorities. A group of 55 male workers (mean age at first employment, 22 years) directly engaged in the manufacture of lewisite was included. Controls (n = 969) were selected from among job titles other than manufacturing (carriers, construction workers, clerks, housekeepers or medical staff). A total of two incident lung cancer cases, both squamous cell carcinomas, occurred in the group exposed to lewisite over the 50-year follow-up (3.6%); while there were 38 lung cancer cases among controls (3.9%). Statistical comparison to control incidence rates was not performed due to the small numbers of subjects and cases, however, there does not appear to be an increase in those exposed. Previous studies of these workers (Yamakido et al., 1996; Shakil et al., 1993; Yamakido et al., 1985; Nishimoto et al., 1983) grouped workers exposed to lewisite with the much larger numbers of workers exposed to mustard gas [480 men in Doi et al. (2011)], diphenylcyanoarsine (178 men), and/or other poison gases produced at the facility; thus, these earlier studies provide little information on the effects of occupational exposure to lewisite.

ANIMAL STUDIES

Oral Exposures

Overview of Animal Oral Exposure Studies

Potentially relevant data for noncancer effects come from a subchronic-duration study in rats exposed to lewisite via intragastric intubation for 13 weeks (Sasser et al., 1996; Sasser et al., 1989a), a subchronic-duration drinking water study in rats (U.S. Army, 1941), a two-generation reproductive toxicity study in rats exposed via intragastric intubation (Sasser et al., 1999; Sasser et al., 1989b), and unpublished developmental toxicity studies in rats and rabbits exposed to lewisite via intragastric intubation (Hackett et al., 1992; Hackett et al., 1987). No chronic-duration or cancer bioassays using oral exposure to lewisite have been identified in the available literature.

Subchronic-Duration Studies

Sasser et al. (1996); Sasser et al. (1989a)

In a 13-week study, ultimately chosen as the principal study, rats were exposed to lewisite (95.8% *trans* isomer, 4% *cis* isomer, and 0.2% unknown compounds) in sesame oil via intragastric intubation was conducted by (Sasser et al., 1996; Sasser et al., 1989a). Groups of 10 male and 10 female Sprague-Dawley (S-D) rats received doses of 0.01, 0.1, 0.5, 1.0, or 2.0 mg/kg-day, 5 days/week for 13 weeks (equivalent to continuous doses of 0.0071, 0.071, 0.36, 0.71, or 1.4 mg/kg-day). The animals were observed daily for mortality and morbidity, and

clinical signs of toxicity were evaluated weekly. Body weight was measured before and at the end of the study, as well as at weekly intervals. Ocular examinations were performed before and after the study in the control and 0.71- and 1.4 mg/kg-day exposure groups. Blood was collected at Week 6 and at the terminal sacrifice for evaluation of hematology endpoints (platelets [PLAT], total and differential leukocyte counts, red blood cell [RBC] and reticulocyte counts [Ret], hemoglobin [Hb], hematocrit [Hct], mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], and mean corpuscular hemoglobin concentration [MCHC]). In addition, blood collected at sacrifice was analyzed for serum chemistry parameters including blood urea nitrogen (BUN), creatinine, total protein, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). At sacrifice or unscheduled death, all animals were subjected to gross necropsy, and the liver, thymus, right kidney, right gonad, heart, brain, and adrenal glands were weighed. Comprehensive histopathology examination (tissues not specified) was limited to the control and highest-dose group; tissues identified as target organs in the high-dose animals were examined microscopically in the lower dose groups. The data were analyzed by analysis of variance (ANOVA) and Tukey's studentized range test.

Mortality among lewisite-treated animals was high (see Table B-1); a total of 28 toxicity-related deaths (or moribund sacrifices) were reported, including two males and three females exposed to 0.36 mg/kg-day, seven males and six females exposed to 0.71 mg/kg-day, and three males and seven females exposed to 1.4 mg/kg-day (Sasser et al., 1996; Sasser et al., 1989a). An additional male of the 0.71-mg/kg-day group died due to an overdose of anesthetic given for blood sampling. Toxicity-related deaths occurred throughout the study, some as early as Week 1 and some as late as Week 13. The cause of death was severe, acute inflammation of the respiratory tract. Signs of toxicity such as dyspnea, drooling, and listlessness occurred 1−2 days prior to death. In survivors, signs of toxicity included drooling, nasal discharge, and mouth breathing among rats exposed to ≥0.36 mg/kg-day. Body weights of surviving rats were not affected by exposure, and ocular examinations did not indicate any treatment-related effects. Statistically significant increases were seen in lymphocytes at Week 6 (but not Week 13) and in platelets at Week 13 in 1.4-mg/kg-day female rats, based on only three or four animals in this group (see Table B-2). No hematological changes were seen in lower-dose females or in males. Changes in serum chemistry parameters were limited to small, statistically significant decreases of uncertain toxicological significance in serum protein, creatinine, ALT, and AST in male rats at Week 13 (see Table B-2). No serum chemistry changes were seen in females. No treatment-related alterations in absolute or relative organ weight were observed at any dose of lewisite.

Grossly visible masses on the mucosal surface of the forestomach were noted in 8/10 males and 4/10 females exposed to 1.4 mg/kg-day and 1/10 males exposed to 0.71 mg/kg-day (Sasser et al., 1996; Sasser et al., 1989a). No gross lesions were reported at doses ≤0.36 mg/kg-day.

Microscopic lesions were observed in the respiratory tract and forestomach (Sasser et al., 1996; Sasser et al., 1989a). Severe, acute inflammatory lesions of the respiratory tract were observed in all animals that died early; tissues affected by the lesions included the lungs, trachea, larynx, and nasal passages (Sasser et al., 1996; Sasser et al., 1989a). The larynx and trachea were noted to be edematous with acute epithelial necrosis and neutrophil infiltration; the lumens were typically filled with exudate. The large intrapulmonary and terminal airways were affected less frequently. Nasal lesions were more severe in the posterior areas than in anterior regions.

The study authors suggested that the respiratory lesions most likely resulted from induced reflux or aspiration of lewisite into the respiratory tract and that volatilization of lewisite from the stomach or esophagus may also have played a role. The location of the most severely affected tissues supports this supposition.

Forestomach lesions noted at the highest dose were characterized by necrosis of the squamous epithelium with neutrophil and macrophage infiltration, hemorrhage, edema, and fibroblast proliferation (Sasser et al., 1996; Sasser et al., 1989a). Among premature deaths, mild acute inflammation of the glandular stomach was noted in 1/10 males and 3/10 females exposed to 1.4 mg/kg-day; the study authors noted that these lesions may not have been detected in other animals that died prematurely because the lesions disappeared due to autolysis. Survivors of the highest exposure, along with two animals that died after 79 days on study, exhibited forestomach ulcerations. The one surviving male exposed to 0.71 mg/kg-day showed epithelial hyperplasia and hyperkeratosis of the forestomach without ulcerations. The 0.36-mg/kg-day dose is a frank effect level (FEL) for mortality (two males and three females) and severe inflammation of the respiratory tract, and the 0.071-mg/kg-day dose is a NOAEL for this study, as no meaningful effects were observed at this dose.

U.S. Army (1941)

In an unpublished study conducted by the Medical Research Division of Edgewood Arsenal (U.S. Army, 1941) for which the full text is not available, rats were exposed for 98−133 days to lewisite (purity 98.6−99.3%) in drinking water. This study lacked information on several aspects of study design and results, as noted below. The strain or sex of rat tested was not reported. Groups of 10 rats were exposed to 0 or 10 ppm lewisite for 98 days, followed by 21 untreated days; additional groups of 10 rats were exposed to 0 or 16 ppm lewisite for 133 days followed by 14 untreated days. The exposure solution was prepared 6 days prior to the start of the experiments, and concentrations in the solution provided to the animals were not quantified analytically. Lewisite in aqueous solution is rapidly hydrolyzed to CVAA and hydrochloric acid, although available data do not provide a quantitative estimate of the rate of hydrolysis (HSDB, 2010). Given the low concentration of lewisite relative to its aqueous solubility [500 mg/L or ppm; HSDB (2010)] and the duration of time between preparation of the solution and administration to the animals, it is very likely that the animals were exposed primarily to the degradation products of lewisite rather than to lewisite itself. As a result, it is not possible to estimate the dose of lewisite, if any, to which the animals were exposed.

The animals were weighed weekly during the study, and water consumption was recorded 3 times/week (U.S. Army, 1941). Upon death or sacrifice, the animals were necropsied, and the kidney, liver, spleen, stomach, and duodenum were examined microscopically. Arsenic content of the liver, kidney, and spleen was measured. The report did not discuss any statistical analyses.

In the 98-day study, four treated animals and two untreated animals were found dead prior to study termination. The cause of death was reported as pulmonary disease (not further detailed) in one control and three treated rats. Causes of the remaining deaths were not determined. In addition to these deaths, there were periodic sacrifices of individual treated and control animals over the course of the study, but it is not clear from the report whether these were moribund sacrifices or sacrifices that were planned. At the end of the treatment period (Day 98), only five treated and four untreated animals remained. In discussing the body-weight

effects in this study, the study authors noted that both controls and treated rats exhibited slight weight loss over the study, and attributed this to the rats' age and the poor conditions of the animal facility (authors noted excessive temperature, humidity, and drafts). In addition, the authors noted that initial weights were highly variable; average initial weights of the treated and control groups were 254 and 233 g, respectively, a difference of 8%. The average body weight of treated rats remained higher than controls over the duration of the study, suggesting little or no effect of exposure on body weight. Average water consumption over the course of the study was ~29% lower among treated rats (77 g/kg-day) than controls (108 g/kg-day). The authors stated that there were no pathology findings related to treatment (data not reported).

In the 133-day exposure study, two controls and one lewisite-treated rat died prematurely. The controls reportedly died from pulmonary disease, and the cause of death in the lewisite-treated rat was not determined. Two rats per group were sacrificed after 98 days (the reason for sacrifice was not reported). Seven treated and six control rats remained at the end of the study. As with the other study, average initial body weights differed by ~8% (183 g in treated rats and 169 g in controls) and weights of treated rats remained higher than that of controls. In addition, average water consumption by treated rats was reduced (89 g/kg-day) compared with controls (120 g/kg-day). Exposure did not result in pathology findings (data not shown). An effect level cannot be determined for this study due to the lack of information on the concentration and/or identity of compounds in the test solution.

Chronic-Duration or Carcinogenicity Studies

No studies have been identified.

Reproductive Studies

Sasser et al. (1999); Sasser et al. (1989b)

Sasser et al. (1999) and Sasser et al. (1989b) conducted a two-generation reproductive study in S-D rats. Groups of 20 male and 25 female adult rats (F0 parents) received lewisite (95.8% *trans* isomer, 4% *cis* isomer, and 0.2% unknown compounds) in sesame oil via intragastric intubation at doses of 0, 0.10, 0.25, or 0.60 mg/kg-day on 5 days/week (females were dosed daily during gestation). These doses are equivalent to continuous doses of 0.071, 0.18, or 0.43 mg/kg-day in males, and doses of 0.076, 0.19, or 0.46 mg/kg-day in females (assuming 5 days/week except during 3 weeks of gestation, when dosing was 7 days/week). The animals were exposed prior to mating (13 weeks), during mating (3 weeks), and after mating until the birth of offspring; dams continued to receive lewisite during lactation. After weaning, randomly selected F1 male and female offspring were exposed to the same doses via intragastric intubation throughout adolescence, mating, and gestation. F0 and F1 male parents were sacrificed when the next generation was born, while F0 and F1 female parents continued exposure during lactation and were sacrificed at weaning of the next generation. F2 pups were sacrificed at weaning. Evaluations of all parental animals included mortality, body weight (measured weekly), gross necropsy, and organ weights (testis, epididymis, ovary, and uterus). Reproductive organs (ovaries, uterus, vagina, testes, seminal vesicles, prostate, and epididymis) of control and high-dose F0 and F1 adults were examined for histopathology; tissues with gross lesions in adults of lower dose groups were also examined for histopathology. Upon delivery, pups were counted, sexed, weighed, and examined for gross abnormalities. Litters were culled to four pups per sex per litter on Postnatal Day (PND) 4. Body weights of pups were recorded on PNDs 4, 14, and 21. The authors reported that statistical analysis of body and organ weights and forestomach lesions was performed using ANOVA followed by Tukey's studentized range test.

Repeated measures on the same animal were analyzed for trends by orthogonal contrast. Growth curves were compared using a nonparametric method (not specified further), while the χ^2 test was used for binary variables.

Mortalities among parental animals occurred at all doses (see Table B-3), with greater mortality in the high-dose group (Sasser et al., 1999; Sasser et al., 1989b). At the high dose, 11 F0 females, 4 F0 males, 18 F1 females, and 6 F1 males died. In the mid-dose group, 4 F0 females, 5 F1 females, and 2 F1 males died. At the low dose, 2 F1 females and 1 F1 male died. A single control female died during parturition due to breach birth. The study authors reported that one high-dose female death also occurred during parturition, and that three other deaths (two F1 females and one F1 male) were attributable to dosing error; however, the distribution of the latter deaths across dose groups was not given. The remaining deaths were attributed to aspiration of the test material into the respiratory tract, as indicated by pulmonary lesions including edema, hemorrhage, acute inflammation of the airways and alveoli, subacute inflammation of the pleura and mediastinal tissues, and presence of foreign material in the lungs at necropsy. The authors postulated that the irritating effect of lewisite administration may have triggered a reflex reaction in the animals, leading to aspiration of the test material.

There were no treatment-related effects on body weight, mating or fertility indices, or reproductive organ weights of F0 or F1 male or female parental animals (Sasser et al., 1999; Sasser et al., 1989b). Likewise, litter size, pup birth weight, sex ratio, numbers of stillbirths, and abnormal pups, and pup weight and survival through weaning were not affected by exposure to lewisite. Histopathology examination did not indicate any treatment-related effects on reproductive organs of parental adults. The only treatment-related microscopic findings consisted of the pulmonary effects noted above as the cause of early mortality (incidences of lesions were not reported). The study authors reported that the high dose was a NOAEL for reproductive toxicity; however, as three F1 deaths occurred at the low dose (0.071 mg/kg-day for males and 0.076 mg/kg-day for females), and it is not clear that the deaths were due to dosing error or breach birth, this dose is considered a FEL.

Developmental Studies

Hackett et al. (1992); Hackett et al. (1987)

The developmental toxicity of lewisite was evaluated in rats and rabbits by Hackett et al. (1992) and Hackett et al. (1987). Lewisite (95.8% *trans* isomer, 4% *cis* isomer, and 0.2% unknown compounds) was administered in sesame oil by intragastric intubation using a dosing needle (rats) or 22-inch feeding tube (rabbits). In the dose-range-finding study in rats, groups of 10 (11 at the high-dose) pregnant CD rats were given daily doses of 0, 0.5, 1.0, 2.0, or 2.5 mg/kg-day lewisite on GDs 6−15. The dose-range-finding study in rabbits employed groups of eight pregnant New Zealand white rabbits receiving daily doses of 0, 0.5, 1.0, 1.5, or 2.0 mg/kg-day on GDs 6−19. Maternal animals were observed for clinical signs of toxicity and weighed regularly (GDs 0, 6−16, and 20 in rats; GDs 0, 6−19, and 30 in rabbits). Upon sacrifice (GD 20 in rats and GD 30 in rabbits), ovaries were examined for corpora lutea, and uteri were removed and evaluated for implantation sites, resorptions, and living and dead fetuses. Maternal Hct, body weights, and uterine weights were measured. Gross necropsies were also performed on maternal animals. Fetuses were weighed, sexed, and examined for gross morphologic defects.

The main developmental study used groups of 25 pregnant CD rats and 18 or 19 (depending on dose) pregnant New Zealand white rabbits exposed on the same schedule as the dose-range-finding study; rats were given doses of 0, 0.5, 1.0, and 1.5 mg/kg-day, while rabbits were given daily doses of 0, 0.07, 0.2, and 0.6 mg/kg-day. The endpoints evaluated in the dose-range-finding study were also assessed in the main developmental study. In addition, fetal crown-rump length and placental weight were recorded. Half of the fetuses were examined for skeletal abnormalities and half for visceral abnormalities. Statistical analysis of continuous data was performed using ANOVA and Dunnett's test; quantal data were analyzed using χ^2 tests (for fetal data) or Fisher's exact test (for litter data).

For the developmental study, which was conducted before the subchronic-duration and reproductive rat toxicity studies by the same research group (Sasser et al., 1999; Sasser et al., 1996; Sasser et al., 1989a, b), deaths were reported separately for dosing trauma and lewisite toxicity. However, in light of the finding in the later studies of lewisite-related toxicity in the respiratory tract from aspiration of the test material, there is some uncertainty in the attribution of deaths in this study, because deaths associated with damage to tissues of the respiratory tract during dosing were apparently automatically categorized as trauma, without consideration of possible toxicity. The study authors acknowledged that assignment of a "probable cause of death" to individual animals using only the gross observations at necropsy was often difficult and may appear to be arbitrary in some cases.

