

Provisional Peer-Reviewed Toxicity Values for  
Guanidine Compounds

(CASRN 113-00-8 Guanidine)  
(CASRN 506-93-4 Guanidine Nitrate)  
(CASRN 50-01-1 Guanidine Chloride)

Superfund Health Risk Technical Support Center  
National Center for Environmental Assessment  
Office of Research and Development  
U.S. Environmental Protection Agency  
Cincinnati, OH 45268

## **AUTHORS, CONTRIBUTORS, AND REVIEWERS**

### **CHEMICAL MANAGER**

Dan D. Petersen, PhD., DABT  
National Center for Environmental Assessment, Cincinnati, OH

### **CONTRIBUTOR**

Scott C. Wesselkamper, PhD  
National Center for Environmental Assessment, Cincinnati, OH

### **PRIMARY INTERNAL REVIEWERS**

Zheng (Jenny) Li, PhD, DABT  
National Center for Environmental Assessment, Washington, DC

Anuradha Mudipalli, MSc, PhD  
National Center for Environmental Assessment, Research Triangle Park, NC

This document was externally peer reviewed under contract to  
Eastern Research Group, Inc.  
110 Hartwell Avenue  
Lexington, MA 02421-3136

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

## TABLE OF CONTENTS

COMMONLY USED ABBREVIATIONS .....	ii
BACKGROUND .....	1
DISCLAIMERS .....	1
QUESTIONS REGARDING PPRTVs.....	1
INTRODUCTION .....	2
REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER).....	6
CASE REPORT SUMMARIES .....	11
Lambert (1966) .....	11
Jimenez-Pabon (1968) .....	11
Vroom and Engel (1969) .....	12
Nakano and Tyler (1970).....	12
Oh (1972).....	12
Oh and Kim (1973).....	12
Cherington (1976).....	13
Henriksson et al. (1977).....	13
Blumhardt et al. (1977).....	13
Streib (1979) .....	13
Jenkyn et al. (1980).....	14
Strieb and Rothner (1981).....	14
Dau and Denys (1982) .....	14
Jablecki (1984).....	14
Bady et al. (1987).....	15
Silbert et al. (1990) .....	15
Oh et al. (1997, 1998).....	15
Tim et al. (1998, 2000) .....	16
GENOTOXICITY DATA .....	16
CATEGORICAL REGRESSION ANALYSIS.....	16
DERIVATION OF PROVISIONAL REFERENCE VALUES .....	20
DERIVATION OF ORAL REFERENCE DOSES .....	22
Derivation of Subchronic Provisional RfD (Subchronic p-RfD) for Guanidine Chloride.....	22
Derivation of Chronic Provisional RfD (chronic p-RfD) Guanidine Chloride.....	24
DERIVATION OF INHALATION REFERENCE CONCENTRATIONS.....	25
DERIVATION OF PROVISIONAL CANCER POTENCY VALUES.....	25
CANCER WEIGHT OF EVIDENCE (WOE) DESCRIPTOR .....	25
APPENDIX A. PROVISIONAL SCREENING VALUES.....	27
CALCULATION OF SCREENING PROVISIONAL ORAL REFERENCES DOSES .....	27
Screening Subchronic Provisional RfD Calculations for Guanidine Compounds.....	27
Screening Chronic Provisional RfD Calculations for Guanidine Compounds.....	28
APPENDIX B. ALTERNATIVE METHODS .....	29
APPENDIX C. CATREG OUTPUT FILES.....	30
APPENDIX D. REFERENCES.....	33

## COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMCL	benchmark concentration lower confidence limit
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
ERC	effective response concentration
ERCL	effective response concentration lower confidence limit
HEC	human equivalent concentration
HED	human equivalent dose
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
LOAEL <sub>ADJ</sub>	LOAEL adjusted to continuous exposure duration
LOAEL <sub>HEC</sub>	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
NOAEL <sub>ADJ</sub>	NOAEL adjusted to continuous exposure duration
NOAEL <sub>HEC</sub>	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
POD	point of departure
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
RfC	inhalation reference concentration
RfD	oral reference dose
UF	uncertainty factor
UF <sub>A</sub>	interspecies uncertainty factor
UF <sub>C</sub>	composite uncertainty factor
UF <sub>D</sub>	database uncertainty factor
UF <sub>H</sub>	intraspecies uncertainty factor
UF <sub>L</sub>	LOAEL-to-NOAEL uncertainty factor
UF <sub>S</sub>	subchronic-to-chronic uncertainty factor
WOE	weight of evidence

## PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR GUANIDINE COMPOUNDS

### 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-duration exposures to substances of concern, to present the major conclusions reached in the hazard identification and derivation of the PPRTVs, and to characterize the overall confidence in these conclusions and toxicity values. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of this substance.

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

### DISCLAIMERS

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

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

### QUESTIONS REGARDING PPRTVs

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

## INTRODUCTION

Several salts of guanidine are used commercially, pharmaceutically, in a variety of laboratory applications, and for other purposes as identified below. Guanidine (see Figure 1 for structure) is a protonated organic base under physiological conditions, sometimes termed guanidinium. Several salts of guanidine (nitrate, sulfate, chloride, acetate, and carbonate) are also commercially available. For example, guanidine nitrate (CASRN 506-93-4) is a high production volume chemical (U.S. EPA, 1990) that is a strong oxidizer and chemically unstable (see Table 1 for chemical properties). It is explosive from friction, heat, or shock, can ignite organic materials on contact, and can emit toxic vapors including nitric acid and nitrogen oxide if heated to decomposition. The chemical is used in the manufacture of explosives, disinfectants, and photographic materials, and as a monopropellant for some model airplane engines. It is important to note that while guanidine nitrate and nitroguanidine are both explosives, they are different chemicals that are at times incorrectly assumed to be the same in some cases in the literature. The physicochemical properties of the most common guanidine compounds are shown in Table 1. Other guanidine salts have differing properties and uses. For example, guanidine hydrochloride, also known as guanidine chloride (CASRN 50-01-1), is used therapeutically to treat several rare neurologic diseases and in the laboratory as a protein denaturant. It is known to have immunosuppressive and other side effects above therapeutic doses.

Guanidine nitrate was considered “slightly” toxic in mice with an oral LD<sub>50</sub> of approximately 1,100 mg/kg (Brown et al., 1988). Other sources listed in the Hazardous Substances Data Bank (HSDB) (NLM, 2008) report slightly different values in the rat (989.6 mg/kg [males], 729.8 mg/kg [females]), and 1,105 mg/kg for male mice and 1,029 mg/kg for female mice (gavage dosing). The acute inhalation toxicity (LC<sub>50</sub>) in the rat (4-hour duration) is also given as >0.853 mg/L (>0.853 mg/m<sup>3</sup>) (Brown et al., 1988). Dermal exposure of rabbits produced only minor dermal irritation at 2 g/kg in the same report.

Guanidine chloride has roughly similar (within a factor of two) acute toxicities to guanidine nitrate. Guanidine chloride is listed as having an oral LD<sub>50</sub> of 475 mg/kg in the rat (treatment conditions not specified) (NLM, 2008). Ecotoxicology data from Brown et al. (1988) reports an EC<sub>50</sub> (48-hour duration) for guanidine nitrate of 70.2 mg/L in *Daphnia magna*, a cladoceran freshwater crustacean, with a lowest-observed-effect concentration (LOEC) of 4.2 mg/L and a no-observed-effect concentration (NOEC) of 2.9 mg/L based on immobilization as the endpoint. In the less sensitive fathead minnow species (*Pimephales promelas*), the LOEC was 424 mg/L and the NOEC was 181 mg/L (96-hour duration).

Available toxicity values for guanidine compounds are summarized in Table 2. There is little information in the literature relative to the potential toxicity of guanidine or guanidine compounds following subchronic- or chronic-duration exposure via the oral or inhalation routes.

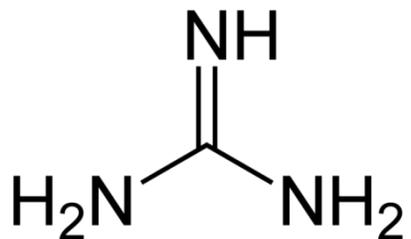


Figure 1. Guanidine Structure

Table 1. Chemical Properties of Guanidine Compounds		
Property (unit)	Guanidine Nitrate (CASRN 506-93-4)	Guanidine Chloride (CASRN 50-01-1)
Molecular Weight (g/mol)	122.08 <sup>a</sup>	95.53 <sup>d, e</sup>
Boiling point (°C)	Decomposes <sup>b</sup>	Decomposes <sup>d</sup>
Melting point (°C)	213–215 <sup>a</sup>	180–185 <sup>d, e</sup>
Density (g/cm <sup>3</sup> )	1.44 <sup>a</sup>	1.34 <sup>d, e</sup> –1.35 <sup>f</sup>
Vapor pressure (Pa at 25°C)	Not available	Not available
pH (aqueous )	8–9.5 <sup>e</sup>	7–8 <sup>d</sup>
Solubility in water (g/100 mL at 20°C)	130 g/L <sup>a, c</sup>	2,000 g/L <sup>d</sup> –2,280 g/L <sup>e, f</sup>
Relative vapor density (air = 1)	Not available	Not available

<sup>a</sup>Source: Chemical Book (no publication date [a], accessed 2-19-2014; guanidine nitrate).

<sup>b</sup>Source: NLM (2008).

<sup>c</sup>Source: CHEMICALLAND21 (no publication date, accessed 2-19-2014; guanidine nitrate).

<sup>d</sup>Source: CHEMICALLAND21 (no publication date, accessed 2-19-2014; guanidine hydrochloride).

<sup>e</sup>Source: Chemical Book (no publication date [b], accessed 2-19-2014; guanidine hydrochloride).

<sup>f</sup>Source: Chemical Book (no publication date [c], accessed 2-19-2014; guanidine hydrochloride).

<b>Table 2. Summary of Available Toxicity Values for Guanidine Nitrate</b>				
<b>Source/ Parameter<sup>a</sup></b>	<b>Value (Applicability)</b>	<b>Notes</b>	<b>Reference</b>	<b>Date Accessed</b>
<b>Noncancer</b>				
ACGIH	NV	NA	ACGIH (2013)	NA
ATSDR	NV	NA	ATSDR (2013)	NA
Cal/EPA	NV	NA	Cal/EPA (2013a,b) <sup>b</sup>	10-17-2013
NIOSH	NV	NA	NIOSH (2010)	NA
OSHA	NV	NA	OSHA (2006, 2011)	NA
IRIS	NV	NA	U.S. EPA (2013)	10-17-2013
Drinking water	NV	NA	U.S. EPA (2012)	NA
Drinking water (Nitroguanidine)	RfD: $1 \times 10^{-1}$ mg/kg-d	Nitroguanidine is a different but structurally related compound, which is also an explosive. The RfD is based on reduced body weights of rats (NOAEL of 316 mg/kg-d and a composite uncertainty factor of 3,000).	U.S. EPA (2012)	NA
HEAST	NV	NA	U.S. EPA (2011)	NA
CARA HEEP	NV	NA	U.S. EPA (1985, 1994)	NA
WHO	NV	NA	WHO	10-17-2013
U.S. Army (Guanidine nitrate)	RfD: $5 \times 10^{-1}$ mg/kg-d	This value is a Performance Standard for guanidine nitrate with a surrogate RfD (based on guanidine chloride) of $5 \times 10^{-1}$ mg/kg-d, intended as a guide in military cleanup activities.	U.S. Army (2012)	NA
NLM	NV	Mononitrate guanidine is included in the Hazardous Substance Data Bank as cited by ACGIH (2010). This citation mentions human health effects (erythrocyte hemolysis in vitro, and respiratory and dermal toxicity in vivo), and nonhuman toxicity and ecotoxicity values, but it does not include human exposure limits.	NLM (2008)	NA
<b>Cancer</b>				
IRIS	NV	NA	U.S. EPA	10-17-2013
HEAST	NV	NA	U.S. EPA (2011)	10-17-2013
IARC	NV	NA	IARC (2013)	NA
NTP	NV	NA	NTP (2011)	NA

<b>Table 2. Summary of Available Toxicity Values for Guanidine Nitrate</b>				
<b>Source/ Parameter<sup>a</sup></b>	<b>Value (Applicability)</b>	<b>Notes</b>	<b>Reference</b>	<b>Date Accessed</b>
Cal/EPA	NV	NA	Cal/EPA (2009, 2013b)	NA
ACGIH	NV	NA	ACGIH (2013)	NA

<sup>a</sup>Sources: Integrated Risk Information System (IRIS) database; Health Effects Assessment Summary Tables (HEAST); International Agency for Research on Cancer (IARC); National Toxicology Program (NTP); California Environmental Protection Agency (Cal/EPA); American Conference of Governmental Industrial Hygienists (ACGIH); Agency for Toxic Substances and Disease Registry (ATSDR); National Institute for Occupational Safety and Health (NIOSH); National Library of Medicine (NLM); Occupational Safety and Health Administration (OSHA); Chemical Assessments and Related Activities (CARA) list; Health and Environmental Effects Profile (HEEP); World Health Organization (WHO).

<sup>b</sup>The Cal/EPA Office of Environmental Health Hazard Assessment (OEHHA) Toxicity Criteria Database (<http://oehha.ca.gov/tcdb/index.asp>) was also reviewed and found to contain no information on guanidine compounds (CASRN 113-00-8 guanidine; CASRN 506-93-4 guanidine nitrate; CASRN 50-01-1 guanidine chloride).

NA = not applicable; NV = not available; NOAEL = no-observed-adverse-effect level; RfD = reference dose.

Literature searches were conducted on sources published from 1900 through September 2013 for studies relevant to the derivation of provisional toxicity values for guanidine (CASRN 113-00-8), guanidine nitrate (CASRN 506-93-4), and guanidine chloride (CASRN 50-01-1). Searches were conducted using EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: AGRICOLA; American Chemical Society; BioOne; Cochrane Library; DOE: Energy Information Administration, Information Bridge, and Energy Citations Database; EBSCO: Academic Search Complete; GeoRef Preview; GPO: Government Printing Office; Informaworld; IngentaConnect; J-STAGE: Japan Science & Technology; JSTOR: Mathematics & Statistics and Life Sciences; NSCEP/NEPIS (EPA publications available through the National Service Center for Environmental Publications [NSCEP] and National Environmental Publications Internet Site [NEPIS] database); PubMed: MEDLINE and CANCERLIT databases; SAGE; Science Direct; Scirus; Scitopia; SpringerLink; TOXNET (Toxicology Data Network): ANEUP, CCRIS, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HEEP, HMTC, HSDB, IRIS, ITER, LactMed, Multi-Database Search, NIOSH, NTIS, PESTAB, PPBIB, RISKLINE, and TRI; TSCATS; Virtual Health Library; Web of Science (searches Current Content database among others); World Health Organization; and Worldwide Science. The following databases outside of HERO were searched for relevant health information: ACGIH, ATSDR, Cal/EPA, EPA IRIS, EPA HEAST, EPA HEEP, EPA OW, EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

### **REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)**

The effects of oral or inhalation exposure to guanidine, guanidine nitrate, and guanidine chloride have not been evaluated in any repeated-dose subchronic or chronic-duration, developmental toxicity, reproductive toxicity, or carcinogenicity studies that are publically available, in either humans or animals. However, in humans, guanidine chloride has been used as an orally administered therapeutic in the management of Lambert-Eaton Myasthenic Syndrome (LEMS), a rare autoimmune disease in which autoantibodies directed against voltage-gated calcium channels at the presynaptic side of the neuromuscular junction result in muscle weakness and autonomic dysfunction (see list of case reports in Table 3). Thus, the occurrence of side effects induced by LEMS treatment with guanidine chloride is used as a basis for the identification of toxicological effects for the derivation of provisional reference doses (p-RfDs) in this PPRTV assessment. Examination of all citations listed within references identified from the initial literature search revealed additional relevant case reports on LEMS patients.

**Table 3. Summary of Potentially Relevant Data in LEMS Patients Treated Therapeutically With Guanidine Chloride**

GC Dosimetry <sup>a</sup>	Effects Observed	NOAEL	LOAEL	Reference	Comments	Notes <sup>b</sup>
<b>Human</b>						
<b>1. Oral</b>						
20–30 mg/kg-d	Paresthesias (mouth)	NDr		Lambert (1966)	<i>N</i> = 1 Short-term-duration	PR; clear clinical and electromyographic (EMG) improvement.
14–42 mg/kg-d	Patient 1: gastrointestinal (GI; frequent bowel movements), central nervous system (CNS; paresthesias , agitation, insomnia, behavioral disturbances  Patient 2: diarrhea and nervous irritability	NDr		Kennedy and Jimenez-Pabon (1968)	<i>N</i> = 2 Durations not classifiable	PR; improvement in strength.
NA	None identified	NDr		Vroom and Engel (1969)	<i>N</i> = 1 Duration not classifiable	PR; no clinical or EMG improvement.
21 mg/kg-d	Atrial fibrillation and hypotension	NDr		Nakano and Tyler (1970)	<i>N</i> = 1 Short-term-duration	PR
12 mg/kg-d	None identified	12 mg/kg-d (subchronic- duration)		Oh (1972)	<i>N</i> = 1 Subchronic-duration	PR; “dramatic” clinical improvement.
12–58 mg/kg-d	Patient 1 = leukopenia and mild thrombocytopenia  Patient 2 = paresthesias of tongue and fingers, confusion, unsteady gait  Patient 3 = none	NDr	12 mg/kg-d (subchronic- duration)	Oh and Kim (1973)	<i>N</i> = 3 Patient 1 = short-term duration;  Patient 2 = subchronic-duration;  Patient 3 = duration not provided.	PR; clinical improvement.

**Table 3. Summary of Potentially Relevant Data in LEMS Patients Treated Therapeutically With Guanidine Chloride**

GC Dosimetry <sup>a</sup>	Effects Observed	NOAEL	LOAEL	Reference	Comments	Notes <sup>b</sup>
21–27 mg/kg-d	Nephrotoxicity (chronic-duration interstitial nephritis, and leukopenia)	NDr	24 mg/kg-d (subchronic)	Cherington (1976)	<i>N</i> = 1 Subchronic-duration	PR
18–19 mg/kg-d	Patient 1 = renal, hepatic, and pancreatic damage, anemia paresthesias  Patient 2 = paresthesias, cold sensations and pancytopenia	NDr	18 mg/kg-d (subchronic-duration)	Herricksson et al. (1977)	<i>N</i> = 2 Patient 1 = subchronic-duration;  Patient 2 = short-term-duration.	PR
14 mg/kg-d	Fibrosing interstitial nephritis	NDr	14 mg/kg-d (chronic-duration)	Blumhardt et al. (1977)	<i>N</i> = 1 Chronic-duration	PR; clinical improvement
30 mg/kg-d	Colicky pain, postprandial fullness, bloating, dark-colored urine	NDr	NDr	Streib (1979)	<i>N</i> = 1 Short-term-duration	PR; also administered physostigmine (dose not provided). EMG improvement.
14–21 mg/kg-d	Leukopenia	NDr	NDr	Jenkyn et al. (1980)	<i>N</i> = 1 Short-term-duration	
12–45 mg/kg-d	Mild GI upset at 20 mg/kg-d; limb and perioral paresthesias at 32 mg/kg-d; nausea, tremors, confusion, agitation at 45 mg/kg-d	12 mg/kg-d (subchronic-duration)	NDr	Streib and Rothner (1981)	<i>N</i> = 3 Subchronic-durations [Patient 2 was previously recorded in Streib (1979)];  Patient 3 received no guanidine	PR; EMG and strength improvement.

**Table 3. Summary of Potentially Relevant Data in LEMS Patients Treated Therapeutically With Guanidine Chloride**

GC Dosimetry <sup>a</sup>	Effects Observed	NOAEL	LOAEL	Reference	Comments	Notes <sup>b</sup>
16–29 mg/kg-d	Numbness, paresthesias, coldness, emesis, renal dysfunction	18 mg/kg-d (subchronic-duration)	16 mg/kg-d (chronic-duration)	Dau and Denys (1982)	<i>N</i> = 5 Patients 1 and 3 = subchronic-duration; Patient 2 = short-term-duration; Patient 4 = chronic-duration; Patient 5 = duration and dose not reported.	PR; other treatments concurrently administered. Partial relief of symptoms.
5 mg/kg-d	None reported	NDr	NDr	Jablecki (1984)	<i>N</i> = 1 Subchronic-duration	PR; slight improvement in strength.
10 mg/kg-d	None reported	NDr	NDr	Bady et al. (1987)	<i>N</i> = 1 Acute-duration	PR; EMG improvement.
5–20 mg/kg-d	Leukopenia	5 mg/kg-d (subchronic-duration)	12 mg/kg-d (subchronic-duration)	Silbert et al. (1990)	<i>N</i> = 1 Subchronic-duration	PR; reduction in muscular weakness.
5–36 mg/kg-d	Fainting spells, nausea, vomiting, epigastric pain, diarrhea, paresthesias (fingers), insomnia	5 mg/kg-d (subchronic-duration) 7 mg/kg-d (chronic-duration)	NDr	Oh et al. (1997)	<i>N</i> = 9 Patient 4 = acute-duration; Patients 1 and 5 = short-term-duration; Patients 2, 3, 6, 7, and 9 = subchronic-duration; Patient 8 = chronic-duration	PR; also administered physostigmine. Clinical and EMG improvement.
6 mg/kg-d	None reported	NDr	NDr	Oh et al. (1998)	<i>N</i> = 1 Short-term-duration	PR; case report on one additional patient from Oh et al. (1997).