In the dose-range-finding study in rats (Hackett et al., 1992; Hackett et al., 1987), deaths attributed to lewisite toxicity occurred at 2.0 mg/kg-day (one rat) and 2.5 mg/kg-day (two rats) (see Table B-4). Additional deaths attributed to dosing trauma included one death at 1.0 mg/kg-day, two deaths at 2.0 mg/kg-day, and one death at 2.5 mg/kg-day. Observations at necropsy of the deaths reported to be toxicity related included gas-filled gastrointestinal tracts containing yellow and bloody fluids. Maternal body weights were decreased with dose by the end of the dosing period (see Table B-4); at the time of sacrifice, a statistical and biological significant decrease in body weight was noted in the 2.5-mg/kg-day group (18% lower than controls). Maternal Hct at sacrifice was not affected by treatment. Among pregnant survivors of the high-dose group, a reduction (not statistically significant) in the percentage of implantations (implantations/corpora lutea) was noted (66 vs. 86% in controls). However, a statistically significant reduction in the number of implantations was noted per dam (mean of 10 per dam in the high-dose group compared with 15 per dam in the controls). A statistically significant increase in the percentage of mid-gestation resorptions was observed at the 2.0-mg/kg-day dose (but not the 2.5-mg/kg-day dose); a higher percentage of early gestation resorptions occurred at the highest dose, but the increase was not statistically significant (see Table B-4). The percentage of litters with resorptions was not affected by treatment. As a result of the decrease in implantations and increase in resorptions at the higher doses, the numbers of live fetuses/litter were significantly lower than controls at 2.0 and 2.5 mg/kg-day (see Table B-4); there were no dead fetuses in any litter at any dose. Fetal body weights in the two highest dose groups (especially in females) were biologically significantly lower than controls (13 and 19% for males and females, respectively), but the differences were not statistically significant, possibly due to the small group sizes. Stunted pups were observed in one litter in each of the 1.0, 2.0, and 2.5-mg/kg-day groups. The only gross abnormality observed in any fetus in the study occurred in the vehicle control group. Based on the effects seen at 2.0 and 2.5 mg/kg-day, the top dose for the main teratology study was set at 1.5 mg/kg-day. The 2.0-mg/kg-day dose is a FEL for mortality; due to uncertainty regarding the possible contribution of toxicity towards the death

attributed to dosing trauma at 1.0 mg/kg-day, as discussed above, the next lower dose of 0.5 mg/kg-day was designated as the NOAEL.

In the main developmental study in rats, one control dam and two dams in the 1.0-mg/kg-day group died due to dosing trauma; no deaths were attributed to lewisite toxicity (Hackett et al., 1992; Hackett et al., 1987). Exposure did not affect maternal body weight, gravid uterine weight, or hematocrit. In addition, there were no treatment-related effects on numbers of implantation sites, percentages of resorptions, numbers of live or dead fetuses per litter, or percentage of live fetuses. Fetal body weight, crown-rump length, sex ratio, and placental weights were not biologically and/or statistically significantly different among the groups. No dose-related increases in the litter incidence of gross, skeletal, or visceral abnormalities or variations were observed. The highest dose in this study (1.5 mg/kg-day) is a NOAEL for developmental and maternal toxicity in rats.

The dose-range-finding study conducted in rabbits (Hackett et al., 1992; Hackett et al., 1987) was hampered by mortality at all dose levels (see Table B-5); dosing trauma was reported to be responsible for deaths of 1/8, 5/8, 1/8, 3/8, and 0/8 animals in the 0-, 0.5-, 1.0-, 1.5-, and 2.0-mg/kg-day groups respectively, while toxicity-related deaths occurred at 1.0 mg/kg-day (6/8 does), 1.5 mg/kg-day (5/8 does), and 2.0 mg/kg-day (8/8 does). Necropsy findings in the deaths attributed to lewisite toxicity (in all affected dose groups) included inflammation and hemorrhage of the mucosa in the pyloric and cardiac regions of the stomach. Duodenal hemorrhage and necrosis and hemorrhagic foci of the cecum were noted in animals of the 1.0-mg/kg-day group that died from lewisite toxicity. No gross lesions were seen in the respiratory tract, esophagus, or thorax of these animals, but it is unclear whether the occurrence of such lesions would have caused a reclassification of these deaths as trauma-related, as suggested by the reported methodology. Evidence of lewisite toxicity observed at necropsy of animals that reportedly died from dosing trauma and in animals that survived to terminal sacrifice included hemorrhage of the gastric mucosa in doses of the 0.5-mg/kg-day group that reportedly died from dosing trauma and inflammation of the gastric mucosa and accumulation of peritoneal or pericardial fluid in 2/3 rabbits at scheduled sacrifice of the 0.5-mg/kg-day group. The lowest dose (0.5 mg/kg-day) is a FEL for hemorrhage of the gastric mucosa. The study authors attributed the high mortality at this dose entirely to dosing trauma, but there is some uncertainty regarding the possible contribution of toxicity to these deaths, as discussed above.

Among surviving rabbits, 3/7 controls, 3/3 rabbits exposed to 0.5 mg/kg-day, and 1/1 rabbit exposed to 1.0 mg/kg-day were pregnant (see Table B-5) (Hackett et al., 1992; Hackett et al., 1987), providing limited data on endpoints other than mortality. Body weights of treated survivors were lower than controls. The available data on other endpoints were too limited to draw any conclusions given the small numbers of pregnant survivors. Based on the toxicity seen in the dose-range-finding study, the authors selected doses of 0, 0.07, 0.2, and 0.6 mg/kg-day for the main teratology study in rabbits.

Maternal mortality was also observed in the main teratology study (see Table B-6), including deaths of 1/19, 5/18, 5/18, and 3/19 animals attributed to dosing trauma, stress, handling trauma, or pregnancy complications in the control, 0.07-, 0.2-, and 0.6-mg/kg-day groups (respectively), as well as deaths of 0/19, 2/18, 6/18, and 11/19 animals attributed to lewisite toxicity in the same dose groups (Hackett et al., 1992; Hackett et al., 1987). Necropsy findings of gastric ulcerations in decedents were similar to those seen in the dose-range-finding

study; no lesions of the respiratory tract were noted. The numbers of pregnant rabbits among survivors in the control through high-dose groups were 9/18, 6/11, 5/7, and 3/5 (pregnant rabbits/survivors). Gestational weight gain among survivors of the high-dose group was statistically significantly lower than controls from GDs 12−20 (data shown graphically); the study authors noted that a large number of animals exposed to the high dose were anorexic during the exposure period. Maternal body weights and weight gain did not differ significantly from controls in the lower dose groups, although the authors noted that qualitatively observed anorexia occurred more frequently in all exposed animals between GDs 20 and 30. Maternal Hct decreased (not statistically significant) with increasing lewisite dose, from 43% in controls to 33% in does exposed to 0.6 mg/kg-day (see Table B-6). The differences did not reach statistical significance, potentially due to the small sample-size of survivors in the higher dose groups. The small numbers of animals and high variability within dose groups limited the conclusions that could be drawn regarding other developmental parameters evaluated in the study. However, some trends with increasing dose were noted, including fewer implantations per corpus luteum (at the high dose only); higher intrauterine mortality; lower placental weights, fetal body weights, and crown-rump lengths; and lower proportions of male fetuses (see Table B-6). A statistically significant increase in the incidence of litters with stunted fetuses was observed in the high-dose group. In addition, the incidences of fetuses (but not litters) with supernumerary ribs and reduced ossification of the pelvis were statistically significantly increased at the high dose. The low dose in this study (0.07 mg/kg-day) is a FEL for mortality due to gastric lesions. However, uncertainties in the attributions of death due to dosing trauma vs toxicity and the small numbers of surviving animals in dose groups makes the interpretation of this study unclear.

Inhalation Exposures

No repeated-dose studies examining effects of lewisite in animals exposed via inhalation for >4 hours on a single day have been identified. Acute studies are presented in Table 5.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Table 4A provides an overview of genotoxicity studies of lewisite and Table 4B provides an overview of other supporting studies on lewisite, including acute toxicity studies, studies of lewisite toxicokinetics, and mechanistic studies of lewisite.

Genotoxicity

Lewisite did not induce mutations in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA102 with or without metabolic activation at concentrations up to those that were cytotoxic (cytotoxicity was noted at 0.01 μg/plate in TA102 and at 5 μg/plate in other strains tested) (U.S. Army, 1989b). Likewise, lewisite was negative for mutation at the HGPRT locus in Chinese hamster ovary (CHO) cells when tested up to cytotoxic concentrations (cytotoxicity was observed at concentrations ≥1 μM) (U.S. Army, 1989a). Lewisite did not induce sister chromatid exchanges at concentrations up to 1 μM, but did induce chromosomal aberrations in CHO cells at concentrations ≥0.50 μM (U.S. Army, 1989a). Lewisite was negative in the *Drosophilla melanogaster* sex-linked recessive lethal assay (Auerbach and Robson, 1947). No evidence of dominant lethal toxicity was observed when male CD rats were exposed via gavage to doses of up to 1.5 mg/kg for 5 consecutive days (Bucci et al., 1993).

Bucci et al. (1993)

Bucci et al. (1993) conducted a study of dominant lethal toxicity in CD rats. Groups of 20 male rats received lewisite in sesame oil via intragastric intubation on 5 consecutive days, and

then were mated to two untreated females per week for 10 consecutive weeks. Doses used in the study were 0, 0.375, 0.75, or 1.5 mg/kg lewisite. Body weights of the male rats were recorded during treatment and subsequent mating. At the end of the mating period, males were sacrificed and the left testis and brain were weighed.[1] Epididymal sperm was evaluated for motility and morphology. In addition, testes were examined microscopically. Student's t-test was used for statistical comparison with control data, and regression analysis was used to assess dose-response relationship. The proportion of females with one or more dead implants was evaluated by the χ^2 test.

Animals of all dose groups survived until scheduled sacrifice (Bucci et al., 1993). Body weights of treated males did not differ from controls during treatment or the subsequent mating period. There were no treatment-related or statistically significant effects on the number or percent of pregnancies, total implants, live or dead fetuses, or early, late, or total resorptions. Furthermore, sperm morphology, testes weight, seminiferous tubule diameter, and testes histopathology did not differ between treated and untreated males; however, it should be noted that these assessments occurred 10 weeks after the end of exposure. Sperm motility was significantly higher in the rats exposed to 1.5 mg/kg-day lewisite, but this effect is not likely to be toxicologically meaningful.

[1]The methods section in Bucci et al. (1993) indicated that the epididymis, seminal vesicles, prostate, and pituitary gland were also weighed, but the results of these organ weights were neither reported nor discussed.

Table 4A. Summary of Lewisite Genotoxicity

Endpoint	Test System	Dose/ Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
Genotoxicity studies in prokaryotic organisms						
Mutation	*S. typhimurium* TA97, TA98, TA100, TA102	1 or 5 µg/plate	−	−	Both plate incorporation and preincubation assays performed. Cytotoxicity observed at higher doses, but nontoxic doses were also tested, so the study is considered adequate.	U.S. Army (1989b)
Genotoxicity studies in mammalian cells—in vitro						
Mutation	CHO cells	2 µM	−	−	Cytotoxicity observed at higher doses, but nontoxic doses were also tested, so the study is considered adequate. Cell survival was ~30% at 1 µM in the absence of S9 and ~100% in the presence of S9.	U.S. Army (1989a)
SCEs	CHO cells	1 µM	−	−		
CAs	CHO cells	0.5 µM	+	−		
Genotoxicity studies—in vivo						
Dominant lethal	CD rats	0, 0.375, 0.75, 1.5 mg/kg	−	NA	Groups of 20 male rats dosed daily by gavage for 5 d and then mated to 2 females/wk for the next 10 wk.	Bucci et al. (1993)
Mutation (sex-linked recessive lethal)	*D. melanogaster*	NR	−	NA		Auerbach and Robson (1947)

[a]Lowest effective dose for positive results, highest dose tested for negative results.
[b]+ = positive; − = negative.

CHO = Chinese hamster ovary (cell line cells); NA = not available; NR = not reported.