<b>Table 3. Summary of Potentially Relevant Data in LEMS Patients Treated Therapeutically With Guanidine Chloride</b>						
<b>GC Dosimetry<sup>a</sup></b>	<b>Effects Observed</b>	<b>NOAEL</b>	<b>LOAEL</b>	<b>Reference</b>	<b>Comments</b>	<b>Notes<sup>b</sup></b>
Not Reported	None reported	NDr	NDr	Tim et al. (1998)	<i>N</i> = 57 Duration not classifiable	PR; 7 of 57 patients received GC with “moderate” improvement in 4 out of 7.
Not Reported	None reported	NDr	NDr	Tim et al. (2000)	<i>N</i> = 73 Durations not classifiable	PR; other treatments concurrently administered. Nine of 73 patients received GC with “marked” improvement in 0%, “moderate” improvement in 56%, “minimal” improvement in 11%, and no improvement in 33%.

<sup>a</sup>Doses are converted to mg/kg-d. If no patient weight was provided in the case reports, a default value of 70 kg was used in the conversion. If a range of doses was used and details not specified, the mean of the range was used. Chronic-duration is defined as greater than 7 years; subchronic-duration is defined as greater than one year; short-term-duration is defined as less than one year; acute duration refers to a single dose.

<sup>b</sup>PR = Peer reviewed, NDr = not determined.

## CASE REPORT SUMMARIES

As stated previously, the effects of oral or inhalation exposure to guanidine compounds have not been evaluated in any repeated-dose subchronic-duration, chronic-duration, developmental toxicity, reproductive toxicity, or carcinogenicity publically available studies conducted in either humans or animals. However, case reports on the treatment of LEMS in humans by oral administration of guanidine chloride are reviewed below. Because LEMS results from a lowering in acetylcholine release, some therapies involve cholinesterase inhibitors, such as pyridostigmine, or potassium channel blockers (e.g., guanidine compounds, 4-aminopyridine, 3,4-diaminopyridine) which increase acetylcholine release. Guanidine inhibits potassium channels by binding within the intracellular pore of the channel, whereby it interacts with its hydrophobic subunit leading to the channel's closure (Kalia and Shwatz, 2011). Given that LEMS is autoimmune in nature; immunosuppressive agents like the corticosteroid prednisone have also been used therapeutically, sometimes in addition to guanidine.

The most important limitation in the use of guanidine compounds in the treatment of LEMS is the occurrence of side effects, which include gastrointestinal effects (including nausea and diarrhea), immunosuppression, and distal paresthesias. Throughout this section and in Table 3 above, the word "symptom" is used to describe the severity of the muscular weakness or other conditions characterized by LEMS, while "side effects" refer to negative effects of the guanidine compound. The current treatment of choice for LEMS appears to be 3,4-diaminopyridine, but guanidine compounds are still occasionally used, in part due to the unavailability of 3,4-diaminopyridine in some countries, including the United States. The available data comes primarily from case reports of individual patients or small groups of LEMS patients that received orally administered guanidine chloride. Only those case reports that accurately report the dosimetry and side effects that might potentially identify points of departure (PODs) or reveal additional side effects are summarized here. The purity of the guanidine chloride used was not reported in any of the case reports, but current products have purities of 99% or greater. The durations that patients were treated with guanidine chloride range from acute (i.e., single doses) to chronic durations. For the LEMS patients, treatment durations greater than 7 years were considered to be chronic-duration, those less than 12 months were considered short-term, and those between 12 months and 84 months were considered to be subchronic durations.

### **Lambert (1966)**

In an early case report, Lambert (1966) reported the treatment of a LEMS patient with oral guanidine chloride in addition to several other potential treatments. Guanidine chloride was used at doses of 20–30 mg/kg-day for at least 13 days, but the definitive treatment duration is unknown. There was a reduction in the patient's neuromuscular weakness with treatment, and the only side effect reported were paresthesias in the mouth that diminished with time. The study author noted that the effect on the neuromuscular transmission peaked 45 to 60 minutes after guanidine chloride administration, while the increase in strength lasted 3 to 4 days after the discontinuation of the guanidine chloride. Neither a no-observed-adverse-effect level (NOAEL) nor a lowest-observed-adverse-effect (LOAEL) is established for this paresthesia because the duration of treatment was short-term.

### **Jimenez-Pabon (1968)**

In a case report of two LEMS patients (both with lung cancer), Kennedy and Jimenez-Pabon (1968) reported treatment with guanidine chloride. Patient 1 was treated with

doses ranging from 14–42 mg/kg-day for approximately 5 months. At 32 mg/kg-day, there was a resolution of the muscle weakness symptoms, but side effects occurred including paresthesias of the mouth and tongue, momentary blurred vision, and an increase in the frequency of bowel movements. Increasing the dose to 42 mg/kg-day caused agitation and more severe central nervous system side effects including agitation, insomnia and behavioral effects. The behavioral symptoms subsided when the dose was reduced to 25 mg/kg-day. Patient 2 was treated with 25 mg/kg-day guanidine chloride for 27 days but saw no resolution of symptoms, while side effects including severe diarrhea and nervous irritability were noted. While these were short-term treatments, an accurate determination of the treatment duration could not be made (the initiation of therapy is given as “July”). Neither a NOAEL nor a LOAEL is established.

#### **Vroom and Engel (1969)**

In a case report from Vroom and Engel (1969), the study authors describe the treatment of a 17-year-old female patient with LEMS with guanidine chloride (no dose reported), which produced minimal resolution of the patient’s muscle weakness. No duration of treatment or side effects were reported. It was noted that prednisone produced a significant improvement at 10 mg/day. Because no dose was reported for the guanidine treatment, neither a NOAEL nor a LOAEL is identified.

#### **Nakano and Tyler (1970)**

In a case report from Nakano and Tyler (1970) reported the treatment of a LEMS patient with 21 mg/kg-day guanidine hydrochloride. Atrial fibrillation and hypotension ensued after 6 days, and the heart rhythm returned to normal when the treatment was discontinued. When guanidine treatment was reinstated 36 hours later, the patient again developed hypotension and atrial fibrillation. Neither a NOAEL nor a LOAEL is established since the observed cardiovascular effects could be considered frank effects, and the treatment duration (6 days) is considered short-term.

#### **Oh (1972)**

A 50-year-old male with LEMS was treated with 12 mg/kg-day guanidine chloride for 2 years in a case report from Oh (1972). No side effects were noted, while the symptoms resolved for the duration of the study. This dose (12 mg/kg-day) is considered a NOAEL, and the treatment is considered of subchronic-duration.

#### **Oh and Kim (1973)**

Data from three LEMS patients were presented in a case report from Oh and Kim (1973). Patient 1 was a 56-year-old male with a 2-month history of fatigability and muscle weakness. The patient was treated with guanidine chloride with a gradual increase in dosage up to 58.3 mg/kg-day in 11 days. Leukopenia ( $2,200$  white blood cells [WBCs]/ $m^3$ ) and mild thrombocytopenia ( $113,000$  thrombocytes/ $m^3$ ) were noted. The dosage was reduced to 29.2 mg/kg-day and blood cell counts returned to normal over the next 3 weeks. The patient was continuously treated with guanidine chloride for approximately 2 months. Patient 2 was a 50-year-old male with a 3-month history of fatigability and muscle weakness, who was treated with 12 mg/kg-day for approximately 2 years. Patient 2 exhibited both peripheral (paresthesias of tongue and finger) and central (confusion and gait disturbances) nervous system side effects. Patient 3 was a 64-year-old male who was treated with 32.8 mg/kg-day guanidine chloride (duration not reported). No side effects were noted. Because the duration of treatment was

short-term for Patient 1, and unknown for Patient 3, neither a NOAEL nor a LOAEL is established from these patients. For Patient 2, a subchronic-duration LOAEL of 12 mg/kg-day is established for peripheral nervous system effects (paresthesias).

#### **Cherington (1976)**

In a case report from Cherington (1976) a 38-year-old male with LEMS was treated with 21–27 (average = 24) mg/kg-day guanidine chloride for 4 years. At that point, guanidine chloride was discontinued because of side effects that included leukopenia (depressed WBC counts), nephrotoxicity (elevated blood urea nitrogen [BUN]), decreased serum calcium (8.2 mg/100 mL) and creatine (17.4 mg/100 mL), and elevated serum phosphate (5.7 mg/100 mL). According to the study author, blood chemistry results obtained prior to the initiation of guanidine chloride treatment were within normal limits. Nephrotoxicity was confirmed by biopsy. A subchronic-duration LOAEL of 24 mg/kg-day is established for leukopenia and nephrotoxicity.

#### **Henriksson et al. (1977)**

A case report from Henriksson et al. (1977) described the treatment of two LEMS patients with guanidine chloride. Patient 1 was treated with 18 mg/kg-day guanidine chloride for 12 months. At that time, renal (i.e., abnormal serum creatine levels), and pancreatic and hepatic (abnormal alkaline phosphatase, gamma glutamate transferase, alanine and aspartate aminotransferases, amylase) and anemia (hemoglobin = 79 g/L) side effects were noted. Patient 2 was treated with 19 mg/kg-day for 7 months, and paresthesias and cold sensations were noted as well as pancytopenia [hemoglobin = 105 g/L,  $60 \times 10^9$ /L thrombocytes,  $2 \times 10^9$ /L leukocytes (30% polymorphonuclear)]. Guanidine treatment was discontinued in both patients because of these side effects. A LOAEL is established for serum chemistry changes indicative of damage to multiple organs.

#### **Blumhardt et al. (1977)**

In a case report published by Blumhardt et al. (1977), a 61-year-old male patient with LEMS was treated chronically with guanidine chloride at 14 mg/kg-day for 7 years. The most notable side effect was interstitial fibrosis in both the cortex and medulla of the kidney. The study authors thought the guanidine chloride was responsible for the nephrosis. A chronic-duration LOAEL of 14 mg/kg-day is established.

#### **Streib (1979)**

In a case report published by Streib (1979), a 62-year-old male patient with LEMS was treated with 30 mg/kg-day guanidine chloride for 10 days, with electromyographic (EMG) improvement. The patient complained of gastrointestinal (GI) symptoms (colicky pain, nausea, postprandial fullness, bloating) and dark-colored urine. Blood chemistry values for total bilirubin, alkaline phosphatase, transaminases; serum glutamic oxaloacetic transaminase (SPOT) and serum glutamic pyruvic transaminase (SGOT), lactate dehydrogenase, and porphobilinogen, which had been within normal limits, became elevated by more than 10%. After discontinuation of the guanidine chloride treatment, the blood chemistry values returned toward pretreatment levels. These observed serum chemistry changes are consistent with liver injury, which has rarely been mentioned in other case reports. No NOAEL or LOAEL is established because of the short-term treatment duration.