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Acute studies	Dogs (breed not reported) were exposed to lewisite vapor at concentrations of 139−384 mg/m^3 for 10 or 30 min in a study assessing the efficacy of BAL treatment. Mortality over 7 d follow-up was assessed and survivors were sacrificed ≥7 d after exposure for gross inspection of the larynx, trachea, and lungs.	22/27 dogs exposed to 0.139 mg/L for 30 min died, most within the first 48 hr. 8/10 dogs exposed to 0.143 mg/L for 30 min died. 4/6 dogs exposed to 0.384 mg/L for 10 min died. Signs of toxicity included retching, vomiting, urination, defecation, respiratory distress, and marked salivation. The cause of early deaths was generally respiratory obstruction; dogs dying later, and some surviving dogs, showed signs of pneumonic consolidation. Treatment with BAL reduced mortality.	Concentrations ≥0.139 mg/L (139 mg/m^3) for 30 min or 0.384 mg/L (384 mg/m^3) for 10 min are acutely lethal to dogs.	Harrison and Ordway (1946)
Acute studies other than oral/inhalation	In a study of skin decontaminants, 4,671 men received dermal applications of 50−400 µg lewisite. The application site was examined 48 hr later and the occurrence and size of blisters and erythemas were recorded.	Results were not reported by dose of lewisite. Across all doses, blisters were evident on skin of 4,331 men treated with lewisite; erythemas were evident in 4,568 men.	Dermal exposure to lewisite at doses between 50 and 400 µg may cause blisters and erythemas.	Thomson et al. (1947)
	Groups of 3 male Dutch rabbits were treated percutaneously with neat lewisite for 6 hr for estimation of the dermal LD_{50}. Animals were observed for up to 30 d. Separate groups of 9 rabbits were treated percutaneously with 10, 14, 20, or 28 mg/kg lewisite and observed for 28 d for comparison with exposed rabbits subsequently treated with chelation therapy.	Authors estimated an LD_{50} of 5.3 mg/kg (95% CI of 3.5−8.5). Deaths typically occurred between 1 and 2 d after exposure. Survivors exhibited significant weight loss over the observation period. Among rabbits treated with 10 mg/kg lewisite, 5/9 had died by 1 d after treatment and 9/9 were dead by 7 d after treatment. Rabbits treated with 10 mg/kg lewisite exhibited focal hepatocellular degeneration, transmural necrosis of the gallbladder, and focal mucosal necrosis of the duodenum. In addition, small bile duct proliferation associated with early portal tract fibrosis was observed in some rabbits (incidence not reported). More severe effects, in addition to sinusoidal congestion of the spleen, kidney congestion, and venous congestion of the lungs, were seen in rabbits receiving doses >10 mg/kg.	The percutaneous LD_{50} in male rabbits is 5.3 mg/kg (95% CI 3.5−8.5). Chelation therapy reduced the severity and extent of pathologic changes seen with percutaneous lewisite exposure.	Inns and Rice (1993)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Acute studies other than oral/inhalation	Groups of 5 male New Zealand white rabbits were given i.v. injections of 1.4, 1.8, 2.0, or 2.4 mg/kg lewisite in propylene glycol. Survivors were sacrificed on D 7; the liver, heart, spleen, kidneys, lungs, stomach, duodenum, and gall bladder were examined microscopically.	Mortality incidences were 0/5, 2/5, 3/5, and 5/5 at 1.4, 1.7, 2.0, and 2.4 mg/kg. Average time to death was 4 hr; all deaths occurred within the first 21 hr. Survivors lost weight for 1−2 d. Histopathology findings in decedents included pulmonary edema and hemorrhage accompanied by lymphocyte infiltration. The biliary epithelium was necrotic in most cases, and adjacent hepatocytes were swollen and sometimes necrotic. 1 animal also exhibited small areas of epithelial necrosis in the duodenum, as well as nuclear debris beneath the epithelium.	The i.v. LD_{50} in male rabbits was 1.8 mg/kg (95% CI 1.6−2.1). Effects were compared with those of sodium arsenite, which had a much higher LD_{50} (7.6 mg/kg) and resulted in histological changes primarily in the kidneys.	Inns et al. (1990); Inns et al. (1988)
	Groups of 3 male New Zealand white rabbits were given i.v. injections of 0.5 mg/kg lewisite in propylene glycol. Groups were sacrificed 6 and 24 hr after dosing for histological examination (organs not specified) and comparison with rabbits treated with sodium arsenite.	Lungs exhibited patchy alveolar edema and hemorrhage; in addition, 2 of the 3 rabbits showed gall bladder damage (inflammatory cell infiltration and/or frank necrosis). Sodium arsenite exposure did not result in histological changes.	Histological changes in the lungs and gall bladder were observed after exposure of rabbits to an LD_{10} dose of lewisite (0.5 mg/kg) but not after an LD_{10} dose of sodium arsenite.	Inns et al. (1988)
	Groups of 12 or 18 male New Zealand white rabbits received single subcutaneous doses of 29.7 µmol/kg lewisite in a study of chelation therapies. Mortality was recorded.	Mortality across both groups was 29/30 rabbits. No other information was provided.	A subcutaneous dose of 29.7 µmol/kg (6.16 mg/kg) lewisite is lethal to rabbits.	Aposhian et al. (1982)
	Lewisite in absolute ethanol was injected subcutaneously into groups of 8 male New Zealand rabbits to obtain lethality data for subsequent pharmacokinetic study. Doses between 0.5 and 5.0 mg/kg were tested; rabbits were observed for 14 d.	The following mortality incidences were observed at doses of 2.0, 2.4, 2.9, 3.5, 4.2, and 5.0 mg/kg: 1/24, 1/16, 3/16, 8/16, 8/16, and 19/24, respectively.	The subcutaneous LD_{50} in male rabbits is 3.79 (95% CI 3.44−4.25) mg/kg.	Snider et al. (1990); U.S. Army (1987)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Acute studies other than oral/inhalation	Groups of 8 female domestic white pigs were exposed (skin only) to saturated lewisite vapor via inverted chambers attached to shaved skin for 6 hr; authors estimated total dose as 0.3 mg/cm^2. Skin injury at various time points after exposure was evaluated by histopathology examination (Rice and Brown, 1999), laser Doppler imaging (Brown et al., 1998), or erythema, skin brightness, skin blueness (cyanosis), and TEWL (Chilcott et al., 2000).	Pig skin exposed to saturated lewisite vapor showed acute inflammation as early as 1 hr after exposure, with coagulative necrosis of the epidermis and papillary dermis by 24 hr after exposure, and necrosis extending into subcutaneous connective and adipose tissue by 48 hr. Laser Doppler imaging and other measurement techniques were useful for clinical evaluation of injury.	Exposure of pig skin to saturated lewisite vapors for 6 hr rapidly causes severe damage to the skin.	Chilcott et al. (2000); Rice and Brown (1999); Brown et al. (1998)
	Groups of 5 SKH-1 hairless mice were exposed (skin only) to saturated lewisite vapor via inverted chambers for 8 min, and subsequently treated with BAL, DMSA, or left untreated. Skin damage was evaluated by macroscopic assessment, skin color measurement, TEWL measured, and histopathology.	Treatment with lewisite vapor resulted in increased redness, decreased brightness, and increased TEWL (statistical analysis not reported). Histopathology findings in lewisite-exposed skin included severe necrosis of the epidermis and upper dermis, alteration of dermis fibers, and massive dermal neutrophil infiltration.	Exposure of mouse skin to saturated lewisite vapors for 8 min causes damage to the skin.	Mouret et al. (2013)
	Groups of 5 male athymic SKH-1 hairless mice were exposed (skin only) to saturated lewisite vapor via inverted chambers for 8 min. Skin damage was evaluated at various time points up to 21 d after exposure by macroscopic assessment, skin color measurement, TEWL, skin elasticity, and histopathology.	TEWL was strongly correlated with macroscopic and histopathology changes over time. Microscopic examination of skin biopsies showed inflammatory cell infiltration and microvesications by D 1 postexposure, with wound closure occurring by D 21.	TEWL measurement provided the best index to track progression of the lesions.	Nguon et al. (2014)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/toxicokinetic				
ADME	Groups of 100 male New Zealand white rabbits received a subcutaneous injection of 2.4 or 3.5 mg/kg lewisite in absolute ethanol; half of the animals were subsequently treated with BAL. Groups of 5 rabbits were sacrificed at 4, 12, 24, 48, and 96 hr after dosing for analysis of arsenic in blood, brain, spinal cord, liver, kidney, fat, testes, lung, injection site skin, and adjacent normal skin.	The blood concentrations of arsenic in lewisite-treated rabbits were highest at the first time point (4 hr postdosing) and declined gradually. Apart from injection site skin, the highest tissue concentrations were in the liver, lung, and kidney, which equilibrated rapidly with blood (peak observed 4−12 hr after dosing; average tissue:blood partition coefficients based on areas under the curve ranged from 7.41−14.50). Brain, spinal cord, and testes tissue concentrations increased between 4 and 96 hr, reflecting slow movement across the blood:tissue barriers (average tissue:blood partition coefficients from 0.59−1.76). Skin and fat exhibited low affinity for arsenic (average tissue:blood partition coefficients from 0.18−2.17).	Clearance of arsenic from blood was estimated to be 112−129 mL/min/kg depending on dose. Volume of distribution was 7.67−12.7 L/kg, reflecting wide distribution. The half-life of arsenic in blood (terminal phase) was 54.7−75 hr. Large difference between arsenic content of injection-site skin and blood suggests that arsenic migration from skin is diffusion rate limited. BAL treatment decreased arsenic tissue-to-blood partitioning and increased clearance of arsenic from blood.	Snider et al. (1990); U.S. Army (1987)
	In a report of a new analytical method for measuring exposure to lewisite, 4 guinea pigs (strain and sex not reported) received a subcutaneous dose of 0.25 mg/kg lewisite. Urine was collected hourly after exposure and analyzed for CVAA. One guinea pig was sacrificed after 24, 72, and 240 hr postexposure; blood was collected and analyzed for the CVAA. One guinea pig was sacrificed after 48 hr for analysis of CVAA in whole blood, globin, and dialyzed globin.	CVAA was detected in urine during the first 12 hr after exposure; by determining bound and unbound CVAA in blood; exposure was detectable 240 hr after exposure.	Exposure to lewisite can be monitored by measuring hemoglobin-bound and unbound CVAA in blood.	Fidder et al. (2000)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
ADME	Groups of 5 male New Zealand white rabbits were given i.v. injections of 0.5 mg/kg lewisite in propylene glycol or sodium arsenite. Survivors were sacrificed on D 7; arsenic in liver and kidney was quantified.	Arsenic concentrations in liver and kidney were 4 µg/g (95% CL ± 1.7) and 5.5 µg/g (±1.4), respectively. Comparable exposure to sodium arsenite yielded 2−5-fold higher liver and kidney concentrations of arsenic than lewisite.	Exposure to lewisite results in arsenic deposition in the liver and kidney.	Inns et al. (1988)
	Groups of 3 male New Zealand white rabbits were given i.v. injections of 1.5 mg/kg lewisite in propylene glycol and sacrificed after 10 min, 30 min, or 2, 6, 24, or 72 hr for measurement of arsenic in lung, liver, kidney, duodenum, stomach, bladder, and brain.	Peak arsenic concentrations in lung, liver, kidney, duodenum, stomach, bladder, and brain were 10.2, 2.7, 2.7, 2.3, 0.7, 0.5, and 0.1 µg/g, respectively. Concentration in the lung peaked at 10 min; those in the liver, kidney, duodenum and stomach peaked at 30 min; and those in the bladder and brain peaked at 6 and 24 hr, respectively.	Arsenic is widely distributed in the body after i.v. exposure to lewisite.	Inns et al. (1988)
	Human blood was exposed to ^{14}C-lewisite at concentrations from 20−0.2 mM for 6 hr. Globin and albumin were isolated and radioactivity measured.	90% of radioactivity in blood was found in erythrocytes, and 25−50% of the radioactivity was associated with globin. No binding to albumin was detected.	The study authors suggested that localization to erythrocytes reflected binding to glutathione, which is present in high levels in erythrocytes.	Fidder et al. (2000)
	Porcine skin flaps were exposed to undiluted lewisite (0.1−150 µL) for 10 min in a Franz cell. Arsenic contents in the skin and receptor medium were measured.	Dose-dependent increases in the arsenic concentration in the skin and medium were reported; the maximum arsenic concentration in skin was observed at 50 µL. Postexposure treatment with decontaminants decreased the arsenic content of the skin and medium.	Lewisite or its arsenic-containing degradation product traverses excised porcine skin in culture.	Haeser et al. (1997)

Table 4B. Other Studies				
Test	**Materials and Methods**	**Results**	**Conclusions**	**References**
Mode of action/mechanistic				
Cell metabolism	Primary human keratinocytes and cells from a keratinocyte-derived line (SCLII) were exposed to 60 μM lewisite for 5 min. For 6 hr after exposure, glucose consumption, lactate production, intracellular ATP content, and tetrazolium reduction (a measure of mitochondrial dehydrogenase activity) were measured, as was lactate dehydrogenase in the supernatant.	Exposure of keratinocytes to lewisite reduced glucose consumption and lactate formation, inhibited hexokinase activity, increased leakage of lactate dehydrogenase, and decreased ATP content.	Lewisite interferes with cell metabolism in keratinocytes in culture.	Kehe et al. (2001)
Skin exposure to lewisite vapor	Yucatan minipigs were exposed (skin only) to saturated lewisite vapor via inverted chambers attached to shaved skin for 24 hr. Exposed skin tissue was excised and evaluated for glycoproteins known to mediate dermo-epidermal attachment (laminin and Type IV collagen).	Degradation of laminin and Type IV collagen, but not Type III collagen, was observed in treated pig skin.	Study authors suggested that damage to laminin and collagen may mediate dermo-epidermal separation seen after vesicant exposure.	Lindsay et al. (2004)
	Groups of 5 male athymic SKH-1 hairless mice were exposed (skin only) to saturated lewisite vapor via inverted chambers for 8 min. Inflammatory cytokines and chemokines in skin were evaluated.	The following cytokines were rapidly upregulated (within 6 hr after exposure): IL-6, CXCL1, CXCL2, and CCL9. In addition, 1 matrix metalloproteinases (MMP-2 and MMP-9) were also upregulated. Expression of MMP-9 was selectively upregulated after exposure. Downregulated cytokines included IL-1ra, IL-1α, and IL-1β.	Study authors suggested that MMP-9 could be effector of vesication process.	Nguon et al. (2014)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Cytotoxicity	Human leukocytes in culture were exposed to lewisite for varying durations up to 20 hr. Flow cytometry was used to evaluate cytotoxicity markers.	At a concentration of $\geq 3 \times 10^{-5}$ M for 1 hr, lewisite reduced cell survival to 20% of control during the first 4 hr after exposure. Exposure to lewisite resulted in a time-and dose-dependent increase in the binding of cytotoxicity markers including annexin V, viaprobe, and propidium iodide.	Cells exposed to lewisite entered the necrotic cell death pathway as early as the first hour after exposure.	Meier (2003); Meier (2004); Meier et al. (1993)

ADME = adsorption, distribution, metabolism, elimination; ATP = adenosine triphosphate; BAL = British Anti-Lewisite; CI = confidence interval; CVAA = cold water vapor atomic absorption; DMSA = meso-2,3-dimercaptosuccinic acid; i.v. = intravenous; LD_{50} = median lethal dose; TEWL = transepidermal water loss.

Acute Toxicity Studies

The acute lethality of lewisite has been estimated in animals exposed via inhalation, oral, dermal, intravenous (i.v.), and subcutaneous routes. Table 5 shows acute lethality data across species and exposure routes. A complete review of the acute lethality and toxicity of lewisite is available in NRC (2013).

Table 5. Acute Lethality Estimates for Lewisite

Exposure Route	Species	Exposure Duration	LC_{50} (mg/m^3) or LD_{50} (mg/kg)	Reference
Inhalation	Rat	9 min	166 mg/m^3	Gates, 1946 as cited in NRC (2013)
	Mouse	10 min	190−200 mg/m^3	Silver and McGrath, 1943 as cited in NRC (2013)
	Guinea pig	9 min	111 mg/m^3	Gates, 1946 as cited in NRC (2013)
		60 min	8 mg/m^3	
	Rabbit	7.5 min	160 mg/m^3	
		60 min	25 mg/m^3	
	Goat	100 min	12.5 mg/m^3	
	Dog	7.5 min	176 mg/m^3	Armstrong, 1923 as cited in NRC (2013)
		15 min	100 mg/m^3	
		30 min	48 mg/m^3	
		60 min	25.4 mg/m^3	
		120 min	11.8 mg/m^3	
		240 min	6.24 mg/m^3	
Oral	Rat	NA	50 mg/kg	U.S. Army, 1974 as cited in NRC (2013)
Dermal	Rabbit	6 hr	5.3 mg/kg (95% CI 3.5−8.5 mg/kg)	Inns and Rice (1993)
	Rat	NR	24 mg/kg	Cameron et al. (1946)
	Guinea pig		12 mg/kg	
	Rabbit		6 mg/kg	
	Dog		15 mg/kg	
	Goat		15 mg/kg	
Subcutaneous	Rat	NA	1 mg/kg	
	Guinea pig		1 mg/kg	
	Rabbit		2 mg/kg	
	Dog		2 mg/kg	
	Rabbit		3.79 mg/kg (05% CI 3.44−4.25 mg/kg)	Snider et al. (1990); U.S. Army (1987)
i.v.	Rabbit	NA	0.5 mg/kg	Cameron et al. (1946)
			1.8 mg/kg (95% CI 1.6−2.1 mg/kg)	Inns et al. (1990); Inns et al. (1988)

i.v. = intravenous; NA = not applicable; NR = not reported

As indicated in Table 5, inhalation of lewisite vapor at concentrations as low as 8 mg/m^3 for 1 hour can be lethal in some species [reviewed by NRC (2013)]. The cause of death in exposed animals is generally respiratory obstruction, with necropsy findings consisting of pulmonary congestion, edema, and hemorrhage, accompanied by inflammation of the entire respiratory tract [reviewed by NRC (2013); see also Harrison and Durlacher (1946)].

The available literature included a single oral median lethal dose (LD_{50}) estimate of 50 mg/kg in rats [U.S. Army (1974) as cited in NRC (2013)]; the primary document is not available for review, and the secondary source provided no additional details beyond the LD_{50} estimate.

Dermal exposure to neat lewisite is lethal to rabbits; Inns and Rice (1993) estimated the LD_{50} to be 5.3 mg/kg (95% confidence interval [CI] of 3.5−8.5 mg/kg). The rabbits in the study showed a profound dose-related weight loss, with some animals losing as much as 72% of initial weight during the 14-day observation period; however, the degree of weight loss was similar in animals that died and those that survived. There were no pathologic effects on the lungs; instead, the animals that died exhibited focal hepatocellular degeneration as well as transmural necrosis of the gallbladder and focal mucosal necrosis of the duodenum. Liver injury was also seen in survivors, in the form of bile duct proliferation and early portal tract fibrosis, with some histopathology findings mimicking cirrhosis (Inns and Rice, 1993).

The localized effects of acute exposure to lewisite (as a liquid or saturated vapor) on skin and eyes of both humans and animals, as well as methods to assess and therapeutic interventions to mitigate this injury, have been well studied (Mouret et al., 2013; Sawyer and Nelson, 2008; Nelson et al., 2006; Lam et al., 2002; Chilcott et al., 2000; Rice and Brown, 1999; Brown et al., 1998; Olajos et al., 1998; Mitcheltree et al., 1989; Hughes, 1947; Thomson et al., 1947; Cameron et al., 1946; Hughes, 1946; Mann et al., 1946). Briefly, direct contact of skin or eyes with lewisite is highly irritating. On skin, depending on the exposure concentration and time since exposure, erythema and edema or burning and blistering are observed [reviewed by Goldman and Dacre (1989)]. Similarly, in the eye, edema and ulceration of the epithelial surfaces may occur shortly after exposure, followed by corneal damage or destruction. It has been estimated that skin vesication and serious corneal damage would occur with lewisite exposure of 1.5 mg/minute/L (equivalent to 1,500 mg/m^3 for 1 minute or 1.5 mg/m^3 for ~17 hours) [reviewed by Goldman and Dacre (1989)].

In rabbits exposed intravenously (1.4−2.4 mg/kg lewisite) in a study of acute lethality and chelation therapy, the lung was the primary target organ, and the cause of death was severe lung damage as shown by pulmonary edema and hemorrhage accompanied by lymphocyte infiltration (Inns et al., 1990; Inns et al., 1988). Perivascular edema and moderate venous congestion, as well as necrosis in the epithelium of the gall bladder and duodenal mucosa, were also seen in decedents. Animals that survived showed multifocal alveolar hemorrhage and lymphocyte infiltration (without pulmonary edema) in addition to liver damage (bile duct proliferation and portal tract fibrosis) and regeneration in the gall bladder and duodenal epithelia (Inns et al., 1990; Inns et al., 1988).

Metabolism/Toxicokinetic Studies

The toxicokinetic behavior of lewisite has been studied in rabbits exposed intravenously (Snider et al., 1990; U.S. Army, 1987), but there are few data on the absorption, distribution,

metabolism, and excretion after exposure via other routes. Reviews suggest that lewisite penetrates skin readily due to its lipophilicity [reviewed by SERDP (1997); Pechura (1993)].

However, as discussed in the introduction, lewisite itself is unstable, so the conditions of exposure will markedly affect the chemical species that contact the body and the disposition of the compounds in the body. For example, lewisite in contact with moist surfaces may undergo hydrolysis to CVAA. Further, under acidic conditions such as in the stomach, lewisite is hydrolyzed to CVAA and hydrochloric acid, and further to lewisite oxide (2-chlorovinyl arsenous oxide) [reviewed by Munro et al. (1999)]. The chemical compound(s) responsible for the vesicant effects of lewisite are not known.

In rabbits exposed to lewisite via i.v. or subcutaneous injection, the highest tissue concentrations (apart from the injection site) were in the liver, lung, and kidney (Snider et al., 1990; Inns et al., 1988; U.S. Army, 1987). Brain, spinal cord, and testes tissue concentrations increased over time, reflecting slow movement across the blood-tissue barriers, and skin and fat exhibited low affinity for arsenic (Snider et al., 1990; U.S. Army, 1987). Arsenic is widely distributed in the body of the rabbits exposed via i.v. or subcutaneous injection (Snider et al., 1990; Inns et al., 1988; U.S. Army, 1987). In the study using subcutaneous exposure (Snider et al., 1990; U.S. Army, 1987) estimated the volume of distribution to be 7.67−12.7 L/kg; clearance of arsenic from blood was estimated to be 112−129 mL/minute/kg, and the half-life (terminal phase) was 54.7−75 hours.

Mode-of-Action/Mechanistic Studies

Acute exposure to high levels of lewisite via dermal or i.v. exposure is believed to result in increased capillary permeability in the skin or lungs (respectively), leading to loss of blood plasma and the characteristic "lewisite shock," a sequence of events mimicking shock in burn victims [reviewed by NRC (2013); Goldman and Dacre (1989)]. Perturbation of osmotic equilibrium can result in the dysfunction of numerous biological system including the lungs, kidneys, cardiovascular, and lymphatic systems.

A comparison of the pathology seen in acute lethality studies of lewisite in rabbits exposed percutaneously (Inns and Rice, 1993) or intravenously (Inns et al., 1990; Inns et al., 1988) shows marked route differences in effect: the liver and gall bladder are the primary targets after dermal exposure, while the lung is primarily affected after i.v. exposure. It has been suggested that the primary injury is to the first capillary bed encountered as lewisite is transported through the body (Inns and Rice, 1993); this would suggest that the liver and gall bladder would be most affected after oral or dermal exposure, while the lung would be affected after inhalation or i.v. exposure.

Lewisite and other arsenicals bind strongly to sulfhydryl groups of functionally important proteins and thiol cofactors, forming stable complexes with critical proteins and enzymes, such as dihydrolipoic acid, keratin, alcohol dehydrogenase, pyruvate dehydrogenase, succinic dehydrogenase, succinic oxidase, and hexokinase [reviewed by NRC (2013); McManus and Huebner (2005); Pechura (1993)]. Inactivation of critical enzymes disrupts cell metabolism. These effects coupled with cell membrane damage lead to cell death and tissue necrosis.

Evidence for disruption of cell metabolism and membrane damage comes from in vitro studies in which exposure to lewisite reduced glucose consumption and lactate formation,

inhibited hexokinase activity, increased leakage of lactate dehydrogenase, and decreased adenosine triphosphate (ATP) content (Kehe et al., 2001; Flohe et al., 1996). Treatment with the chelating agents meso-2,3-dimercaptosuccinic acid (DMSA) or 2,3-dimercapto-1-propane-sulphonic acid (DMPS) immediately after lewisite exposure prevented effects on glucose, lactate, and lactate dehydrogenase (Kehe et al., 2001). Incubation of lewisite (60−600 μmol/L) for 5 minutes with pure hexokinase, a key enzyme in glucose metabolism, was shown to result in inhibition (40−100% inhibited) of hexokinase activity (Flohe et al., 1996). Decreases in glucose utilization and increased lactate dehydrogenase leakage were noted in isolated perfused porcine skin flaps exposed to lewisite concentrations ranging from 0.07−5.0 mg/mL (King et al., 1992; Monteiro-Riviere et al., 1990).

Several studies have demonstrated the efficacy of chelation therapy (British Anti Lewisite [BAL] or dimercaprol; DMSA; and DMPS) in mitigating the lethal effects of lewisite (Inns and Rice, 1993; Inns et al., 1990; Aposhian et al., 1984; Aposhian et al., 1982; Harrison and Ordway, 1946). Chelating agents have also been shown to be effective against lewisite-induced skin and eye injury in humans and rabbits (Mouret et al., 2013; Hughes, 1947; Thomson et al., 1947; Hughes, 1946). The chelating agents may reduce absorption of lewisite and/or may inhibit the interaction of lewisite with key thiol-containing macromolecules.

Limited data are available on the toxicity of lewisite degradation products and impurities. Based primarily on lethality data comparisons, reviews have suggested that the impurities known as lewisite-2 and lewisite-3 are of similar or lower toxicity than lewisite-1 (NRC, 2013; Munro et al., 1999; Lindberg et al., 1997).

DERIVATION OF PROVISIONAL VALUES

Tables 6 and 7 present summaries of the derived noncancer and cancer references values, respectively.

Table 6. Summary of Noncancer Reference Values for Lewisite (CASRN 541-25-3)

Toxicity Type (units)	Species/Sex	Critical Effect	p-Reference Value	POD Method	POD	UF_C	Principal Study
Subchronic p-RfD (mg/kg-d)	Rat/females	Mortality	5×10^{-6}	$BMDL_{01}$	0.0049	1,000	Sasser et al., 1996; Sasser et al., 1989a
Chronic p-RfD (mg/kg-d)	Rat/females	Mortality	5×10^{-6}	$BMDL_{01}$	0.0049	1,000	Sasser et al., 1996; Sasser et al., 1989a
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

BMDL = benchmark dose lower confidence limit; NDr = not determined.

Table 7. Summary of Cancer Reference Values for Lewisite (CASRN 541-25-3)

Toxicity Type	Species/Sex	Tumor Type	Cancer Value	Principal Study
p-OSF	NDr			
p-IUR	NDr			

NDr = not determined

DERIVATION OF ORAL REFERENCE DOSES

Information on the effects of oral exposure to lewisite is available from a subchronic-duration study in rats (Sasser et al., 1996; Sasser et al., 1989a), a two-generation reproductive toxicity study of rats (each generation exposed for 23 weeks) (Sasser et al., 1999; Sasser et al., 1989b), and developmental toxicity studies in rats and rabbits (Hackett et al., 1992; Hackett et al., 1987). A study of drinking water exposure (U.S. Army, 1941) is not considered suitable due to the rapid hydrolysis of lewisite in water and the lack of analytical data on the test solutions used. In addition, a dominant lethal toxicity study in male rats (Bucci et al., 1993) was not considered usable because the exposure duration was only 5 days. All of the potential key studies administered lewisite in sesame oil via intragastric intubation at adjusted daily doses ranging from 0.007−2.5 mg/kg-day. LOAELs were not identified in any of the potential key studies; the only effect levels identified were FELs for mortality (see Table 3A). FELs based on mortality were identified as low as 0.07 mg/kg-day in rabbits exposed during gestation (in the main developmental toxicity study) and in rats in the two-generation reproductive study. In the subchronic-duration study of rats, the FEL was 0.36 mg/kg-day for mortality; in the developmental dose-range-finding study in rats, the FEL for mortality was 2.0 mg/kg-day. In the developmental dose-range-finding study in rabbits, the lowest dose tested (0.5 mg/kg-day) was

considered a FEL based on gastric hemorrhage in does. High mortality at this dose was attributed entirely to dosing trauma by the study authors, but there is some uncertainty regarding the possible contribution of toxicity to these deaths; deaths attributed to lewisite toxicity due to gastric hemorrhage occurred at the next higher dose of 1.0 mg/kg-day. No deaths were seen in rats exposed to 0.071 or 0.0071 mg/kg-day in the subchronic-duration study, or in the preliminary and main developmental toxicity studies of rats at doses up to 0.5 and 1.5 mg/kg-day, respectively.

Deaths in the subchronic-duration and two-generation reproductive toxicity studies in rats were attributed to severe inflammatory lesions of the respiratory tract; the authors postulated that these lesions resulted from induced regurgitation or accidental deposition of lewisite into the pharynx and subsequent aspiration (Sasser et al., 1999; Sasser et al., 1996; Sasser et al., 1989a, b). The location of the lesions (posterior nasal passages, larynx, trachea, and large intrapulmonary airways) was consistent with this hypothesis; the terminal airways and parenchyma were less frequently affected. In contrast, deaths in the developmental toxicity studies in both rats and rabbits (Hackett et al., 1992; Hackett et al., 1987) were attributed to inflammatory lesions and hemorrhage in the stomach. However, there is some uncertainty in the attribution of deaths in this study because deaths associated with damage to tissues of the respiratory tract during dosing were apparently automatically categorized as dosing trauma without consideration of possible toxicity. The authors acknowledged that assignment of a "probable cause of death" to individual animals was often difficult and may appear to be arbitrary in some cases.

The mode of administration in all of the pertinent studies (Sasser et al., 1999; Sasser et al., 1996; Sasser et al., 1992; Sasser et al., 1989a, b) was intragastric intubation of a bolus dose of lewisite. This exposure route leads to direct contact of stomach tissues to high concentrations of lewisite. Hackett et al. (1987) suggested that the higher mortality of rabbits (compared to rats) in the developmental toxicity study may have been partly due to the higher test material concentrations (and consequent stronger vesicant effect on the gastric tissues in contact with the administered solution) in the solutions administered to rabbits than in rats. However, comparison of FELs on the basis of measured lewisite concentration in the test solution does not support this hypothesis, as rabbit deaths were seen at a lower measured concentration of lewisite (0.11−0.22 mg/mL) than the concentration that yielded no rat deaths (0.47 mg/mL). The study authors also suggested that use of a 22-inch feeding tube for the rabbit studies, rather than the dosing needle used for the rat studies, may have contributed to the higher number of dosing trauma-related deaths in the rabbit studies.

The lowest effect levels in any study were freestanding FELs of 0.07 mg/kg-day in the two-generation study of rats (Sasser et al., 1999; Sasser et al., 1989b) and the developmental toxicity study in rabbits (Hackett et al., 1992; Hackett et al., 1987). However, benchmark dose modelling of the dose-response curves is the favored approach, and this yields several alternative PODs (see Table 8 below).

The subchronic study in rats is selected as the principal study for deriving a subchronic p-RfD for lewisite (Hackett et al., 1992; Hackett et al., 1987). Based on the available toxicity data for lewisite, it is apparent that mortality is a common effect of oral exposure (see Table 3A). As noted previously, the lowest effect levels in any study of lewisite (see Table 3A) were freestanding frank effect levels (FELs) of 0.07 mg/kg-day for mortality and stomach and/or

respiratory tract lesions in the two-generation study of rats (Sasser et al., 1999; Sasser et al., 1989b) and the developmental toxicity study in rabbits (Hackett et al., 1992; Hackett et al., 1987). Examining the PODs calculated from the lewisite database as a whole (see Table 8), only one dose lower than the FEL of 0.07 mg/kg-day is available; the subchronic-duration study of rats (Sasser et al., 1996; Sasser et al., 1989b) included a group exposed to 0.007 mg/kg-day in which no deaths or other adverse effects were seen (see Table 3A). Data sets for mortality from the available toxicity studies for lewisite were selected to derive potential PODs via BMD modeling (see Table 8, and Appendix C for model outputs). The dose-range-finding studies in rats and rabbits (Hackett et al., 1992; Hackett et al., 1987) were not considered for subchronic p-RfD derivation because there are more comprehensive, definitive studies that tested more animals (Hackett et al., 1992; Hackett et al., 1987). Confidence in the rabbit data is reduced because of uncertainties in the attribution of mortalities (e.g., dosing trauma) and the short duration of exposure. BMDs and BMDLs from the best fitting models for the selected dichotomous data sets are presented in Table 8.

Table 8. Possible Subchronic PODs for Lewisite[a]

Effect	Sex/Species	Duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	$BMDL_{01}$	Study
Mortality	Males/Rat	Subchronic 13 wk	0.071	0.36	NF	Sasser et al. (1996); Sasser et al. (1989a)
Mortality	**Females/Rat**	**Subchronic 13 wk**	**0.071**	**0.36**	**0.0049**	**Sasser et al. (1996); Sasser et al. (1989a)**
Mortality	F0 Females/Rat	Repro 23 wk	0.076	0.19	0.007	Sasser et al. (1996); Sasser et al. (1989a)
Mortality	F0 Males/Rat	Repro 23 wk	0.18	0.43	0.0155	Sasser et al. (1996); Sasser et al. (1989a)
Mortality	F1 Males/Rat	Repro 23 wk	NDr	0.071	0.0069	Sasser et al. (1999); Sasser et al. (1989b)
Mortality	F1 Females/Rat	Repro 23 wk	NDr	0.076	0.0052	Sasser et al. (1999); Sasser et al. (1989b)
Mortality	Females/Rat	Developmental Range Finding 10 d	0.5	2.0	Not run	Hackett et al. (1987); Hackett et al. (1992)
Mortality	Females/Rabbit	Developmental Range Finding 14 d	NDr	0.5	Not run	Hackett et al. (1987); Hackett et al. (1992)
Mortality	Females/Rabbit	Developmental Range Finding 14 d	NDr	0.07	0.002	Hackett et al. (1987); Hackett et al. (1992)

[a]NDr = not determinable; NF = no fit.

Derivation of a Subchronic p-RfD

The $BMDL_{01}$ of 0.0049 mg/kg-day in rats exposed to lewisite in the subchronic study (Sasser et al., 1996; Sasser et al., 1989a) was selected as the POD for the subchronic p-RfD derivation. The critical effect for the subchronic p-RfD is mortality due to lesions triggered by

the test chemical. These lesions reflect portal-of-entry effects. Because available dosimetric scaling approaches (body weight and skin surface area scaling) may not be appropriate for portal-of-entry effects, a dosimetric adjustment of the POD was not used, following EPA guidance (U.S. EPA, 2011b). The subchronic p-RfD for lewisite was derived as follows:

$$\textbf{Subchronic p-RfD} = BMDL_{01} \div UF_C$$
$$= 0.0049 \text{ mg/kg-day} \div 1{,}000$$
$$= \mathbf{5 \times 10^{-6} \text{ mg/kg-day}}$$

Table 9 summarizes the uncertainty factors (UFs) for the subchronic p-RfD for lewisite.

Table 9. Uncertainty Factors for the Subchronic p-RfD for Lewisite

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied to account for uncertainty in extrapolating from animals to humans, in the absence of information to assess species differences in toxicokinetic and toxicodynamic characteristics of lewisite and in the absence of a rationale to support use of HED for a POD based on portal-of-entry effects.
UF_H	10	A UF_H of 10 is applied to account for human variability in susceptibility, in the absence of information to assess toxicokinetic and toxicodynamic variability of lewisite in humans.
UF_D	10	A UF_D of 10 is applied to account for deficiencies and uncertainties in the database, especially the lack of identification of a critical effect other than mortality associated with severe stomach and/or respiratory lesions and uncertainty in relative sensitivity of rabbit compared to rat.
UF_L	1	A UF_L of 1 is applied because the POD is a BMDL, not a LOAEL.
UF_S	1	A UF_S of 1 is applied because the principal study is a subchronic study.
UF_C	1,000	Composite UF = $UF_A \times UF_H \times UF_D \times UF_L \times UF_S$.

BMDL = benchmark dose lower confidence limit; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; POD = point-of-departure.

The confidence descriptors for the subchronic p-RfD are explained in Table 10.

Table 10. Confidence Descriptors for Subchronic p-RfD for Lewisite

Confidence Categories	Designation	Discussion
Confidence in study	M	The principal study included appropriate numbers of animals in exposure and control groups for meaningful statistical analyses and assessment of a wide range of toxicological endpoints (clinical signs, body weight, food consumption, hematology, serum chemistry, urinalysis, selected organ weights, and comprehensive gross and microscopic pathology). The major factor restricting confidence in the principal study is the failure to identify endpoints other than mortality.
Confidence in database	M	Confidence in the database is medium. The database for noncancer effects of lewisite consists of a subchronic study in rats, and reproductive and developmental toxicity studies in rats and rabbits. There are no pertinent human data.
Confidence in subchronic p-RfC	M	The overall confidence in the subchronic p-RfD is medium.

M = medium.

Derivation of Chronic p-RfD

The $BMDL_{01}$ of 0.0049 mg/kg-day in rats exposed to lewisite in the subchronic study (Sasser et al., 1996; Sasser et al., 1989a) was also selected as the POD for chronic p-RfD derivation. As with the subchronic p-RfD, a dosimetric adjustment of the POD was not used. A comparison of the potential PODs between the 13-week rat subchronic study and the 23-week rat reproductive study (see Table 8) reveals that there was no change in POD associated with increased duration of exposure. As mortality is the most sensitive endpoint, and the chronic (23-week) is slightly less sensitive than the subchronic (13-week) endpoint, no additional uncertainty is incorporated to account for duration for use of a subchronic study in the chronic RfD derivation.

The chronic p-RfD for lewisite is thus derived as follows:

$$\textbf{Chronic p-RfD} = BMDL_{01} \div UF_C$$
$$= 0.0049 \text{ mg/kg-day} \div 1{,}000$$
$$= \mathbf{5 \times 10^{-6} \text{ mg/kg-day}}$$

Table 11 summarizes the UFs for the chronic p-RfD for lewisite.

Table 11. Uncertainty Factors for the Chronic p-RfD for Lewisite

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied to account for uncertainty in extrapolating from animals to humans, in the absence of information to assess species differences in toxicokinetic and toxicodynamic characteristics of lewisite and in the absence of a rationale to support use of HED for a POD based on portal-of-entry effects.
UF_H	10	A UF_H of 10 is applied to account for human variability in susceptibility, in the absence of information to assess toxicokinetic and toxicodynamic variability of lewisite in humans.
UF_D	10	A UF_D of 10 is applied to account for deficiencies and uncertainties in the database, especially the lack of identification of a critical effect other than mortality associated with severe forestomach and/or respiratory lesions and uncertainty in relative sensitivity of rabbit compared to rat.
UF_L	1	A UF_L of 1 is applied because the POD is a BMDL, not a LOAEL.
UF_S	1	A UF_S of 1 is applied because there is no apparent change in POD when exposure duration increases (see Table 8).
UF_C	1,000	Composite UF = $UF_A \times UF_H \times UF_D \times UF_L \times UF_S$.