### **Jenkyn et al. (1980)**

In a case report from Jenkyn et al. (1980), a 62-year-old male patient with LEMS was treated with guanidine chloride initially at 14 mg/kg-day. The patient reported a significant subjective increase in muscle strength. Two months later, the patient complained of increasing weakness, and the dose was increased to 21 mg/kg-day. Two months following this dosage increase, however, mild leukopenia was noted and the dose was reduced to 18 mg/kg-day. The latter dosing regime was continued for another 5 months. No NOAEL or LOAEL is established because of the short-term durations of exposure.

### **Strieb and Rothner (1981)**

The treatment of three LEMS patients with guanidine chloride is described a series of case reports from Strieb and Rothner (1981). Patient 1 was treated with several different doses of guanidine chloride, with differing side-effects noted at each dose. The patient was initially treated with 20 mg/kg-day, and mild gastrointestinal upset was noted. At 32 mg/kg-day, peripheral paresthesias (limb and perioral) were noted. At 45 mg/kg-day, nausea was constant, coarse tremors developed, and the patient became confused and agitated. The dose was subsequently reduced to 12 mg/kg-day and was maintained for 3 years. Patient 2 was previously described in Streib (1979), and a third patient was not treated with guanidine chloride. A subchronic-duration NOAEL of 12 mg/kg-day is established for Patient 1.

### **Dau and Denys (1982)**

In a series of case reports on five LEMS patients, Dau and Denys (1982) report the use of plasmapheresis and plasmapheresis combined with immunosuppressive drugs in addition to guanidine chloride. Patient 1 was treated with 18 mg/kg-day for 18 months without significant side effects. Patient 2 was treated with 29 mg/kg-day for 6 months, but treatment was discontinued after acral paresthesias developed. Patient 3 (also described in Cherington [1976]) was treated with 24 mg/kg-day but developed interstitial nephritis after 4 years of guanidine chloride therapy. Patient 4 was treated with 16 mg/kg-day guanidine chloride for 12 years, and side effects included mild nausea that was treatable with antacids. Patient 5 was treated with guanidine chloride (dose not reported) for 1 month, with side effects that included nausea, paresthesias, and cold extremities. A subchronic-duration NOAEL of 18 mg/kg-day is established for Patient 1. The limited duration (acute) of treatment for Patient 2 precludes the establishment of a NOAEL or LOAEL. A subchronic-duration LOAEL of 24 mg/kg-day was previously characterized for Patient 3 in Cherington et al. (1976). A chronic-duration LOAEL of 16 mg/kg-day is established for Patient 4. No NOAEL or LOAEL is established for Patient 5 because of the incomplete reporting of dosimetry.

### **Jablecki (1984)**

In a case report published by Jablecki (1984), a 50-year-old female LEMS patient was treated with guanidine hydrochloride for 6 months. The initial dose was 5 mg/kg-day, and this was slowly increased to an unreported dose. Slight clinical improvement in strength was reported over the 6 month treatment; however, therapy was discontinued due to hemoptysis (likely due to oat-cell carcinoma of lung). Because of the incomplete reporting of dosimetry, no NOAEL or LOAEL is established.

**Bady et al. (1987)**

In a case report published by Bady et al. (1987), a 4-year-old female with LEMS was treated acutely (single dose) with 10 mg/kg guanidine chloride. Increased muscle tone was reported with no side effects. Because of the acute treatment duration, no NOAEL or LOAEL is established.

**Silbert et al. (1990)**

A 60-year old female patient with LEMS was treated chronically with guanidine chloride for approximately 5 years (Silbert et al., 1990). This case is particularly interesting since several doses were tried, and a dose response in the severity of side effects observed. In this case report, the patient was treated initially with 10 mg/kg-day. Resolution of the muscle weakness ensued, and the dose was increased to 20 mg/kg-day, with muscle power returning to baseline levels. Five weeks later, however, blood tests revealed leukopenia ( $3.5 \times 10^9/L$  WBCs,  $2.27 \times 10^9/L$  neutrophils,  $0.91 \times 10^9/L$  lymphocytes), and the dose was reduced to 12 mg/kg-day. The repeat blood test again revealed leukopenia one week later ( $2.8 \times 10^9/L$  WBCs,  $1.14 \times 10^9/L$  neutrophils,  $1.23 \times 10^9/L$  lymphocytes). Therapy was discontinued, and a repeat blood test conducted 4 days later showed normal WBC counts ( $6.6 \times 10^9/L$ ). A 10 mg/kg dose of guanidine chloride was reinstated every other day (5 mg/kg-day). Therapy was continued for approximately 5 years without the return of side effects until the patient's death (unrelated to therapy).

This particular case report was notable in that it revealed an apparently steep dose-response curve within a single patient, allowing the determination of doses where chronic-duration guanidine chloride treatment elicited no or negligible negative side effects, but retention of the positive therapeutic effects and relief of LEMS symptoms. A subchronic-duration NOAEL of 5 mg/kg-day (i.e., 10 mg/kg given on alternate days) is established. A subchronic-duration LOAEL of 12 mg/kg-day is established for leukopenia.

**Oh et al. (1997, 1998)**

A series of case reports is presented involving nine LEMS patients treated with guanidine chloride was published by Oh et al. (1997). The duration of treatment ranged from 3 to 102 months. Patient 1 (a 46-year-old female) received 5 mg/kg-day for 20 months, with no reported side effects. Patient 2 (a 26-year-old female) initially received 27 mg/kg-day for 10 months, but this dose was reduced because of a fainting spell. Patient 2 continued treatment with 5 mg/kg-day for another 10 months, but this dose was discontinued because of nausea. Patient 3 (a 53-year-old female) received 21 mg/kg-day, but the dose was reduced to 5 mg/kg-day due to nausea and vomiting. The duration of treatment for Patient 3 was 55 months. Patient 4 (a 58-year-old male) was initially treated with doses of 21, 27, and 36 mg/kg-day, but the dosage was reduced to below 15 mg/kg-day within 3 months because of paresthesias of the fingers, insomnia, and diarrhea. Treatment of Patient 4 continued for approximately 4 years. Patient 5 (a 70-year-old male) was treated with 5 mg/kg-day for 5 months, but treatment was discontinued because of nausea. Patient 6 (a 75-year-old male) was treated with 7 mg/kg-day for 12 months and experienced some abdominal pain. Patient 7 (a 59-year-old female) was treated with 10 mg/kg-day for 57 months with no side effects. Patient 8 (a 36-year-old female) received the longest treatment duration with 7 mg/kg-day for 102 months, with relief of LEMS symptoms and no reported side effects. Patient 9 (53-year-old male) was treated for 36 months with 9 mg/kg-day, with resolution of muscular weakness but the appearance of paresthesias. From these nine cases, a subchronic-duration NOAEL of

5 mg/kg-day is established for Patient 1, a subchronic-duration NOAEL of 11 mg/kg-day is established for Patient 7, and a chronic-duration NOAEL of 7 mg/kg-day is established for Patient 8. A subsequent review by Oh et al. (1998) added one additional patient treated with 6 mg/kg-day guanidine chloride for 7 months with no side effects. However, the duration is considered short-term for this additional patient, thus no NOAEL or LOAEL is established.

**Tim et al. (1998, 2000)**

The reviews by Tim et al. (1998, 2000) incompletely report the data, and neither guanidine chloride treatment duration nor dose could be determined. As such, no NOAELs or LOAELs are established.

**GENOTOXICITY DATA**

In the Ames test, guanidine nitrate has tested negative at 5,000 µg/plate in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 with or without metabolic activation. The chemical was also negative for chromosomal aberrations in hamster fibroblasts (NLM, 2008). Guanidine chloride was also negative in the Hamster chromosome aberration test at 5 mg/ml, and in the Ames test in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1527, and TA1538 at up to 5,000 µg/plate with or without metabolic activation (NLM, 2008).

**CATEGORICAL REGRESSION ANALYSIS**

Since guanidine compounds act at the cholinergic neuromuscular junction when used therapeutically, the methods used by Dourson et al. (1997) on categorical regression of aldicarb (a carbamate insecticide that causes a similar elevation of acetylcholine) were followed as an example since the constellations of side effects are similar. Dourson et al. (1997) used four severity grades to characterize the observed effects, including severe effects, moderate, mild, and none (no effects), with corresponding assigned values of 4, 3, 2, and 1, respectively. Using these methods in the aldicarb IRIS assessment (U.S. EPA, 1993), the oral RfD was based on a POD for a Level 2 effect (sweating), which was considered a NOAEL. For this PPRTV assessment, the severity scores associated with the various side effects following guanidine chloride treatment of LEMS patients are shown in Table 4.

<b>Table 4. Description of Severity Scores Associated with Side Effects Following Guanidine Chloride Treatment of LEMS Patients</b>		
<b>Category</b>	<b>Side Effects</b>	<b>Potential POD Groups</b>
Category 1 (None)	No effects.	NOAELs
Category 2 (Mild)	Serum chemistry changes <10% or within one standard deviation.	NOAELs
Category 3 (Moderate)	Larger (>10%) serum chemistry changes, twitching, blurred vision, watery eyes, excess salivation, hyperactivity, mild leukopenia, sweating, clamminess, paresthesias, renal histopathology, or mild gastrointestinal effects including irritation managed by antacids.	LOAELs
Category 4 (Severe)	Atrial fibrillation; hypotension; severe leukopenia; severe gastrointestinal effects including unmanageable nausea, vomiting, or diarrhea; or central nervous system effects including disorientation, confusion or seizures.	LOAELs or frank effect levels (FELs), depending on severity.

Table 5 summarizes the oral doses given to LEMS patients in the case reports. The duration of treatment in these cases ranges from acute to chronic-duration (single doses to 120 months). The most common side effects included gastrointestinal effects in 7 of 35 patients and various paresthesias in 7 of 35 patients. Other less common side effects included renal effects (in 3 of 35 patients) and various blood anemias and dyscrasias (most commonly leukopenia), (in 5 of 35 patients). All of these observed side effects occurred at doses at or above 10 mg/kg-day. A review of the available data reveals an apparent dose dependence of the side effects following treatment, with no effects to mild effects observed between 5–10 mg/kg-day, various mild to moderate effects between 10–30 mg/kg-day, and more severe effects  $\geq 30$  mg/kg-day. In particular, the results of Silbert et al. (1990) showing immunosuppression and Streib and Rothner (1981) showing gastrointestinal and peripheral and central nervous system effects indicate an increasing severity of these side effects with increasing dose within individual patients.

Because much of the human data shown in Table 5 are from case reports of individual patients with varying treatment durations, a conventional dose-response analysis of the data was not possible. However, the overall approach used in categorical regression is useful in characterizing and visualizing the data. In some of the clinical cases, LEMS patients were started on a low dose of guanidine chloride and then titrated up in dose until side effects became significant. Thus, there is sometimes a description of a dose-dependent decrease in LEMS symptoms and a concomitant increase in side effects within the available data from a single patient. In the cases where the study authors carefully describe what side effects occurred at what dose, more than one severity score may be identified for an individual patient.