BMDL = benchmark dose lower confidence limit; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; POD = point-of-departure.

The confidence descriptors for the chronic p-RfD are explained in Table 12.

Table 12. Confidence Descriptors for Chronic p-RfD for Lewisite

Confidence Categories	Designation	Discussion
Confidence in study	M	The principal study included appropriate numbers of animals in exposure and control groups for meaningful statistical analyses and assessment of a wide range of toxicological endpoints (clinical signs, body weight, food consumption, hematology, serum chemistry, urinalysis, selected organ weights, and comprehensive gross and microscopic pathology). The major factor restricting confidence in the principal study is the failure to identify endpoints other than mortality
Confidence in database	M	Confidence in the database is medium. The database for noncancer effects of lewisite consists of a subchronic study in rats, and reproductive and developmental toxicity studies in rats and rabbits. There are no pertinent human data.
Confidence in subchronic p-RfC	M	The overall confidence in the chronic p-RfD is medium.

M = medium.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No studies of humans or animals exposed to lewisite via inhalation for >4 hours on a single day have been identified in the available literature, precluding derivation of inhalation RfCs.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Table 13 identifies the cancer weight-of-evidence (WOE) descriptor for lewisite.

Table 13. Cancer WOE Descriptor for Lewisite

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 animal studies to support this.
"Suggestive Evidence of Carcinogenic Potential"	NS	NA	There are no suitable animal studies to support this.
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	**NA**	**The available human study on the potential carcinogenicity of lewisite (Doi et al., 2011) lacks exposure information. There are no chronic-duration or carcinogenicity studies of animals exposed to lewisite.**
"Not Likely to Be Carcinogenic to Humans"	NS	NA	There are no suitable animal studies to support this.

NA = not applicable; NS = not selected.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

There are no suitable carcinogenicity studies of lewisite in humans or animals; thus, neither cancer provisional oral slope factor (p-OSF) nor provisional inhalation unit risk (p-IUR) values have been derived.

APPENDIX A. SCEENING VALUES

There are no screening values derived.

APPENDIX B. DATA TABLES

Table B-1. Mortality in Male and Female Sprague-Dawley Rats Exposed to Lewisite by Gastric Intubation for 13 Weeks[a]

Endpoint	Dose (mg/kg BW)					
	0	**0.0071**	**0.071**	**0.36**	**0.71**	**1.4**
Males	0/10	0/10	0/10	2/10	8/10[b]	3/10
Females	0/10	0/10	0/10	3/10	6/10	7/10

[a]Sasser et al. (1996); Sasser et al. (1989a)
[b]Includes one rat that died from anesthetic overdose.

Table B-2. Selected Hematology and Serum Chemistry Changes in Male and Female Sprague-Dawley Rats Exposed to Lewisite by Gastric Intubation for 13 Weeks[a]

Endpoint	Dose (mg/kg BW)					
	0	0.0071	0.071	0.36	0.71	1.4
Hematology						
Males, Week 6						
Number evaluated	10	10	10	8	8	9
Lymphocytes (× 103/µL)	12.15 ± 1.18[b]	12.05 ± 1.28	10.48 ± 0.60	11.19 ± 1.48	11.95 ± 1.11	11.99 ± 0.91
Platelets (× 10^3/µL)	786 ± 31	805 ± 39	800 ± 21	817 ± 48	764 ± 40	829 ± 35
Males, Week 13						
Number evaluated	10	10	10	8	1	7
Lymphocytes (× 10^3/µL)	8.53 ± 0.77	7.34 ± 0.71	6.02 ± 0.39	6.88 ± 0.74	5.81	5.52 ± 0.71
Platelets (× 10^3/µL)	820 ± 25	916 ± 46	849 ± 31	1,073 ± 100	802	833 ± 32
Females, Week 6						
Number evaluated	10	10	10	8	5	4
Lymphocytes (× 10^3/µL)	8.03 ± 0.53	8.27 ± 0.69	9.05 ± 0.64	9.92 ± 1.19	8.24 ± 0.81	12.03 ± 0.60*
Platelets (× 10^3/µL)	836 ± 28	762 ± 33	848 ± 26	763 ± 30	780 ± 34	964 ± 171
Females, Week 13						
Number evaluated	10	10	10	7	4	3
Lymphocytes (× 10^3/µL)	5.16 ± 0.81	4.61 ± 0.66	3.86 ± 0.52	5.80 ± 1.10	3.75 ± 1.28	5.03 ± 0.74
Platelets (× 10^3/µL)	898 ± 39	831 ± 47	966 ± 58	901 ± 94	928 ± 78	1,319 ± 164*
Serum chemistry						
Males, Week 13						
Number evaluated	10	10	10	8	2	7
Protein (g/dL)	7.4 ± 0.11	7.4 ± 0.13	7.1 ± 0.08	7.1 ± 0.14	7.1 ± 0.10	6.7 ± 0.05*
Creatinine (mg/dL)	1.3 ± 0.030	1.2 ± 0.045	1.1 ± 0.034	1.0 ± 0.044*	1.0 ± 0.200*	0.9 ± 0.052*
AST (IU)	94 ± 6	83 ± 3*	82 ± 4*	83 ± 4*	114 ± 38	83 ± 4*
ALT (IU)	41 ± 4.2	33 ± 1.4	31 ± 1.9	32 ± 2.9	30 ± 3.0	21 ± 3.1*
Females, Week 13						
Number evaluated	10	10	9	7	4	3
Protein (g/dL)	7.9 ± 0.18	7.9 ± 0.16	7.9 ± 0.19	7.8 ± 0.09	8.0 ± 0.25	7.2 ± 0.10

Table B-2. Selected Hematology and Serum Chemistry Changes in Male and Female Sprague-Dawley Rats Exposed to Lewisite by Gastric Intubation for 13 Weeks[a]

Creatinine (mg/dL)	1.4 ± 0.065	1.4 ± 0.065	1.3 ± 0.056	1.2 ± 0.044	1.2 ± 0.091	1.2 ± 0.067
AST (IU)	121 ± 20	98 ± 9	107 ± 10	108 ± 15	162 ± 42	96 ± 9
ALT (IU)	62 ± 15.6	39 ± 7.2	53 ± 10.3	47 ± 10.1	83 ± 27.8	29 ± 4.8

[a]Sasser et al. (1996); Sasser et al. (1989a)
[b]Mean ± SE.
[*]Significantly different from control value by Tukey's test ($p < 0.05$), as reported by study authors.

ALT = alanine aminotransferase; AST = aspartate aminotransferase

Table B-3. Mortality in Adult Male and Female Sprague-Dawley Rats Exposed to Lewisite by Gastric Intubation for 23 Weeks[a,b]

Endpoint	Dose (mg/kg BW)			
	0	**0.071−0.076**	**0.18−0.19**	**0.43−0.46**
F0 parents				
Males	0/20	0/20	0/20	4/20
Females	1/25	0/25	4/25	11/25
F1 parents				
Males	0/20	1/20	2/20	6/20
Females	0/25	2/25	5/25	18/25

[a]Sasser et al. (1999); Sasser et al. (1996); Sasser et al. (1989b)
[b]The authors indicated that one control and one high-dose F0 female died during parturition, and one F1 male and two F1 females of unspecified dose groups died due to dosing error; the remaining deaths were attributed to toxicity.

Table B-4. Mortality and Other Effects in Dose-Range-Finding Developmental Study of Rats Exposed to Lewisite by Gastric Intubation on GDs 6−15[a]

Endpoint	Dose (mg/kg BW)				
	0	**0.5**	**1.0**	**2.0**	**2.5**
Mortality due to dosing error	0/10	0/10	1/10	2/10	1/11
Mortality due to lewisite exposure (number deaths/number dosed)	0/10	0/10	0/10	1/10	2/11
Mortality due to lewisite exposure corrected for other causes of death (number of deaths/[number dosed − number dying from other causes])	0/10	0/10	0/9	1/8	2/10
Pregnant rats among survivors	6/10	9/10	8/9	6/7	5/8
Maternal body weight at GD 20 (g)	382 ± 10.5[b]	375 ± 11.4	371 ± 9.7	339 ± 21.9	314 ± 32.8*
Number of implantations/dam	15 ± 1.2	14 ± 1.0	16 ± 1.1	13 ± 1.7	10 ± 3.1*
Implantations/corpora lutea (%)	86 ± 6.8	91 ± 2.7	81 ± 5.4	74 ± 8.9	66 ± 18.3
Early resorptions (%)	6.5 ± 3.0	13.6 ± 7.6	4.3 ± 1.9	5.5 ± 3.4	24.0 ± 19.2
Mid gestation resorptions (%)	0	0	0.7 ± 0.6	28 ± 16.4*	1.3 ± 1.3
Total resorptions (%)	6.5 ± 3.0	13.6 ± 7.6	5.0 ± 2.1	34.6 ± 16.2	25.3 ± 19.0
Live fetuses/litter	15 ± 1.5	12 ± 1.4	15 ± 0.9	8 ± 2.4*	8 ± 3.1*
Fetal body weight, male (g)	3.29 ± 0.08	3.60 ± 0.06	3.37 ± 0.12	3.47 ± 0.12	2.86 ± 0.65
Fetal body weight, female (g)	3.22 ± 0.12	3.35 ± 0.08	3.22 ± 0.10	2.98 ± 0.38	2.62 ± 0.58

[a]Hackett et al. (1992); Hackett et al. (1987)
[b]Mean ± SE.
*Statistically significant ($p \leq 0.05$) compared with control by Duncan's multiple range test conducted by study authors.

Table B-5. Mortality in Dose-Range-Finding Developmental Study of Rabbits Exposed to Lewisite by Gastric Intubation on GDs 6−19[a]

Endpoint	0	0.5	1.0	1.5	2.0
Mortality due to dosing error	1/8	5/8	1/8	3/8	0/8
Mortality due to lewisite exposure (number deaths/number dosed)	0/8	0/8	6/8	5/8	8/8
Mortality due to lewisite exposure corrected for other causes of death (number of deaths/[number dosed − number dying from other causes])	0/7	0/3	6/7	5/5	8/8
Pregnant rabbits among survivors	3/7	3/3	1/1	0/0	0/0

[a]Hackett et al. (1992); Hackett et al. (1987)

Table B-6. Mortality and Other Selected Endpoints in Main Developmental Study of Rabbits Exposed to Lewisite by Gastric Intubation on GDs 6−19[a]

Endpoint	Dose (mg/kg body weight)			
	0	**0.07**	**0.2**	**0.6**
Mortality due to dosing error, stress, handling trauma, or pregnancy complications	1/19	5/18	5/18	3/19
Mortality due to lewisite exposure (number deaths/number dosed)	0/19	2/18	6/18	11/19
Mortality due to lewisite exposure corrected for other causes of death (number of deaths/[number dosed − number dying from other causes])	0/18	2/13	6/13	11/16
Pregnant rabbits among survivors	9/18	6/11	5/7	3/5
Maternal hematocrit (%)	43 ± 0.9[b]	42 ± 3.0	37 ± 1.7	33 ± 0
Implantations/corpora lutea (%)	74.4 ± 6.7	84.9 ± 7.4	100.8 ± 5.0*	57.0 ± 7.2
Total resorptions per litter (%)	11.0 ± 5.9	33.0 ± 16.6	23.1 ± 19.5	34.7 ± 19.3
Fetal body weight, male (g)	44.2 ± 2.4	46.8 ± 8.2	41.4 ± 2.6	38.7 ± 7.9
Fetal body weight, female (g)	44.1 ± 3.0	45.9 ± 5.2	41.1 ± 2.0	38.6 ± 9.3
Crown-rump length, male (mm)	98 ± 3.0	97 ± 4.3	94 ± 2.1	91 ± 3.7
Crown-rump length, female (mm)	98 ± 3.4	96 ± 2.9	93 ± 3.0	89 ± 7.0
Placental weight, male (g)	5.54 ± 0.47	5.12 ± 1.39	4.32 ± 0.41	4.80 ± 0.70
Placental weight, female (g)	5.30 ± 0.46	5.74 ± 1.84	4.58 ± 0.56	4.97 ± 0.97
Sex ratio (% male)	52 ± 5.7	31 ± 16.3	42 ± 11.1	33 ± 17.6
Stunted fetuses (litters with stunted fetuses)	0/63 (0/9)	0/23 (0/4)	2/39 (2/4)	5/16[c] (2/3*
Fetuses (litters) with supernumerary ribs	28/63 (8/9)	6/23 (2/4)	11/39 (2/4)	13/16* (3/3)
Fetuses (litters) with reduced ossification of the pelvis	0/63 (0/9)	0/23 (0/4)	0/39 (0/4)	3/16* (1/3)

[a]Hackett et al. (1992); Hackett et al. (1987)
[b]Mean ± SE.
*Statistically significant ($p < 0.05$) difference from control incidence by Fisher's exact test conducted by study authors.

APPENDIX C. BENCHMARK DOSE MODELING

MODELING PROCEDURE FOR DICHOTOMOUS DATA

The benchmark dose (BMD) modeling of dichotomous data was conducted with the EPA's Benchmark Dose Software (BMDS) (Version 2.6). For mortality data, all of the dichotomous models (i.e., Gamma, Multistage, Logistic, Log-logistic, Probit, Log-probit, and Weibull models) available within the software were fit using a benchmark response (BMR) of 1% extra risk based on the EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012b). Adequacy of model fit was judged based on the χ^2 goodness-of-fit *p*-value ($p > 0.1$), magnitude of scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. Among all models providing adequate fit, the lowest benchmark dose lower confidence limit (BMDL) was selected if the BMDLs estimated from different models varied greater than threefold; otherwise, the BMDL from the model with the lowest Akaike's information criterion (AIC) was selected as a potential point of departure (POD) from which to derive a provisional oral reference dose (p-RfD).

For mortality data in male rats treated with lewisite for 13 weeks (Sasser et al., 1996; Sasser et al., 1989a), no model provided adequate fit to the data as shown in Table C-1.

Table C-1. Model Data for Mortality from Male Rats Exposed to Lewisite for 13 Weeks[a]

Model Name	AIC	*p*-value	Specified Effect	Risk Type	BMD	BMDL
Gamma	46.6223	**0.0333**	0.01	Extra risk	0.015172	0.00985486
Logistic	57.4655	**0.0004**	0.01	Extra risk	0.0563967	0.035462
LogLogistic	47.4174	**0.0325**	0.01	Extra risk	0.0128316	0.00586856
LogProbit	46.5792	**0.0257**	0.01	Extra risk	0.0857313	0.0586518
Multistage	46.6223	**0.0333**	0.01	Extra risk	0.015172	0.00985486
Multistage	46.6223	**0.0333**	0.01	Extra risk	0.015172	0.00985486
Probit	56.6455	**0.0005**	0.01	Extra risk	0.0521553	0.0334274
Weibull	46.6223	**0.0333**	0.01	Extra risk	0.015172	0.00985486

[a]Sasser et al. (1996); Sasser et al. (1989a)

AIC = Akaike's information criterion; BMD = benchmark dose; BMDL = benchmark dose lower confidence limit

For mortality data in female rats treated with lewisite for 13 weeks (Sasser et al., 1996; Sasser et al., 1989a), all models provided adequate fit by the χ^2 goodness-of-fit criteria (see Table C-2). Among the models with acceptable fits, there was more than a three-fold variation in BMDL values. Therefore, the model with lowest BMDL was selected (LogLogistic). The plot of the LogLogistic Model is shown in Figure C-1, while the text output from the model run follows.

Table C-2. Model Data for Mortality from Female Rats Exposed to Lewisite for 13 Weeks[a]

Model Name	AIC	*p*-value	Specified Effect	Risk Type	BMD	BMDL
Gamma	43.5714	0.8577	0.01	Extra risk	0.0262234	0.00712875
Logistic	50.336	0.1441	0.01	Extra risk	0.0474142	0.0278098
LogLogistic	**42.9525**	**0.9389**	**0.01**	**Extra risk**	**0.0378891**	**0.00492191**
LogProbit	40.6884	0.9838	0.01	Extra risk	0.064487	0.043871
Multistage	41.9246	0.9349	0.01	Extra risk	0.010406	0.00694656
Multistage	41.9246	0.9349	0.01	Extra risk	0.010406	0.00694656
Probit	49.6021	0.1669	0.01	Extra risk	0.0453868	0.0267235
Weibull	43.7222	0.8519	0.01	Extra risk	0.0187729	0.00704865

[a]Sasser et al. (1996); Sasser et al. (1989a)

AIC = Akaike's information criterion; BMD = benchmark dose; BMDL = benchmark dose lower confidence limit.