**Table 5. Severity Scoring of Guanidine Chloride Case Reports**

<b>Dosimetry<sup>a</sup></b>	<b>Effects Observed</b>	<b>Severity Score<sup>b</sup></b>	<b>Reference</b>
10–30 mg/kg-d, 13 d	Paresthesias (mouth and tongue),	3 at 30 mg/kg-d	Lambert (1966)
Patient 1: 14–42 mg/kg-d, 5 mo Patient 2: 25 mg/kg-d, 27 d	GI distress, paresthesias, agitation, insomnia forgetfulness Severe diarrhea, nervous irritability	3 at 32mg/kg-d, 4 at 42 mg/kg-d 4 at 25 mg/kg-d	Kennedy and Jimenez-Pabon (1968)
NA	None identified	NA	Vroom and Engel (1969)
21 mg/kg-d, 1 wk	Atrial fibrillation and hypotension	4 at 21 mg/kg-d	Nakano and Tyler (1970)
12 mg/kg-d, 24 mo	None identified	1 at 12 mg/kg-d	Oh (1972)
12–58 mg/kg-d, 2–24 mo	Patient 1: Leukopenia, thrombocytopenia. High dose = 11 d, low dose = 2 mo Patient 2: Paresthesias at 2 yr, confusion, unsteady gait P3: None	Patient 1: 4 at 58 mg/kg-d, 3 at 29 mg/kg-d Patient 2: 4 at 12 mg/kg-d Patient 3: 1 at 32.8 mg/kg-d	Oh and Kim (1973)
21–27 mg/kg-d 48 mo	Chronic interstitial nephritis and leukopenia	3 at 24 mg/kg-d	Cherington (1976)
Patient 1: 18 mg/kg-d, 12 mo Patient 2: 19 mg/kg-d, 7 mo	Renal, pancreatic, anemia, hepatic damage Bone marrow damage (pancytopenia)	Patient 1: 4 at 18 mg.kg-d Patient 2: 4 at 19 mg/kg-d	Henriksson et al. (1977)
14 mg/kg-d, 84 mo	Fibrosing interstitial nephritis	3 at 14 mg/kg-d	Blumhardt et al. (1977)
30 mg/kg-d, 10 d	Colicky pain, postprandial fullness, bloating, dark-colored urine; elevated liver enzymes	3 at 30 mg/kg-d	Streib (1979)
14 mg/kg-d, 2 mo 18 mg/kg-d, 5 mo 21 mg/kg-d, 2 mo	Mild leukopenia at 21 mg/kg-d	1 at 14 mg/kg-d 1 at 18 mg/kg-d 3 at 21 mg/kg-d	Jenkyn et al. (1980)
12–45 mg/kg-d, 36 mo 12 mg/kg-d, 36 mo	Mild GI upset at 20 mg/kg-d, limb and perioral paresthesias at 32 mg/kg-d, nausea, tremors, confusion, agitation at 45 mg/kg-d	1 at 12 mg/kg-d 3 at 20 mg/kg-d 3 at 32 mg/kg-d 4 at 45 mg/kg-d	Streib and Rothner (1981)

**Table 5. Severity Scoring of Guanidine Chloride Case Reports**

Dosimetry <sup>a</sup>	Effects Observed	Severity Score <sup>b</sup>	Reference
18–29 mg/kg-d, 6–144 mo	Patient 1: No side effects reported at 18 mo Patient 2: Numbness, paresthesia, coldness at 6 mo Patient 3: Chronic interstitial nephritis and leukopenia at 48 mo Patient 4: Emesis at 144 mo Patient 5: Dose not reported at 1 mo	Patient 1: 1 at 18 mg/kg-d Patient 2: 3 at 32 mg/kg-d Patient 3: 3 at 24 mg/kg-d (same patient reported in Cherington [1976]) Patient 4: 3 at 16 mg/kg-d Patient 5: unable to score	Dau and Denys (1982)
5 mg/kg-d initially, 6 mo	None Reported	Unable to score	Jablecki (1984)
10 mg/kg-d, 2 hr	None Reported	1 at 10 mg/kg-d	Bady et al. (1987)
5 mg/kg-d, 60 mo 10 mg/kg-d, 1 wk 12 mg/kg-d, 1 wk 20 mg/kg-d, 5 wk	Leukopenia	1 at 5 mg/kg-d 1 at 10 mg/kg-d 3 at 12 mg/kg-d 3 at 20 mg/kg-d	Silbert et al. (1990)
5–36 mg/kg-d, 3–102 mo	Patient 1: None at 20 mo Patient 2: Fainting, nausea at 10 mo Patient 3: GI at 55 mo Patient 4: Paresthesias, GI at 48 mo Patient 5: GI 5 at mo Patient 6: GI 12 at mo Patient 7: None at 57 mo Patient 8: None at 102 mo Patient 9: Paresthesia at 36 mo	Patient 1: 1 at 5 mg/kg-d, 1 at 3 mg/kg-d Patient 2: 4 at 27 mg/kg-d, 4 at 5 mg/kg-d Patient 3: 4 at 21 mg/kg-d, 3 at 5 mg/kg-d Patient 4: 4 at 36 mg/kg-d, 3 at 11 mg/kg-d Patient 5: 4 at 5 mg/kg-d Patient 6: 4 at 5 mg/kg-d, 4 at 7 mg/kg-d Patient 7: 1 at 7 mg/kg-d, 1 at 11 mg/kg-d Patient 8: 1 at 7 mg/kg-d Patient 9: 3 at 9 mg/kg-d	Oh et al. (1997)
6 mg/kg-d, 7 mo	None at 7 mo	1 at 6 mg/kg-d	Oh et al. (1998)
None reported	None reported	Unable to score	Tim et al. (1998)
None reported	None reported	Unable to score	Tim et al. (2000)

<sup>a</sup>Doses are converted to mg/kg-d. If no patient weight was provided in the case reports, a default value of 70 kg was used in the conversion. If a range of doses were used, and details were not specified, the mean of the range was used.

<sup>b</sup>Represents a severity score (as indicated in Table 4) for a given patient at a given dose in mg/kg-d.

It is important to note that there is a negative correlation in the data between severity and treatment duration, because in patients with severe side effects, the drug was discontinued or the dose lowered, and the durations were therefore shorter. However, those patients with minimal side effects continued to receive guanidine chloride for years. This affects the dose-response modeling of the human data as will be discussed below. The observed side effects however, appear relatively quickly (in days), compared to onset in months to years with many chemicals. There did not appear to be a difference in the constellation of symptoms relative to the duration of treatment.

While significant scatter is present in the dose-response data, the Cloglog model of the categorical regression (CatReg) software (U.S. EPA, Version 3.0 [build 12/07/11] software running on R Version 3.0.0, see Appendix C for additional details) does provide a statistically significant fit to the data. All the severity scores and durations listed in Table 5 were used in the categorical regression model. The CatReg software generated estimates for subchronic (12-month) and chronic (84-month) durations separately. All the models provide effective response concentration ( $ERC_{10}$ ) values very close to one another, and the choice of the best fitting model is made by the lowest Akaike's Information Criterion (AIC), which in this case is the Cloglog model with linear dose for both subchronic and chronic-duration guanidine chloride treatment durations. For subchronic-duration treatment, the Cloglog model provides an estimated effective response concentration at a benchmark response (BMR) of 10% ( $ERC_{10}$ ; the CatReg equivalent of a  $BMD_{10}$ ) of 4 mg/kg-day and a lower 95% confidence bound on this  $ERC_{10}$  ( $ERCL_{10}$ ; the CatReg equivalent of a  $BMDL_{10}$ ) of 2 mg/kg-day. For chronic-duration guanidine chloride treatment, the Cloglog model also provides an  $ERC_{10}$  and  $ERCL_{10}$  of 4 mg/kg-day and 2 mg/kg-day, respectively.

## DERIVATION OF PROVISIONAL REFERENCE VALUES

Tables 6 and 7 present summaries of noncancer and cancer reference values for guanidine chloride, respectively.

**Table 6. Summary of Reference Values for Guanidine Chloride**

<b>Toxicity Type (units)</b>	<b>Species/ Sex</b>	<b>Critical Effect</b>	<b>p-Reference Value</b>	<b>POD Method</b>	<b>POD</b>	<b>UF<sub>C</sub></b>	<b>Principal Study</b>
Subchronic p-RfD (mg/kg-d)	Human/Both	NA	$2 \times 10^{-2}$	ERCL <sub>10</sub>	2 mg/kg-d	100	NA
Chronic p-RfD (mg/kg-d)	Human/Both	NA	$2 \times 10^{-2}$	ERCL <sub>10</sub>	2 mg/kg-d	100	NA
Subchronic p-RfC (mg/m <sup>3</sup> )	NDr						
Chronic p-RfC (mg/m <sup>3</sup> )	NDr						

NDr = not determined, NA = not applicable.

**Table 7. Summary of Cancer Values for Guanidine Compounds**

<b>Toxicity Type</b>	<b>Species/Sex</b>	<b>Tumor Type</b>	<b>Cancer Value</b>	<b>Principal Study</b>
p-OSF (mg/kg-d) <sup>-1</sup>	NDr			
p-IUR (mg/m <sup>3</sup> )	NDr			

NDr = not determined.

## DERIVATION OF ORAL REFERENCE DOSES

Several case reports identify doses of guanidine chloride that are associated with negative side effects including anemia and blood dyscrasias, peripheral and central nervous system symptoms, gastrointestinal pain, and other frank effects. These same reports identify doses for which no such side effects are noted, and these serve as potential PODs in the derivation of p-RfD values for guanidine chloride. As stated above, limitations in the available data preclude development of cancer and noncancer provisional toxicity values for any other guanidine compounds. However, based on molecular weight adjustments and stoichiometric calculations, p-RfDs derived for guanidine chloride can be used as the basis for the derivation of screening toxicity values for other guanidine compounds when portal-of-entry effects are not expected. Thus, summaries of screening subchronic and chronic p-RfDs for guanidine nitrate and guanidine are presented in Tables A-1 and A-2, respectively. For systemic effects, since guanidine compounds are expected to ionize in the blood, the toxicities of the chemicals would be due to guanidine (and not the particular salt), thus the toxicities of the guanidine compounds would be expected to be similar on a molar basis. An exception may exist for guanidine nitrate, because nitrate has intrinsic biological effects. However, a chronic RfD for nitrate itself (U.S. EPA, 1991) is listed in IRIS as 1.6 mg/kg-day. The derivation for guanidine chloride presented below results in subchronic and chronic p-RfDs that are approximately two orders of magnitude lower, which would be protective of any potential health effects from nitrate itself.