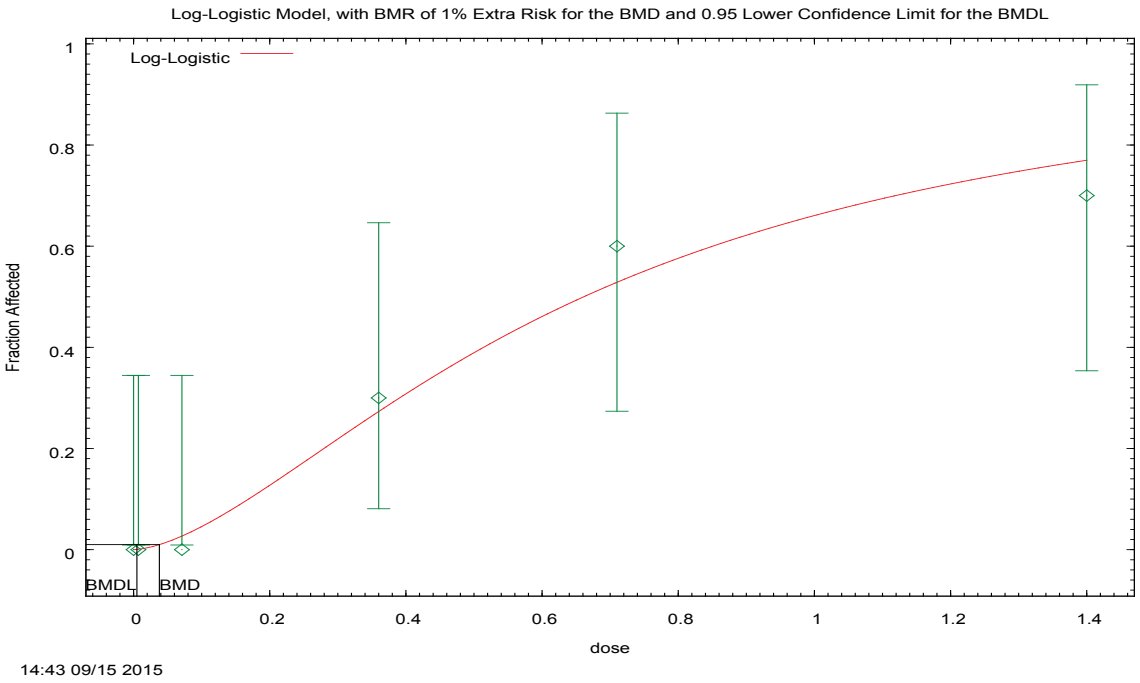

Figure C-1. LogLogistic Model of Mortality Data from Female Rats Treated with Lewisite for 13 Weeks (Sasser et al., 1996; Sasser et al., 1989a).

Text output from the benchmark dose response modeling software for the chosen model for the POD:

```
================================================================
        Logistic Model. (Version: 2.14; Date: 2/28/2013)
        Input Data File: C:/Users/DPETERSE/Desktop/BMDS260/Data/lnl_Lewisite Rat
Female 13 wk_Lnl-BMR01-Restrict.(d)
        Gnuplot Plotting File:  C:/Users/DPETERSE/Desktop/BMDS260/Data/lnl_Lewisite
Rat Female 13 wk_Lnl-BMR01-Restrict.plt
                                          Mon Sep 28 08:20:30 2015
 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 6
   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
```

User has chosen the log transformed model

Default Initial Parameter Values
background = 0
intercept = 0.315332
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	0.55
slope	0.55	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	NA		
intercept	0.66621	0.469779	-0.25454	1.58696
slope	1.60745	0.561096	0.507721	2.70718

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-18.9474	6			
Fitted model	-19.4762	2	1.05767	4	0.9009
Reduced model	-34.7949	1	31.695	5	<.0001

AIC: 42.9525

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	10.000	0.000
0.0071	0.0007	0.007	0.000	10.000	-0.083
0.0710	0.0270	0.270	0.000	10.000	-0.526
0.3600	0.2737	2.737	3.000	10.000	0.187
0.7100	0.5289	5.289	6.000	10.000	0.451
1.4000	0.7698	7.698	7.000	10.000	-0.524

Chi^2 = 0.80 d.f. = 4 P-value = 0.9389

```
   Benchmark Dose Computation

Specified effect =           0.01

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =      0.0378891

            BMDL =     0.00492191
```

For mortality data in F1 male rats treated with lewisite in a reproductive study (Sasser et al., 1996; Sasser et al., 1989a), all models provided adequate fit by the χ^2 goodness-of-fit criteria (see Table C-3). Among the models with acceptable fits, there was more than a three-fold variation in BMDL values. Therefore, the model with lowest BMDL was selected (LogLogistic). The plot of the LogLogistic Model is shown in Figure C-2, and the text output of the model run follows.

Table C-3. Model Data for Mortality from F1 Male Rats Exposed to Lewisite in a Reproductive Study[a]

Model Name	AIC	*p*-value	Specified Effect	Risk Type	BMD	BMDL
Gamma	49.4889	0.9467	0.01	Extra risk	0.0211277	0.00817782
Logistic	50.5704	0.6919	0.01	Extra risk	0.0520948	0.0290168
LogLogistic	**49.5109**	**0.9362**	**0.01**	**Extra risk**	**0.0228707**	**0.00693045**
LogProbit	50.392	0.6482	0.01	Extra risk	0.0687452	0.0444701
Multistage	49.447	0.9659	0.01	Extra risk	0.0177185	0.00820791
Multistage	49.4238	0.9771	0.01	Extra risk	0.0166431	0.00822471
Probit	50.3887	0.7292	0.01	Extra risk	0.0468449	0.02598
Weibull	49.4821	0.9497	0.01	Extra risk	0.020814	0.00818271

[a]Sasser et al. (1996); Sasser et al. (1989a)

AIC = Akaike's information criterion; BMD = benchmark dose; BMDL = benchmark dose lower confidence limit.

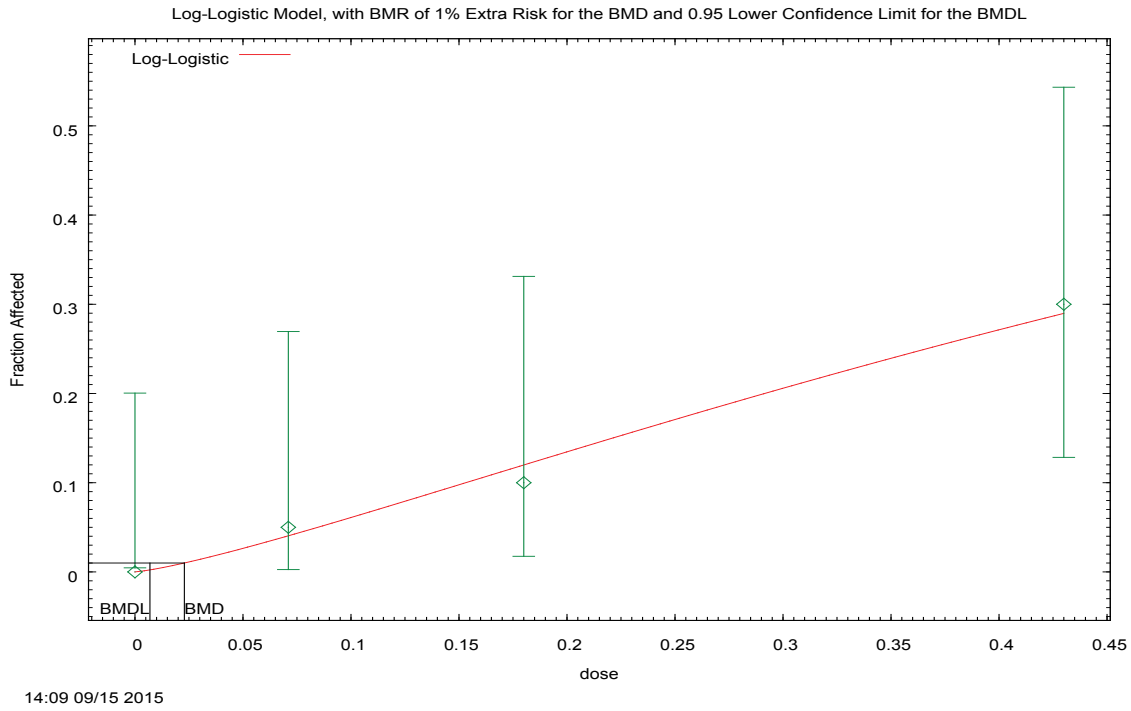

Figure C-2. LogLogistic Model of Mortality Data from F1 Male Rats Treated with Lewisite in a Reproductive Study (Sasser et al., 1996; Sasser et al., 1989a)

Text output from the benchmark dose response modeling software for the chosen model:

```
 ======================================================================
        Logistic Model. (Version: 2.14; Date: 2/28/2013)
        Input Data File: C:/Users/DPETERSE/Desktop/BMDS260/Data/lnl_Lewisite F1 Males
23 wk_Lnl-BMR01-Restrict.(d)
        Gnuplot Plotting File:  C:/Users/DPETERSE/Desktop/BMDS260/Data/lnl_Lewisite
F1 Males 23 wk_Lnl-BMR01-Restrict.plt
                                          Fri Sep 25 10:23:01 2015
 ======================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 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
```

User has chosen the log transformed model

Default Initial Parameter Values
background = 0
intercept = 0.016284
slope = 1.16026

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	0.91
slope	0.91	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	NA		
intercept	0.167448	0.891442	-1.57975	1.91464
slope	1.26064	0.618007	0.0493673	2.47191

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-22.6893	4			
Fitted model	-22.7555	2	0.132415	2	0.9359
Reduced model	-28.1368	1	10.8952	3	0.01231

AIC: 49.5109

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	20.000	0.000
0.0710	0.0404	0.809	1.000	20.000	0.217
0.1800	0.1198	2.396	2.000	20.000	-0.273
0.4300	0.2898	5.795	6.000	20.000	0.101

Chi^2 = 0.13 d.f. = 2 P-value = 0.9362

```
   Benchmark Dose Computation

Specified effect =           0.01

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =      0.0228707

            BMDL =     0.00693045
```

For mortality data in F1 female rats treated with lewisite in a reproductive study (Sasser et al., 1996; Sasser et al., 1989a), all models provided adequate fit by the χ^2 goodness-of-fit criteria (see Table C-4). Among the models with acceptable fits, there was more than a three-fold variation in BMDL values. Therefore, the model with lowest BMDL was selected (Multistage 3rd Degree). The plot of the Multistage 3rd Degree Model is shown in Figure C-3.

Table C-4. Model Data for Mortality from F1 Female Rats Exposed to Lewisite in a Reproductive Study[a]

Model Name	AIC	*p*-value	Specified Effect	Risk Type	BMD	BMDL
Gamma	73.3461	0.6877	0.01	Extra risk	0.0310001	0.00668997
Logistic	74.4998	0.5709	0.01	Extra risk	0.0287477	0.0168832
LogLogistic	73.6338	0.5867	0.01	Extra risk	0.0343067	0.0105866
LogProbit	74.1361	0.4624	0.01	Extra risk	0.0428324	0.0249112
Multistage	72.8949	0.8648	0.01	Extra risk	0.017047	0.00548079
Multistage	**72.6466**	**0.9798**	**0.01**	**Extra risk**	**0.0107259**	**0.00519058**
Probit	74.0341	0.6485	0.01	Extra risk	0.0268908	0.0153729
Weibull	73.0866	0.781	0.01	Extra risk	0.0265364	0.00688619

[a]Sasser et al. (1996); Sasser et al. (1989a)

AIC = Akaike's information criterion; BMD = benchmark dose; BMDL = benchmark dose lower confidence limit.

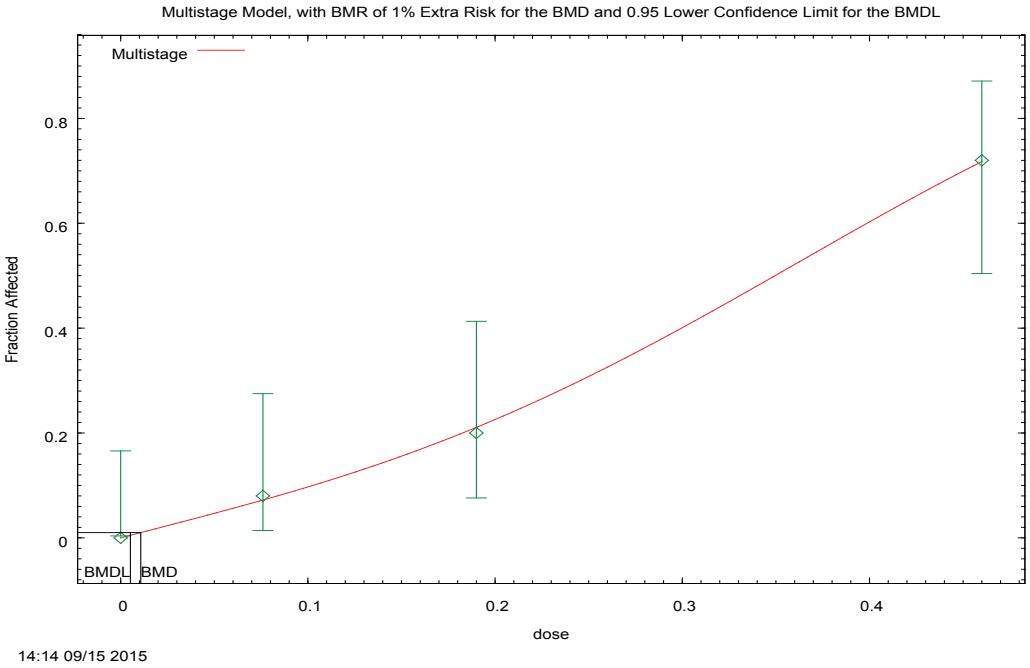

Figure C-3. Multistage Model of Mortality Data from F1 Female Rats Treated with Lewisite in a Reproductive Study (Sasser et al., 1996; Sasser et al., 1989a)

Text output from the benchmark dose response modeling software for the chosen model:

```
=================================================================
        Multistage Model. (Version: 3.4;  Date: 05/02/2014)
 Input Data File: C:/Users/DPETERSE/Desktop/BMDS260/Data/mst_Lewisite Rat F1 Females
23 wk_mst3-BMR01-Restrict.(d)
        Gnuplot Plotting File:  C:/Users/DPETERSE/Desktop/BMDS260/Data/mst_Lewisite
Rat F1 Females 23 wk_mst3-BMR01-Restrict.plt
                                          Mon Sep 28 12:47:08 2015
 =================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3
```

```
 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =   0.00547098
                        Beta(1) =     0.843746
                        Beta(2) =            0
                        Beta(3) =      9.03136


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(2)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                Beta(1)      Beta(3)

   Beta(1)            1        -0.67

   Beta(3)        -0.67            1


                                Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0              NA
        Beta(1)         0.936033         0.497796          -0.0396283
1.91169
        Beta(2)                0              NA
        Beta(3)          8.57316           4.3191             0.10788
17.0384

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -34.3031         4
   Fitted model        -34.3233         2     0.0403374      2           0.98
  Reduced model        -56.2335         1       43.8608      3         <.0001

           AIC:         72.6466


                                Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size          Residual
  ---------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000      25.000          0.000
    0.0760     0.0722         1.804     2.000      25.000          0.151
    0.1900     0.2107         5.268     5.000      25.000         -0.132
```

```
    0.4600      0.7178         17.944     18.000       25.000         0.025

 Chi^2 = 0.04       d.f. = 2         P-value = 0.9798


   Benchmark Dose Computation

Specified effect =           0.01

Risk Type        =       Extra risk

Confidence level =           0.95

             BMD =       0.0107259

            BMDL =      0.00519058

            BMDU =       0.0663656

Taken together, (0.00519058, 0.0663656) is a 90      % two-sided confidence
interval for the BMD
```

For mortality data in F0 male rats treated with lewisite for 23 weeks in a reproductive study (Sasser et al., 1996; Sasser et al., 1989a), all models provided adequate fit by the χ^2 goodness-of-fit criteria (see Table C-5). Among the models with acceptable fits, there was more than a three-fold variation in BMDL values. Therefore, the model with lowest BMDL was selected (Quantal Linear). The plot of the Quantal Linear Model is shown in Figure C-4, and the text output from the model run follows.

Table C-5. Model Data for Mortality from F0 Male Rats Exposed to Lewisite for 23 weeks in a Reproductive Study[a]

Model Name	AIC	*p*-value	Specified Effect	Risk Type	BMD	BMDL
Gamma	22.0184	1	0.01	Extra risk	0.287832	0.0612538
Logistic	24.0161	1	0.01	Extra risk	0.385147	0.090797
LogLogistic	22.0161	1	0.01	Extra risk	0.359789	0.0612721
LogProbit	24.0161	1	0.01	Extra risk	0.330848	0.0859863
Multistage	23.6451	0.8228	0.01	Extra risk	0.101073	0.0243082
Multistage	22.6824	0.9505	0.01	Extra risk	0.157315	0.0322242
Probit	24.0161	1	0.01	Extra risk	0.354374	0.0822947
Weibull	22.0161	1	0.01	Extra risk	0.361966	0.0594456
Quantal Linear	**23.0259**	**0.4577**	**0.01**	**Extra risk**	**0.032012**	**0.0155141**

[a]Sasser et al. (1996); Sasser et al. (1989a)

AIC = Akaike's information criterion; BMD = benchmark dose; BMDL = benchmark dose lower confidence limit.