Potential POD Type	Potential POD Value	Duration	Reference
ERCL <sub>10</sub>	2 mg/kg-d <sup>a</sup>	Subchronic	All available data used-12-mo duration
NOAEL	5 mg/kg-d	Subchronic	Oh et al. (1997)—Patient 1
NOAEL	5 mg/kg-d	Subchronic	Silbert et al. (1990)
NOAEL	6 mg/kg-d	Subchronic	Oh et al. (1997)—Patient 9
NOAEL	11 mg/kg-d	Subchronic	Oh and Kim (1973)—Patient 7
LOAEL	12 mg/kg-d	Subchronic	Silbert et al. (1990)
LOAEL	12 mg/kg-d	Subchronic	Oh and Kim (1973)—Patient 2
ERCL <sub>10</sub>	2 mg/kg-d	Chronic	All available data used-84-mo duration
NOAEL	7 mg/kg-d	Chronic	Oh et al. (1997)—Patient 8
LOAEL	14 mg/kg-d	Chronic	Blumhardt et al. (1977)

<sup>a</sup>ERCL<sub>10</sub> = lower 95% confidence bound on the ERC<sub>10</sub>.

### Derivation of Subchronic Provisional RfD (Subchronic p-RfD) for Guanidine Chloride

Although using case reports is not ideal because of the small sample size of LEMS patients evaluated (*N* ranging from 1–9), the identification and reporting of side effects is somewhat subjective, relatively few endpoints are evaluated (however, in some cases clinical chemistry or pathologies are reported), and the durations of exposure are varied. However, the case reports on treatment of LEMs patients with guanidine chloride provide the only data available on guanidine compounds. The case reports identifying potential PODs are chosen

because of relatively clear descriptions of the dosimetry, LEMS symptoms and side effects, and the treatment durations. Following subchronic-duration treatment with guanidine chloride, potential PODs include NOAELs of 5 mg/kg-day established from both Oh et al. (1997) and Silbert et al. (1990), a NOAEL of 6 mg/kg-day established from Oh et al. (1997), and the categorical regression subchronic (12-month) ERCL<sub>10</sub> value of 2 mg/kg-day generated from a meta-analysis of all the human data. LOAELs of 12 mg/kg-day are also potential PODs established from both the Oh and Kim (1973) and the Silbert et al. (1990) data. The categorical regression ERCL<sub>10</sub> value of 2 mg/kg-day is chosen as the POD because the categorical regression uses all of the available data in the collective dose response, uses a dose-response model (compared to a single point estimate from a NOAEL), provides a statistical basis for estimating the confidence interval of the POD, and is the most sensitive potential POD.

The subchronic p-RfD for guanidine chloride, based on the subchronic ERCL<sub>10</sub>, is derived as follows:

$$\begin{aligned} \text{Subchronic p-RfD} &= \text{ERCL}_{10} \div \text{UF}_C \\ &= 2 \text{ mg/kg-day} \div 100 \\ &= 2 \times 10^{-2} \text{ mg/kg-day} \end{aligned}$$

The composite uncertainty factor (UF<sub>C</sub>) for the subchronic p-RfD for guanidine chloride is 100, as explained in Table 9 below.

<b>Table 9. Uncertainty Factors for Subchronic p-RfD for Guanidine Chloride</b>		
UF	Value	Justification
UF <sub>A</sub>	1	A UF <sub>A</sub> of 1 has been applied for interspecies extrapolation to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability) because human data is used.
UF <sub>D</sub>	10	A UF <sub>D</sub> of 10 has been applied because there are no acceptable two-generation reproductive toxicity or developmental toxicity studies via the oral route.
UF <sub>H</sub>	10	A UF <sub>H</sub> of 10 has been applied for inter-individual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of guanidine chloride in humans.
UF <sub>L</sub>	1	A UF <sub>L</sub> of 1 has been applied for LOAEL-to-NOAEL extrapolation because the POD is an ERCL <sub>10</sub> .
UF <sub>S</sub>	1	A UF <sub>S</sub> of 1 has been applied because a subchronic-duration (12 mo) estimate generated by the CatReg software was utilized.
UF <sub>C</sub>	100	Composite uncertainty factor (UF <sub>C</sub> = UF <sub>A</sub> × UF <sub>D</sub> × UF <sub>H</sub> × UF <sub>L</sub> × UF <sub>S</sub> )

The confidence in the subchronic p-RfD for guanidine chloride is low as explained in Table 10 below.

<b>Confidence Categories</b>	<b>Designation<sup>a</sup></b>	<b>Discussion</b>
Confidence in studies	L	The case reports all evaluated small numbers of subjects.
Confidence in database	L	The database for repeat-dose oral exposures includes only clinical studies, and no reproductive or developmental toxicity studies are available.
Confidence in subchronic p-RfD <sup>b</sup>	L	The overall confidence descriptor is low.

<sup>a</sup>L= low; M= medium; H= high.

<sup>b</sup>The overall confidence cannot be greater than the lowest entry in the table.

### Derivation of Chronic Provisional RfD (chronic p-RfD) Guanidine Chloride

A chronic p-RfD is derived in an analogous manner to the subchronic p-RfD, except that the potential PODs include a LOAEL of 14 mg/kg-day from Blumhardt et al. (1977), a NOAEL of 7 mg/kg-day from Oh et al. (1997) (Patient 8), chronic (84-month) ERCL<sub>10</sub> value of 2 mg/kg-day. Ultimately, the categorical regression ERCL<sub>10</sub> value of 2 mg/kg-day is chosen as the POD for the identical rationale applied to the derivation of the subchronic p-RfD as outlined above.

The chronic p-RfD for guanidine chloride, based on the chronic ERCL<sub>10</sub>, is derived as follows:

$$\begin{aligned}
 \text{Chronic p-RfD} &= \text{ERCL}_{10} \div \text{UF}_C \\
 &= 2 \text{ mg/kg-day} \div 100 \\
 &= 2 \times 10^{-2} \text{ mg/kg-day}
 \end{aligned}$$

The composite uncertainty factor (UF<sub>C</sub>) for the chronic p-RfD for guanidine chloride is 100, as explained in Table 11 below.

<b>UF</b>	<b>Value</b>	<b>Justification</b>
UF <sub>A</sub>	1	A UF <sub>A</sub> of 1 has been applied for interspecies extrapolation to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability) because human data is used.
UF <sub>D</sub>	10	A UF <sub>D</sub> of 10 has been applied because there are no acceptable two-generation reproductive toxicity or developmental toxicity studies via the oral route.
UF <sub>H</sub>	10	A UF <sub>H</sub> of 10 has been applied for inter-individual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of guanidine chloride in humans.
UF <sub>L</sub>	1	A UF <sub>L</sub> of 1 has been applied for LOAEL-to-NOAEL extrapolation because the POD is an ERCL <sub>10</sub> .
UF <sub>S</sub>	1	A UF <sub>S</sub> of 1 has been applied because a chronic-duration (84 mo) estimate generated by the CatReg software was utilized.
UF <sub>C</sub>	100	Composite uncertainty factor (UF <sub>C</sub> = UF <sub>A</sub> × UF <sub>D</sub> × UF <sub>H</sub> × UF <sub>L</sub> × UF <sub>S</sub> )

The confidence in the chronic p-RfD for guanidine chloride is low as explained in Table 12 below.

<b>Table 12. Confidence Descriptors for Chronic p-RfD of Guanidine Chloride</b>		
<b>Confidence Categories</b>	<b>Designation<sup>a</sup></b>	<b>Discussion</b>
Confidence in studies	L	The case reports all evaluated small numbers of subjects.
Confidence in database	L	The database for repeat-dose oral exposures includes only clinical studies, and no reproductive or developmental toxicity studies are available.
Confidence in chronic p-RfD <sup>b</sup>	L	The overall confidence descriptor is low.

<sup>a</sup>L = low; M= medium; H= high.

<sup>b</sup>The overall confidence cannot be greater than the lowest entry in the table.

As stated previously, screening toxicity values for guanidine nitrate and guanidine are derived based on molecular weight adjustments and stoichiometric calculations (see Appendix A). A computational approach to identifying additional potential surrogates for guanidine nitrate was attempted, but no satisfactory surrogates were found (see Appendix B).

#### **DERIVATION OF INHALATION REFERENCE CONCENTRATIONS**

No studies have been identified to derive either subchronic or chronic p-RfCs for any guanidine compounds.

#### **DERIVATION OF PROVISIONAL CANCER POTENCY VALUES**

A paucity of data on the carcinogenicity of any guanidine compound precludes the derivation of either provisional oral slope factors (p-OSFs) or inhalation unit risks (p-IUR).

#### **CANCER WEIGHT OF EVIDENCE (WOE) DESCRIPTOR**

Because no carcinogenicity data are available for any guanidine compounds, the Cancer WOE descriptor for guanidine compounds is “*Inadequate Information to Assess the Carcinogenic Potential*” for both oral and inhalation routes of exposure (see Table 13).

<b>Table 13. Cancer WOE Descriptor for Guanidine Compounds</b>			
<b>Possible WOE Descriptor</b>	<b>Designation</b>	<b>Route of Entry (Oral, Inhalation, or Both)</b>	<b>Comments</b>
“Carcinogenic to Humans”	NS	NA	NA
“Likely to Be Carcinogenic to Humans”	NS	NA	NA
“Suggestive Evidence of Carcinogenic Potential”	NS	NA	NA
<b>“Inadequate Information to Assess Carcinogenic Potential”</b>	<b>Selected</b>	<b>Both</b>	<b>No studies on the carcinogenic potential of guanidine compounds in animals or humans via the oral or inhalation route are available in the literature.</b>
“Not Likely to Be Carcinogenic to Humans”	NS	NA	NA

NS = not selected; NA = not applicable.

## APPENDIX A. PROVISIONAL SCREENING VALUES

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

### CALCULATION OF SCREENING PROVISIONAL ORAL REFERENCES DOSES

The subchronic and chronic p-RfDs derived for guanidine chloride are used as the basis for calculating the screening subchronic and chronic p-RfDs for additional guanidine compounds. Because the toxicity of the various guanidine salts is expected to be due to guanidine itself, the toxicity of such salts would be directly related to the fraction of the molecular weight contributed from guanidine. Thus, based on molecular weight adjustments to the subchronic and chronic p-RfDs derived for guanidine chloride (molecular weight = 95.53 g/mol) in this PPRTV assessment the resulting screening subchronic and chronic p-RfDs are summarized in Table A-1 and Table A-2, respectively, for guanidine nitrate and guanidine (see calculations below).

#### Screening Subchronic Provisional RfD Calculations for Guanidine Compounds

##### Guanidine Nitrate:

$$\begin{aligned}
 \text{Screening Subchronic p-RfD} &= \text{Subchronic p-RfD for Guanidine Chloride} \times \\
 \text{for Guanidine Nitrate} & \quad (\text{MW of Guanidine Nitrate} \div \text{MW of} \\
 & \quad \text{Guanidine Chloride}) \\
 &= 2 \times 10^{-2} \text{ mg/kg-day} \times (122.08 \text{ g/mol} \div \\
 & \quad 95.53 \text{ g/mol}) \\
 &= \mathbf{3 \times 10^{-2} \text{ mg/kg-day}}
 \end{aligned}$$

##### Guanidine:

$$\begin{aligned}
 \text{Screening Subchronic p-RfD} &= \text{Subchronic p-RfD for Guanidine Chloride} \times \\
 \text{for Guanidine} & \quad (\text{MW of Guanidine} \div \text{MW of Guanidine Chloride}) \\
 &= 2 \times 10^{-2} \text{ mg/kg-day} \times (59.07 \text{ g/mol} \div 95.53 \text{ g/mol}) \\
 &= \mathbf{1 \times 10^{-2} \text{ mg/kg-day}}
 \end{aligned}$$

MW = molecular weight

<b>Table A-1. Molecular Weights and Screening Subchronic p-RfDs for Guanidine Compounds</b>			
<b>Compound</b>	<b>Fraction as Guanidine (%)</b>	<b>Molecular Weight (g/mol)</b>	<b>Subchronic Screening p-RfD (mg/kg-day)</b>
Guanidine Nitrate	48	122.08	$3 \times 10^{-2}$
Guanidine	100	59.07	$1 \times 10^{-2}$

### Screening Chronic Provisional RfD Calculations for Guanidine Compounds

Guanidine Nitrate:

$$\begin{aligned}
 \text{Screening Chronic p-RfD for Guanidine Nitrate} &= \text{Chronic p-RfD for Guanidine Chloride} \times \\
 &\quad (\text{MW of Guanidine Nitrate} \div \text{MW of Guanidine Chloride}) \\
 &= 2 \times 10^{-2} \text{ mg/kg-day} \times (122.08 \text{ g/mol} \div 95.53 \text{ g/mol}) \\
 &= \mathbf{3 \times 10^{-2} \text{ mg/kg-day}}
 \end{aligned}$$

Guanidine:

$$\begin{aligned}
 \text{Screening Chronic p-RfD for Guanidine} &= \text{Chronic p-RfD for Guanidine Chloride} \times \\
 &\quad (\text{MW of Guanidine} \div \text{MW of Guanidine Chloride}) \\
 &= 2 \times 10^{-2} \text{ mg/kg-day} \times (59.07 \text{ g/mol} \div 95.53 \text{ g/mol}) \\
 &= \mathbf{1 \times 10^{-2} \text{ mg/kg-day}}
 \end{aligned}$$

MW = molecular weight

<b>Table A-2. Molecular Weights and Screening Chronic p-RfDs for Guanidine Compounds</b>			
<b>Compound</b>	<b>Fraction as Guanidine (%)</b>	<b>Molecular Weight (g/mol)</b>	<b>Chronic Screening p-RfD (mg/kg-day)</b>
Guanidine Nitrate	48	122.08	$3 \times 10^{-2}$
Guanidine	100	59.07	$1 \times 10^{-2}$

## **APPENDIX B. ALTERNATIVE METHODS**

Several public databases (e.g., DSSTox, ChemIDplus) were screened for chemicals in support of a computational approach to derive potential provisional reference values. The standard approach involves the identification of a surrogate chemical with sufficient structural and sufficient in vivo outcome similarities on which to base an extrapolation (Wang et al., 2012). In this case, no chemicals were identified with >80% structural similarity to guanidine nitrate that possessed repeated-dose toxicity information for comparison. Thus, a computational toxicological surrogate-based approach was not feasible.

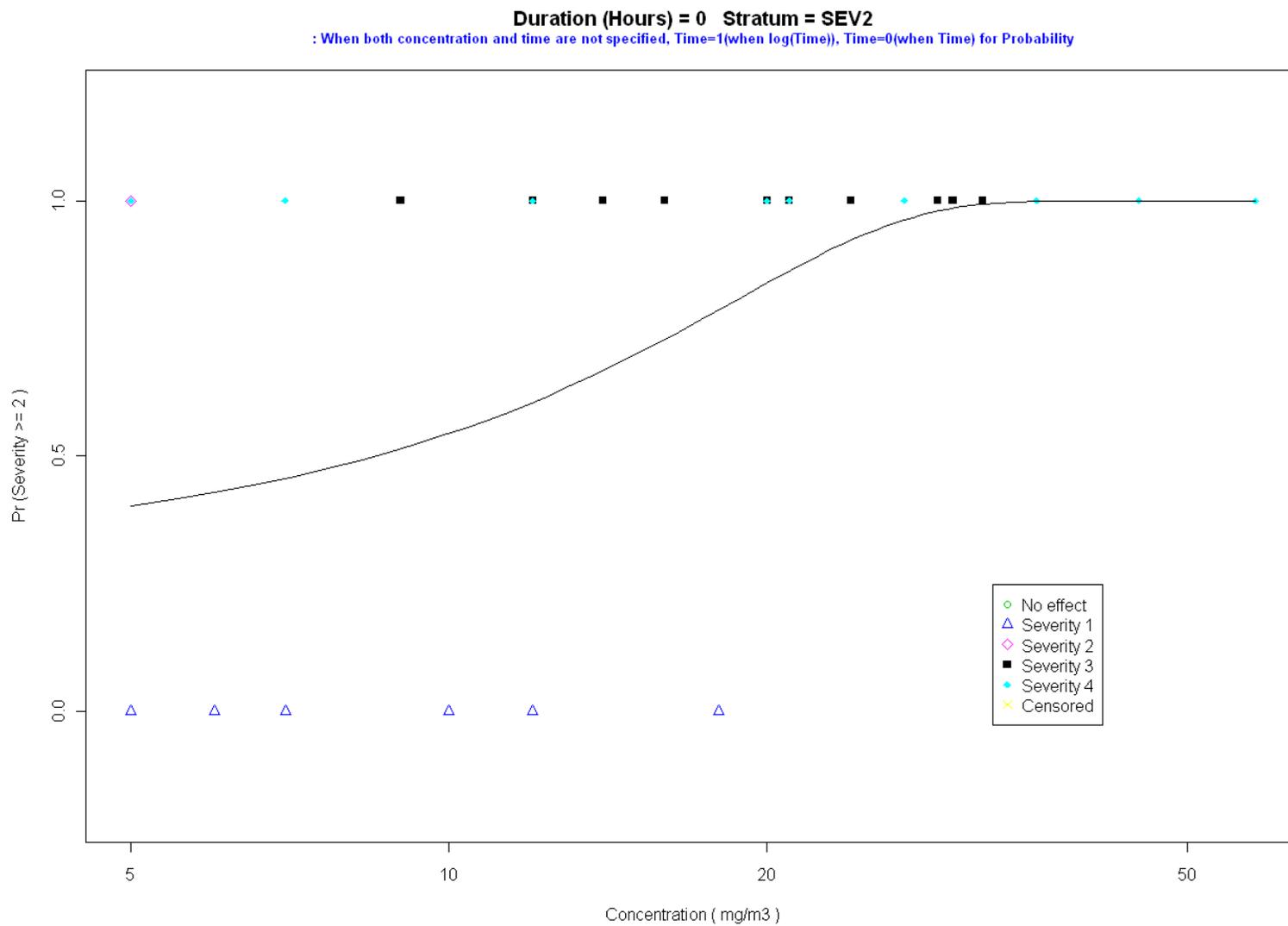
### APPENDIX C. CATREG OUTPUT FILES

The CatReg Version 3.0 (build 12/07/11) software running on R Version 3.0.0 was used to model the guanidine chloride dose-response. The model form was Cumulative Odds, the Extra Risk Option was used, the benchmark response (BMR) was set to 0.10 (10%), the confidence interval was set to 95%, and linear dose and log time were used. Table C-1 shows the model fitting data. All of the models ran correctly when the time function was deleted from the data. However, when the time functions were added, the CatReg software generated ERC<sub>10</sub> and ERCL<sub>10</sub> values for the subchronic and chronic durations of interest with only the Cloglog model. The Cloglog model with the lowest Akaike's Information Criterion (AIC) score is deemed the best fit. The model outputs for 12 months are used for the subchronic p-RfD derivation for guanidine chloride, while 84 months is used for the chronic p-RfD derivation.

<b>Table C-1. CatReg Modeling Results</b>				
<b>Model Name</b>	<b>AIC</b>	<b>ERC<sub>10</sub> Value<sup>a</sup></b>	<b>ERCL<sub>10</sub> Value<sup>b</sup></b>	<b>p-Value</b>
<b>CatReg Modeling Results (no time specified)</b>				
Logit Linear Dose	77.70715	2.87	1.68	≤0.05
Probit Linear Dose	78.01757	3.12	1.95	≤0.05
<b>Cloglog Linear Dose</b>	<b>74.15734</b>	<b>3.65</b>	<b>1.92</b>	<b>≤0.05</b>
Logit Log Dose	78.77464	2.0924	0.6773	≤0.05
Probit Log Dose	79.42696	2.0637	0.6521	≤0.05
Cloglog Log Dose	75.40959	2.5387	0.9352	≤0.05
<b>CatReg Subchronic Modeling Results (12-mo)<sup>c</sup></b>				
Logit Linear	No Fit	No Fit	No Fit	No Fit
Probit Linear Dose	No Fit	No Fit	No Fit	No Fit
<b>Cloglog Linear Dose</b>	<b>72.84863</b>	<b>3.7305</b>	<b>2.2125</b>	<b>≤0.05</b>
Logit Log Dose	No Fit	No Fit	No Fit	No Fit
Probit Log Dose	No Fit	No Fit	No Fit	No Fit
Cloglog Log Dose	No Fit	No Fit	No Fit	No Fit
<b>CatReg Chronic Modeling Results (84 mo)<sup>c</sup></b>				
Logit Linear	No Fit	No Fit	No Fit	No Fit
Probit Linear Dose	No Fit	No Fit	No Fit	No Fit
<b>Cloglog Linear Dose</b>	<b>72.84863</b>	<b>3.7233</b>	<b>1.7731</b>	<b>≤0.05</b>
Logit Log Dose	No Fit	No Fit	No Fit	No Fit
Probit Log Dose	No Fit	No Fit	No Fit	No Fit
Cloglog Log Dose	No Fit	No Fit	No Fit	No Fit

<sup>a</sup>ERC<sub>10</sub> = estimated effective response concentration at a benchmark response (BMR) of 10%.

<sup>b</sup>ERCL<sub>10</sub> = the lower 95% confidence bound on the ERC<sub>10</sub>.<sup>c</sup>Model fits are reported at 12-month (subchronic) and 84-month (chronic) time periods.



**Figure C-1. CatReg plot of Cloglog model run**

The dose-response curve is steep, the time-response models indicate a relatively small change in ERC<sub>10</sub> values with time. Table C-2 shows the effects of time on the ERC values, with time in months from subchronic to chronic durations.