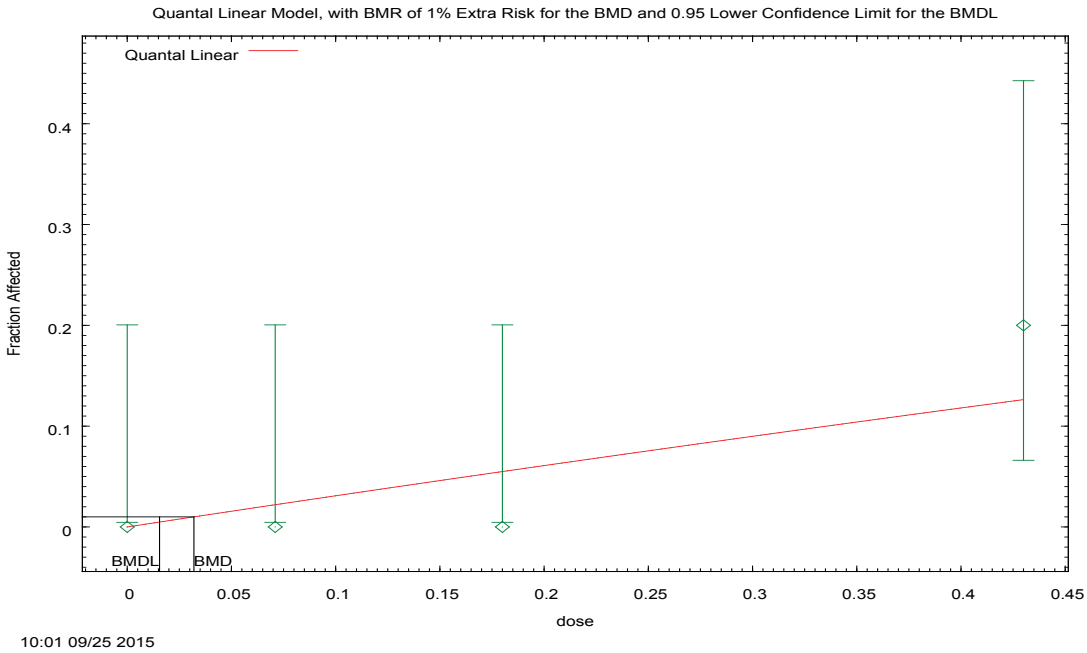

Figure C-4. Quantal-Linear Model of the 23 Week F0 Male Rat Data (Sasser et al., 1996; Sasser et al., 1989a)

Text output from the benchmark dose response modeling software for the chosen model:

```
 ================================================================================
        Quantal Linear Model using Weibull Model (Version: 2.16;  Date: 2/28/2013)
        Input Data File: C:/Users/DPETERSE/Desktop/BMDS260/Data/qln_Lewisite Rate F0
Males 23wk_Qln-BMR01.(d)
        Gnuplot Plotting File:  C:/Users/DPETERSE/Desktop/BMDS260/Data/qln_Lewisite
Rate F0 Males 23wk_Qln-BMR01.plt
                                                Fri Sep 25 10:01:39 2015
 ================================================================================

 BMDS_Model_Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose)]


   Dependent variable = Effect
   Independent variable = Dose

   Total number of observations = 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



                  Default Initial (and Specified) Parameter Values
                     Background =    0.0454545
                          Slope =     0.491416
                          Power =            1   Specified
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Power
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Slope
Slope	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	NA		
Slope	0.313956	0.157097	0.00605107	0.62186

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-10.008	4			
Fitted model	-12.0129	1	4.00977	3	0.2604
Reduced model	-15.8812	1	11.7463	3	0.008305

AIC: 26.0259

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	20.000	0.000
0.0710	0.0220	0.441	0.000	20.000	-0.671
0.1800	0.0549	1.099	0.000	20.000	-1.078
0.4300	0.1263	2.526	4.000	20.000	0.992

Chi^2 = 2.60 d.f. = 3 P-value = 0.4577

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.032012

BMDL = 0.0155141

For mortality data in F0 female rats treated with lewisite for 23 weeks in a reproductive study (Sasser et al., 1996; Sasser et al., 1989a), all models provided adequate fit by the χ^2 goodness-of-fit criteria (see Table C-6). Among the models with acceptable fits, there was more than a three-fold variation in BMDL values. Therefore, the model with lowest BMDL was selected (Quantal-Linear). The plot of the Quantal Linear Model is shown in Figure C-5. The text output from the chosen model follows.

Table C-6. Model Data for Mortality from F0 Female Rats Exposed to Lewisite in a Reproductive Study[a]

Model Name	AIC	*p*-value	Specified Effect	Risk Type	BMD	BMDL
Gamma	73.1496	0.2001	0.01	Extra risk	0.0668565	0.0131271
Logistic	72.0049	0.3369	0.01	Extra risk	0.0408858	0.0244533
LogLogistic	73.1796	0.2004	0.01	Extra risk	0.062954	0.0150912
LogProbit	72.7821	0.2356	0.01	Extra risk	0.0781818	0.0425468
Multistage	71.3586	0.4105	0.01	Extra risk	0.060283	0.0104785
Multistage	71.3586	0.4105	0.01	Extra risk	0.060283	0.010126
Probit	71.8411	0.3598	0.01	Extra risk	0.0364795	0.0218079
Weibull	73.354	0.1858	0.01	Extra risk	0.057685	0.0116653
Quantal-Linear	**74.3328**	**0.1685**	**0.01**	**Extra Risk**	**0.0107542**	**0.00701412**

[a]Sasser et al. (1996); Sasser et al. (1989a)

AIC = Akaike's information criterion; BMD = benchmark dose; BMDL = benchmark dose lower confidence limit.

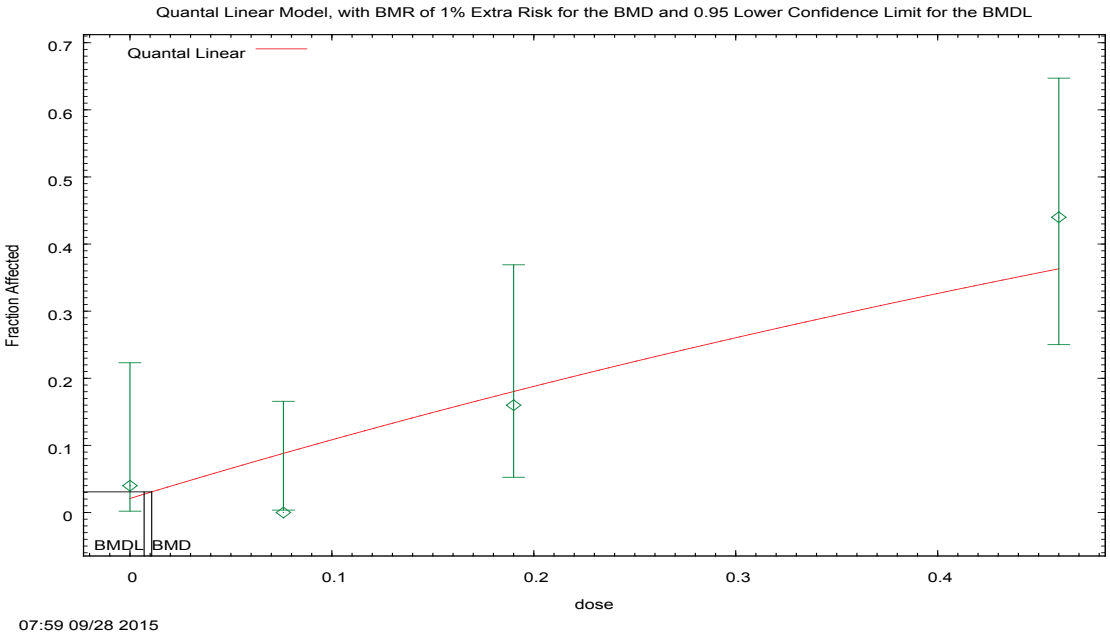

Figure C-5. Quantal-Linear Model of the 23 Week F0 Female Rat Data (Sasser et al., 1996; Sasser et al., 1989a)

Text output from the benchmark dose response modeling software for the chosen model:

```
================================================================
        Quantal Linear Model using Weibull Model (Version: 2.16;  Date: 2/28/2013)
        Input Data File: C:/Users/DPETERSE/Desktop/BMDS260/Data/qln_Lewisite Rat F0
Female 23 wk_Qln-BMR01.(d)
        Gnuplot Plotting File:  C:/Users/DPETERSE/Desktop/BMDS260/Data/qln_Lewisite
Rat F0 Female 23 wk_Qln-BMR01.plt
                                          Mon Sep 28 07:59:57 2015
 ================================================================

 BMDS_Model_Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose)]


   Dependent variable = Effect
   Independent variable = Dose

   Total number of observations = 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


                  Default Initial (and Specified) Parameter Values
                     Background =    0.0740741
                          Slope =      1.11049
                          Power =            1   Specified
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Power
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Slope
Background	1	-0.23
Slope	-0.23	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0210426	0.0206976	-0.0195239	0.0616091
Slope	0.934553	0.266503	0.412217	1.45689

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-32.3386	4			
Fitted model	-35.1664	2	5.65565	2	0.05914
Reduced model	-43.967	1	23.2568	3	<.0001

AIC: 74.3328

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0210	0.526	1.000	25.000	0.660
0.0760	0.0882	2.204	0.000	25.000	-1.555
0.1900	0.1803	4.508	4.000	25.000	-0.264
0.4600	0.3631	9.078	11.000	25.000	0.799

Chi^2 = 3.56 d.f. = 2 P-value = 0.1685

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0107542

BMDL = 0.00701412

For mortality data in female rabbits treated with lewisite during gestation in a developmental study (Sasser et al., 1996; Hackett et al., 1992; Sasser et al., 1989a; Hackett et al., 1987), several models provided adequate fit by the χ^2 goodness-of-fit criteria (see Table C-7). Among the models with acceptable fits, there was more than a three-fold variation in BMDL values. Therefore, the model with lowest BMDL was selected (Loglogistic). The plot of the Loglogistic Model is shown in Figure C-6. The text output of the model follows.

Table C-7. Model Data for Mortality in Female Rabbits Exposed to Lewisite in a Reproductive Study[a]

Model Name	AIC	*p*-value	Specified Effect	Risk Type	BMD	BMDL
Gamma	51.7504	0.8506	0.01	Extra risk	0.00442511	0.00304123
Logistic	59.8177	0.0701	0.01	Extra risk	0.0171768	0.0109278
LogLogistic	**53.1903**	**0.9013**	**0.01**	**Extra risk**	**0.00451531**	**0.00162205**
LogProbit	52.7548	0.5767	0.01	Extra risk	0.0256647	0.0180007
Multistage	51.7504	0.8506	0.01	Extra risk	0.00442511	0.00304123
Multistage	51.7504	0.8506	0.01	Extra risk	0.00442511	0.00304123
Probit	59.3935	0.079	0.01	Extra risk	0.0158485	0.010443
Weibull	51.7504	0.8506	0.01	Extra risk	0.00442511	0.00304123
Quantal-Linear	51.7504	0.8506	0.01	Extra Risk	0.00442511	0.00304123

[a]Hackett et al. (1992); Hackett et al. (1987)

AIC = Akaike's information criterion; BMD = benchmark dose; BMDL = benchmark dose lower confidence limit

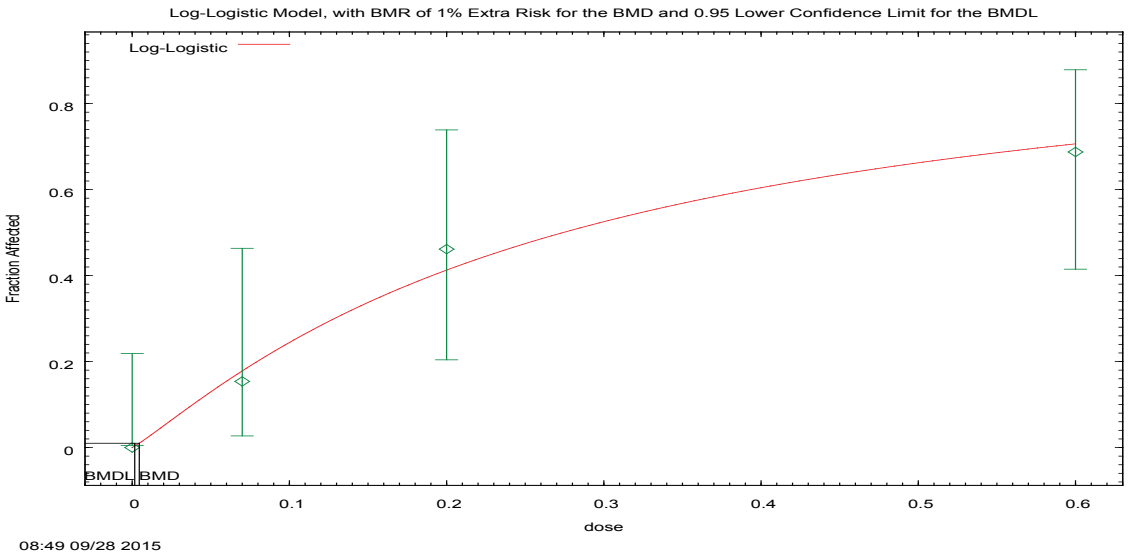

Figure C-6 LogLogistic Model of the 6-19 day Female Rabbit Data (Hackett et al., 1992; Hackett et al., 1987)

Text output from the benchmark dose response modeling software for the chosen model:

```
=============================================================================
         Logistic Model. (Version: 2.14; Date: 2/28/2013)
         Input Data File: C:/Users/DPETERSE/Desktop/BMDS260/Data/lnl_Lewisite Rabbit
Female 13 corrected_Lnl-BMR01-Restrict.(d)
         Gnuplot Plotting File:  C:/Users/DPETERSE/Desktop/BMDS260/Data/lnl_Lewisite
Rabbit Female 13 corrected_Lnl-BMR01-Restrict.plt
                                                Mon Sep 28 08:49:08 2015
 =============================================================================

 BMDS_Model_Run
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   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 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



   User has chosen the log transformed model


                  Default Initial Parameter Values
                     background =            0
                      intercept =       1.4883
                          slope =      1.15813


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -background
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

              intercept        slope

 intercept            1         0.86

     slope         0.86            1



                                  Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     background                0              NA
      intercept          1.45129         0.681821             0.11495
2.78764
```

```
          slope          1.11965         0.415855            0.304587
1.93471

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -24.491         4
   Fitted model        -24.5951         2      0.208309      2          0.9011
  Reduced model        -37.4599         1       25.9378      3         <.0001

           AIC:         53.1903


                                  Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size          Residual
  ---------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000      18.000          0.000
    0.0700     0.1786         2.321     2.000      13.000         -0.233
    0.2000     0.4132         5.372     6.000      13.000          0.354
    0.6000     0.7067        11.307    11.000      16.000         -0.169

 Chi^2 = 0.21      d.f. = 2        P-value = 0.9013


   Benchmark Dose Computation

Specified effect =           0.01

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =     0.00451531

            BMDL =     0.00162205
```

APPENDIX D. REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). (2015). 2015 TLVs and BEIs. Based on the documentation of the threshold limit values for chemical substances and physical agents and biological exposure indices [TLV/BEI]. Cincinnati, OH. http://www.acgih.org/forms/store/ProductFormPublic/2015-tlvs-and-beis

Aposhian, HV; Carter, DE; Hoover, TD; Hsu, CA; Maiorino, RM; Stine, E. (1984). DMSA, DMPS, and DMPA--as arsenic antidotes. Fundam Appl Toxicol 4: S58-S70. http://dx.doi.org/10.1016/0272-0590(84)90138-6

Aposhian, HV; Mershon, MM; Brinkley, FB; Hsu, CA; Hackley, BE. (1982). Anti-lewisite activity and stability of meso-dimercaptosuccinic acid and 2,3-dimercapto-1-propanesulfonic acid. Life Sci 31: 2149-2156. http://dx.doi.org/10.1016/0024-3205(82)90108-4

ATSDR (Agency for Toxic Substances and Disease Registry). (2015). Minimal risk levels (MRLs). April 2015. Atlanta, GA: Agency for Toxic Substances and Disease Registry (ATSDR). Retrieved from http://www.atsdr.cdc.gov/mrls/index.asp

Auerbach, C; Robson, JM. (1947). Tests of chemical substances for mutagenic action. Proc Roy Soc Edinb B Biol 62: 284-291.

Brown, RF; Rice, P; Bennett, NJ. (1998). The use of laser Doppler imaging as an aid in clinical management decision making in the treatment of vesicant burns. Burns 24: 692-698. http://dx.doi.org/10.1016/S0305-4179(98)00105-3

Bucci, TJ; Parker, RM; Dacre, JC; Denny, KH. (1993). Developmental toxicity (dominant lethal mutation) study on agent lewisite: Dominant lethal study of lewisite in male rats. Frederick, MD: U.S. Army Medical Research and Materiel Command. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=ADA290671

Cal/EPA (California Environmental Protection Agency). (2011). Hot spots unit risk and cancer potency values. Appendix A. Sacramento, CA: Office of Environmental Health Hazard Assessment. http://www.oehha.ca.gov/air/hot_spots/2009/AppendixA.pdf

Cal/EPA (California Environmental Protection Agency). (2014). All OEHHA acute, 8-hour and chronic reference exposure levels (chRELs) as of June 2014. Sacramento, CA: Office of Health Hazard Assessment. http://www.oehha.ca.gov/air/allrels.html

Cal/EPA (California Environmental Protection Agency). (2015a). Chemicals known to the state to cause cancer or reproductive toxicity August 25, 2015. (Proposition 65 list). Sacramento, CA: California Environmental Protection Agency, Office of Environmental Health Hazard Assessment. http://oehha.ca.gov/prop65/prop65_list/files/P65single060614.pdf

Cal/EPA (California Environmental Protection Agency). (2015b). OEHHA toxicity criteria database [Database]. Sacramento, CA: Office of Environmental Health Hazard Assessment. Retrieved from http://www.oehha.ca.gov/tcdb/index.asp

Cameron, GR; Carleton, HM; Short, RH. (1946). Pathological changes induced by lewisite and allied compounds. J Pathol Bacteriol 58: 411-422. http://dx.doi.org/10.1002/path.1700580311

Chilcott, RP; Brown, RF; Rice, P. (2000). Non-invasive quantification of skin injury resulting from exposure to sulphur mustard and Lewisite vapours. Burns 26: 245-250.

Doi, M; Hattori, N; Yokoyama, A; Onari, Y; Kanehara, M; Masuda, K; Tonda, T; Ohtaki, M; Kohno, N. (2011). Effect of mustard gas exposure on incidence of lung cancer: a longitudinal study. Am J Epidemiol 173: 659-666. http://dx.doi.org/10.1093/aje/kwq426

Fidder, A; Noort, D; Hulst, AG; de Jong, LP; Benschop, HP. (2000). Biomonitoring of exposure to lewisite based on adducts to haemoglobin. Arch Toxicol 74: 207-214. http://dx.doi.org/10.1007/s002040000117

Flohe, S; Kraus, M; Haeser, C; Hannemann, M; Kreppel, H; Szinicz, L. (1996). In vitro studies on the effects of 2-chlorovinyldi-chloroarsine lewisite. Naunyn Schmiedebergs Arch Pharmacol 353: R125.

Goldman, M; Dacre, JC. (1989). Lewisite: its chemistry, toxicology, and biological effects [Review]. Rev Environ Contam Toxicol 110: 75-115. http://dx.doi.org/10.1007/978-1-4684-7092-5_2

Hackett, PL; Sasser, LB; Rommereim, RL; Buschbom, RL; Kalkwarf, DR; Dacre, JC. (1992). Developmental toxicity studies of lewisite in rats and rabbits [Abstract]. Toxicologist 12: 198.

Hackett, PL; Sasser, LB; Rommereim, RL; Cushing, JA; Buschbom, RL; Kalkwarf, DR. (1987). Teratology studies of lewisite and sulfur mustard agents: Effects of lewisite in rats and rabbits. (PNL-6408). Frederick, MD: U.S. Army Medical Research and Development Command. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=ADA198423

Haeser, C; Kehe, K; Kreppel, H; Szinicz, L. (1997). Effects of various decontaminants on skin damage and penetration by 2-chlorovinyldichloroarsine in vitro [Abstract]. Naunyn Schmiedebergs Arch Pharmacol 355: 608.

Harrison, HE; Durlacher, SH. (1946). The treatment by 2,3-dimercaptopropanol (BAL) of the systemic toxic effects of skin contamination with lewisite and phenyldichlorarsine. J Pharmacol Exp Ther 87: 81-84.

Harrison, HE; Ordway, NK. (1946). Poisoning from inhalation of the vapors of lewisite and phenyldichlorarsine; its pathology in the dog and treatment with 2,3-dimercaptopropanol (BAL). J Pharmacol Exp Ther 87: 76-80.

HSDB (Hazardous Substances Data Bank). (2010). Lewisite, CASRN 541-25-3 [Fact Sheet]. Bethesda, MD: National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~6K88SO:1

Hughes, WF. (1946). Clinical uses of 2,3-dimercaptopropanol (BAL); the treatment of lewisite burns of the eye with BAL. J Clin Invest 25: 541-548. http://dx.doi.org/10.1172/JCI101736

Hughes, WF. (1947). Treatment of lewisite burns of the eye with dimercaprol (BAL). Arch Ophthalmol 37: 25-41. http://dx.doi.org/10.1001/archopht.1947.00890220030004

IARC (International Agency for Research on Cancer). (2015). IARC Monographs on the evaluation of carcinogenic risk to humans. Geneva, Switzerland: International Agency for Research on Cancer, WHO. http://monographs.iarc.fr/ENG/Monographs/PDFs/index.php

Inns, RH; Bright, JE; Marrs, TC. (1988). Comparative acute systemic toxicity of sodium arsenite and dichloro(2-chlorovinyl)arsine in rabbits. Toxicology 51: 213-222. http://dx.doi.org/10.1016/0300-483X(88)90151-5

Inns, RH; Rice, P. (1993). Efficacy of dimercapto chelating agents for the treatment of poisoning by percutaneously applied dichloro(2-chlorovinyl)arsine in rabbits. Hum Exp Toxicol 12: 241-246. http://dx.doi.org/10.1177/096032719301200307

Inns, RH; Rice, P; Bright, JE; Marrs, TC. (1990). Evaluation of the efficacy of dimercapto chelating agents for the treatment of systemic organic arsenic poisoning in rabbits. Hum Exp Toxicol 9: 215-220. http://dx.doi.org/10.1177/096032719000900403

Kehe, K; Flohé, S; Krebs, G; Kreppel, H; Reichl, FX; Liebl, B; Szinicz, L. (2001). Effects of Lewisite on cell membrane integrity and energy metabolism in human keratinocytes and SCL II cells. Toxicology 163: 137-144. http://dx.doi.org/10.1016/s0300-483x(01)00389-4

King, JR; Riviere, JE; Monteiro-Riviere, NA. (1992). Characterization of lewisite toxicity in isolated perfused skin. Toxicol Appl Pharmacol 116: 189-201. http://dx.doi.org/10.1016/0041-008x(92)90298-7

Lam, DG; Rice, P; Brown, RF. (2002). The treatment of Lewisite burns with laser debridement---'lasablation'. Burns 28: 19-25.

Lindberg, G; Runn, P; Winter, S; Faellman, A. (1997). Basic Information on Lewisite. A Chemical Warfare Agent with Effects Similar to Mustard Gas (Grundlaeggande Data om Lewisit. Ett Kemiskt Stridsmedel med Egenskaper Liknande Senapsgas). Lindberg, G; Runn, P; Winter, S; Faellman, A. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=PB97209803

Lindsay, CD; Hambrook, JL; Brown, RF; Platt, JC; Knight, R; Rice, P. (2004). Examination of changes in connective tissue macromolecular components of large white pig skin following application of Lewisite vapour. J Appl Toxicol 24: 37-46. http://dx.doi.org/10.1002/jat.942

Mann, I; Pirie, A; Pullinger, BD. (1946). A study of lewisite lesions of the eyes of rabbits. Am J Ophthalmol 29: 1215-1227.

McManus, J; Huebner, K. (2005). Vesicants [Review]. Crit Care Clin 21: 707-718, vi. http://dx.doi.org/10.1016/j.ccc.2005.06.005

Meier, H. (2003). A comparison of cytotoxicity induced by sulfur mustard and lewisite [Abstract]. Toxicologist 72: 154.

Meier, HL. (2004). The use of flow cytometry to determine the effects of the vesicating agents sulfur mustard (SM) and lewisite (L) on human T cells (CD3+) [Abstract]. Toxicologist 78: 357.

Meier, HL; Kelly, SA; Corun, CM. (1993). A comparison of cytotoxicity of lewisite and sulfur mustard in human lymphocyte preparations [Abstract]. FASEB J 7: A37.

Mitcheltree, LW; Mershon, MM; Wall, HG; Pulliam, JD; Manthei, JH. (1989). Microblister formation in vesicant-exposed pig skin. Cutan Ocul Toxicol 8: 309-319. http://dx.doi.org/10.3109/15569528909062934

Monteiro-Riviere, NA; King, JR; Riviere, JE. (1990). Cutaneous toxicity of mustard and lewisite on the isolated perfused porcine skin flap (pp. 144). Monteiro-Riviere, NA; King, JR; Riviere, JE.

Mouret, S; Wartelle, J; Emorine, S; Bertoni, M; Nguon, N; Cléry-Barraud, C; Dorandeu, F; Boudry, I. (2013). Topical efficacy of dimercapto-chelating agents against lewisite-induced skin lesions in SKH-1 hairless mice. Toxicol Appl Pharmacol 272: 291-298. http://dx.doi.org/10.1016/j.taap.2013.06.012

Munro, NB; Talmage, SS; Griffin, GD; Waters, LC; Watson, AP; King, JF; Hauschild, V. (1999). The sources, fate, and toxicity of chemical warfare agent degradation products [Review]. Environ Health Perspect 107: 933-974.

NAS (National Academy of Sciences). (1999). Review of the U.S. Army's health risk assessments for oral exposure to six chemical-warfare agents. Washington, DC: National Academy Press. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=PB2000103122

Nelson, P; Hancock, JR; Sawyer, TW. (2006). Therapeutic effects of hypothermia on Lewisite toxicity. Toxicology 222: 8-16. http://dx.doi.org/10.1016/j.tox.2005.12.026

Nguon, N; Cléry-Barraud, C; Vallet, V; Elbakdouri, N; Wartelle, J; Mouret, S; Bertoni, M; Dorandeu, F; Boudry, I. (2014). Time course of lewisite-induced skin lesions and inflammatory response in the SKH-1 hairless mouse model. Wound Repair Regen 22: 272-280. http://dx.doi.org/10.1111/wrr.12147

NIOSH (National Institute for Occupational Safety and Health). (2015). NIOSH pocket guide to chemical hazards. Index of chemical abstracts service registry numbers (CAS No.). Atlanta, GA: Center for Disease Control and Prevention, U.S. Department of Health, Education and Welfare. http://www.cdc.gov/niosh/npg/npgdcas.html

Nishimoto, Y; Yamakido, M; Shigenobu, T; Onari, K; Yukutake, M. (1983). Long-term observation of poison gas workers with special reference to respiratory cancers. J UOEH 5: 89-94.

NRC (National Research Council). (2013). Acute exposure guideline levels for selected airborne chemicals: Volume 15. Washington (DC): National Academies Press (US).

NTP (National Toxicology Program). (2014). Report on carcinogens. Thirteenth edition. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service. http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html

Olajos, EJ; Olson, CT; Salem, H; Singer, AW; Hayes, TL; Menton, RG; Miller, TL; Rosso, T; Maciver, B. (1998). Evaluation of neutralized chemical agent identification sets (CAIS) for skin injury with an overview of the vesicant potential of agent degradation products. J Appl Toxicol 18: 409-420.

OSHA (Occupational Safety & Health Administration). (2006). Table Z-1 limits for air contaminants. Occupational safety and health standards, subpart Z, toxic and hazardous substances. (OSHA standard 1910.1000). Washington, DC: U.S. Department of Labor. http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9992

Pechura, CM. (1993). The health-effects of mustard gas and lewisite. JAMA 269: 453. http://dx.doi.org/10.1001/jama.1993.03500040015008

Rice, P; Brown, RF. (1999). The development of Lewisite vapour induced lesions in the domestic, white pig. Int J Exp Pathol 80: 59-67.

Sasser, LB; Buschbom, RL; Dacre, JC. (1992). Two generation reproduction study of lewisite in rats [Abstract]. Toxicologist 12: 198.

Sasser, LB; Cushing, JA; Kalkwarf, DR; Mellick, PW; Buschbom, RL. (1989a). Toxicology studies on lewisite and sulfur mustard agents: Subchronic toxicity study of lewisite in rats. (ADA217886. PNL-6860). Richland, WA: Pacific Northwest Laboratory. http://www.osti.gov/scitech/servlets/purl/1086505

Sasser, LB; Cushing, JA; Kalkwarf, DR; Mellick, PW; Buschbom, RL. (1989b). Toxicology studies on lewisite and sulfur mustard agents: Two-generation reproduction study of lewisite in rats: Final report. (PNL-6978). Richmond, VA: Pacific Northwest Laboratory. http://www.osti.gov/scitech/biblio/1086599

Sasser, LB; Cushing, JA; Lindenmeier, CW; Mellick, PW; Dacre, JC. (1999). Two-generation reproduction study of lewisite in rats. J Appl Toxicol 19: 229-235.

Sasser, LB; Cushing, JA; Mellick, PW; Kalkwarf, DR; Dacre, JC. (1996). Subchronic toxicity evaluation of lewisite in rats. J Toxicol Environ Health 47: 321-334. http://dx.doi.org/10.1080/009841096161672

Sawyer, TW; Nelson, P. (2008). Hypothermia as an adjunct therapy to vesicant-induced skin injury. Eplasty 8: e25.

SERDP (Strategic Environmental Research and Development Program). (1997). Final report of SERDP project #770. Development of military IRIS system for the hazard identification and risk assessment/characterization of defense related pollutants. (ER-770). Alexandria, VA: Environmental Security Technology Certification Program (ESTCP). http://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Risk-Assessment/ER-770

Shakil, FA; Kuramoto, A; Yamakido, M; Nishimoto, Y; Kamada, N. (1993). Cytogenetic abnormalities of hematopoietic tissue in retired workers of the Ohkunojima poison gas factory. Hiroshima J Med Sci 42: 159-165.

Snider, TH; Wientjes, MG; Joiner, RL; Fisher, GL. (1990). Arsenic distribution in rabbits after Lewisite administration and treatment with British anti-Lewisite (BAL). Fundam Appl Toxicol 14: 262-272. http://dx.doi.org/10.1016/0272-0590(90)90206-y

Thomson, JF; Savit, J; Goldwasser, E. (1947). Tests of 2,3-dimercaptopropanol and related dithiols for decontamination of lewisite on human skin. J Pharmacol Exp Ther 89: 1-13.

U.S. Army. (1941). MD(EA) memorandum report 18. Purification of water contaminated with lewisite. A toxicological study of water containing 10 ppm and 16 ppm of lewisite. (ADE471864). Edgewood Arsenal, MD: Medical Research Division.

U.S. Army. (1987). Multiple animal studies for medical chemical defense program in soldier/ patient decontamination and drug development on task 84-4: Tissue distribution of arsenic in the rabbit following subcutaneous administration of lewisite with or without british anti-lewisite therapy. (ADB117112). http://www.dtic.mil/cgi-bin/GetTRDoc?Location=U2&doc=GetTRDoc.pdf&AD=ADB117112

U.S. Army. (1989a). Toxicology studies of lewisite and sulfur mustard agents: Genetic toxicity of lewisite (L) in Chinese hamster ovary cells. (ADA206449; PNL-6922). Frederick, MD: U.S. Army Medical Research and Development Command. http://www.dtic.mil/cgi-bin/GetTRDoc?Location=U2&doc=GetTRDoc.pdf&AD=ADA216449

U.S. Army. (1989b). Toxicology studies on lewisite and sulfur mustard agents: mutagenicity of lewisite in the salmonella histidine reversion assay. (ADA213146. PNL-6872). Frederick, MD: U.S. Army Medical Research and Development Command. http://www.dtic.mil/cgi-bin/GetTRDoc?Location=U2&doc=GetTRDoc.pdf&AD=ADA213146

U.S. Army. (2000). Chronic toxicological criteria for chemical warfare compounds. Aberdeen Proving Ground, MD: Army Center for Health Promotion and Preventive Medicine. http://tera.org/iter/OTSG2000.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2002). A review of the reference dose and reference concentration processes. (EPA/630/P-02/002F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=51717

U.S. EPA (U.S. Environmental Protection Agency). (2011a). Health effects assessment summary tables (HEAST). Washington, DC: U.S. Environmental Protection Agency, Office of Emergency and Remedial Response. http://epa-heast.ornl.gov/

U.S. EPA (U.S. Environmental Protection Agency). (2011b). Recommended use of body weight 3/4 as the default method in derivation of the oral reference dose. (EPA/100/R11/0001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/raf/publications/interspecies-extrapolation.htm

U.S. EPA (U.S. Environmental Protection Agency). (2012a). 2012 Edition of the drinking water standards and health advisories [EPA Report]. (EPA/822/S-12/001). Washington, DC: Office of Water. http://water.epa.gov/action/advisories/drinking/upload/dwstandards2012.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2012b). Benchmark dose technical guidance. (EPA/100/R-12/001). Washington, DC: Risk Assessment Forum. http://www.epa.gov/raf/publications/pdfs/benchmark_dose_guidance.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2015). Integrated risk information system (IRIS) [Database]. Washington, DC: U.S. Environmental Protection Agency, Integrated Risk Information System. Retrieved from http://www.epa.gov/iris/

WHO (World Health Organization). (2015). Online catalog for the Environmental Health Criteria (EHC) monographs. Geneva, Switzerland: World Health Organization (WHO). http://www.who.int/ipcs/publications/ehc/en/

Yamakido, M; Ishioka, S; Hiyama, K; Maeda, A. (1996). Former poison gas workers and cancer: Incidence and inhibition of tumor formation by treatment with biological response modifier N-CWS. Environ Health Perspect 104: 485-488.

Yamakido, M; Shigenobu, T; Kamitsuna, A; Nishimoto, Y; Yukutake, M. (1985). The causes of death in the retired workers of Okuno-Jima poison gas factory Japan [Abstract]. Jpn J Med 24: 394.