<b>Table C-2. ERC<sub>10</sub> at Various Times at Severity Score 2</b>			
<b>Duration</b>	<b>ERC<sub>10</sub></b>	<b>Lower Bound on ERC<sub>10</sub></b>	<b>Upper Bound on ERC<sub>10</sub></b>
12 mo	3.7305	2.2125	5.2485
20 mo	3.7286	2.1642	5.2930
40 mo	3.7260	2.0152	5.4368
60 mo	3.7245	1.8917	5.5573
84 mo	3.7233	1.7731	5.6734

## APPENDIX D. REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). (2010) Threshold limit values for chemical substances and physical agents and biological exposure indices. ACGIH, Cincinnati, OH. As cited in HSDB (Hazardous Substances Data Bank). Available online at <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Accessed on October 4, 2010.

ACGIH (American Conference of Governmental Industrial Hygienists). (2013) 2013 TLVs and BEIs. Based on the documentation of the threshold limit values for chemical substances and physical agents & biological exposure indices. ACGIH, Cincinnati, OH.

Aguirre Engineers, Inc. (Aguirre). (1998) U.S Army property transfer environmental baseline survey report, Sunflower Army Ammunition Plant, Kansas. Prepared for U.S. Army Industrial Operations Command. Contract No. DAAA09-G-0001.001. August 1998.

ATSDR (Agency for Toxic Substances and Disease Registry). (2013) Minimal risk levels (MRLs) for hazardous substances. ATSDR, Atlanta, GA. Available online at [http://www.atsdr.cdc.gov/mrls/pdfs/atsdr\\_mrls\\_july\\_2013.pdf](http://www.atsdr.cdc.gov/mrls/pdfs/atsdr_mrls_july_2013.pdf).

Bady, B., Chauplannaz, G., and Carrier, H. (1987) Congenital Lambert-Eaton myasthenic syndrome. *J Neurol Neurosurg Psych* 50:476–478.

Blumhardt, LD., Joekes, A.M., Marshall, J., et al. (1977) Guanidine treatment and impaired renal function in the Lambert-Eaton syndrome. *British Medical Journal* 9:946–947.

Brown, L.D., Hiatt, G.F., Wheeler, CR; et al. (1988) Acute oral toxicity of guanidine nitrate in mice. Letterman Army Inst of Research Presido of San Francisco CA. July 1988. Available online at <http://www.dtic.mil/dtic/tr/fulltext/u2/a197827.pdf>.

Cal/EPA (California Environmental Protection Agency). OEHHA toxicity criteria database. Office of Environmental Health Hazard Assessment, Sacramento, CA. Available online at <http://www.oehha.ca.gov/risk/ChemicalDB/index.asp>. Accessed on 10-17-2013.

Cal/EPA (California Environmental Protection Agency). (2009) Hot spots unit risk and cancer potency values. Appendix A. Office of Environmental Health Hazard Assessment, Sacramento, CA. Available online at [http://oehha.ca.gov/air/hot\\_spots/2009/AppendixA.pdf](http://oehha.ca.gov/air/hot_spots/2009/AppendixA.pdf).

Cal/EPA (California Environmental Protection Agency). (2013a) All OEHHA Acute, 8-hour and Chronic Reference Exposure Levels (chREs) as on August 2013. Office of Environmental Health Hazard Assessment, Sacramento, CA. Available online at <http://www.oehha.ca.gov/air/allrels.html>.

Cal/EPA (Environmental Protection Agency). (2013b) Current proposition 65 list [11/08/13]. Office of Environmental Health Hazard Assessment, Sacramento, CA. Available online at [http://oehha.ca.gov/prop65/prop65\\_list/Newlist.html](http://oehha.ca.gov/prop65/prop65_list/Newlist.html).

Chemical Book. (a) Guanidine nitrate (506-93-4). Available online at [http://www.chemicalbook.com/ProductChemicalPropertiesCB9150870\\_EN.htm](http://www.chemicalbook.com/ProductChemicalPropertiesCB9150870_EN.htm). Accessed on 2-19-2014.

Chemical Book. (b) Guanidine hydrochloride (50-01-1). Available online at [http://www.chemicalbook.com/ChemicalProductProperty\\_EN\\_CB6677329.htm](http://www.chemicalbook.com/ChemicalProductProperty_EN_CB6677329.htm). Accessed on 2-19-2014.

Chemical Book. (c) Guanidine hydrochloride (50-01-1). Available online at [http://www.chemicalbook.com/ProductMSDSDetailCB6677329\\_EN.htm](http://www.chemicalbook.com/ProductMSDSDetailCB6677329_EN.htm). Accessed on 2-19-2014.

CHEMICALLAND21. Guanidine hydrochloride. Available online at <http://www.chemicaland21.com/industrialchem/organic/GUANIDINE%20HYDROCHLORIDE.htm>. Accessed on 2-19-2014.

CHEMICALLAND21. Guanidine nitrate. Available online at <http://www.chemicaland21.com/industrialchem/organic/GUANIDINE%20NITRATE.htm>. Accessed on 2-19-2014.

Cherington, M. (1976) Guanidine and Germin in Lambert-Eaton Syndrome. *Neurol* 26(26):944–946.

Dau, P.C. and Denys, E.H. (1982) Plasmapheresis and Immunosuppressive Drug Therapy in the Eaton-Lambert Syndrome. *Ann Neurol* 11:570–575.

Dourson, M., Teuschler, L., Durkin, P., et al. (1997) Categorical regression of toxicity data: a case study using aldicarb. *Regul Toxicol Pharmacol* 25:121–129.

Europeana Chemicals Bureau. IUCID Dataset. Available online at <http://esis.jrc.ec.europa.eu/>.

Henriksson, K.G., Nilson, O., Rosen, I., et al. (1977) Clinical neurophysiological and morphological findings in Eaton Lambert Syndrome. *Acta Neurol Scandinav* 56(2):117–140.

IARC (International Agency for Research on Cancer). (2013) Monographs on the evaluation of carcinogenic risks to humans. Lyon, France: IARC. Available online at <http://monographs.iarc.fr/ENG/Monographs/vol103/mono103-B02-B03.pdf>.

Jablecki, C. (1984) Lambert-Eaton myasthenic syndrome. *Muscle Nerve* 7(3):250–257.

Jenkyn, L.R., Brooks, P.L., Forcier, et al. (1980) Remission of the Lambert-Eaton syndrome and small cell anaplastic carcinoma of the lung induced by chemotherapy and radiotherapy. *Cancer* 46(5):11234–1127.

Kalia, L. and Swartz, K. (2011) Elucidating the molecular basis of action of a classic drug: guanidine compounds as inhibitors of voltage-gated potassium channels. *Mol Pharmacol* 80(6):1085–1095.

Kennedy, W.R. and Jimenez-Pabon, E. (1968) The myasthenic syndrome associated with small-cell carcinoma of the lung (Lambert-Eaton Syndrome). *Neurol* 18(8):757–766.

Lambert, E.H. (1966) Defects of neuromuscular transmission in syndromes other than myasthenia gravis. *Ann N Y Acad Sci* 135(1):367–384.

Nakano, K.K. and Tyler, H.R. (1970) Cardiovascular complications of guanidine therapy in the myasthenic syndrome. *Neurol* 20(40):408.

NIOSH (National Institute for Occupational Safety and Health). (2010) NIOSH pocket guide to chemical hazards. Index of chemical abstracts service registry numbers (CAS No.). Center for Disease Control and Prevention, U.S. Department of Health, Education and Welfare, Atlanta, GA. Available online at <http://www.cdc.gov/niosh/npg/npgdcas.html>.

NLM (National Library of Medicine). Guanidine hydrochloride. Available online at <http://chem.sis.nlm.nih.gov/chemidplus/rn/50-01-1>. Accessed on 2-29-2014.

NLM (National Library of Medicine). (2008) Guanidine, mononitrate. Available online at <http://toxnet.nlm.nih.gov/>. Accessed on 2-14-2014.

NTP (National Toxicology Program). (2011) Report on carcinogens, 12<sup>th</sup> edition. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC. Available online at <http://ntp.niehs.nih.gov/ntp/roc/twelfth/roc12.pdf>.

Oh, S.J. (1972) The Eaton-Lambert syndrome. *Arch Neurol* 27:91–94.

Oh, S.J. and Kim, K.W. (1973) Guanidine hydrochloride in the Lambert-Eaton syndrome. *Neurol* 23(10):1084–1090.

Oh, S.J., Kim, D.S., Head, T.C., et al. (1997) Low dose guanidine and pyridostigmine: relatively safe and effective long-term symptomatic therapy in Lambert-Eaton syndrome. *Muscle Nerve* 20(9):1146–1152.

Oh, S.J., Kim, D.S., Kwon, K.H., et al. (1998) Wide spectrum of symptomatic treatment in Lambert-Eaton myasthenic syndrome. *Ann N Y Acad Sci* 841:827–831.

OSHA (Occupational Safety and Health Administration). (2006) Air contaminants: occupational safety and health standards for shipyard employment, subpart Z, toxic and hazardous substances. U.S. Department of Labor, Washington, DC. OSHA Standard 1915.1000. Available online at [http://www.osha.gov/pls/oshaweb/owadisp.show\\_document?p\\_table=STANDARDS&p\\_id=10286](http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10286). Accessed on October 4, 2010.

- OSHA (Occupational Safety and Health Administration). (2011) Air contaminants: occupational safety and health standards for shipyard employment, subpart Z, toxic and hazardous substances. U.S. Department of Labor, Washington, DC; OSHA Standard 1915.1000. Available online at [https://www.osha.gov/pls/oshaweb/owadisp.show\\_document?p\\_table=STANDARDS&p\\_id=10286](https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10286).
- Silbert, P.L, Hankey, G.J., and Barr, A.L. (1990) Successful alternative day guanidine therapy following guanidine-induced neutropenia in the Lambert-Eaton myasthenic syndrome. *Muscle Nerve* 13(4):360–361.
- Streib, E.W. (1979) Reversible hepatic dysfunction owing to guanidine treatment. *Arch Neurol* 36(6):386.
- Streib, E.W. and Rothener, A, D. (1981) Eaton-Lambert myasthenic syndrome: long-term treatment of three patients with prednisone. *Ann Neurol* 10(5):448–453.
- Tim, R.W., Massey, J.M., and Sanders, D.B. (1998) Lambert-Eaton Myasthenic Syndrome (LEMS). Clinical and electrodiagnostic features and response to therapy in 59 patients. *Ann NY Acad Sci* 841:823–826.
- Tim, R.W., Massey, J.M., and Sanders, D.B. (2000) Lambert-Eaton Myasthenic syndrome: Electrodiagnostic findings and response to treatment. *Neurol* 54(11):2176–2178.
- U.S. Army. (2012) Development of a Guanidine Nitrate Performance Standard: Former Sunflower Army Ammunition Plant, DeSoto Kansas. 6/22/2012 p 1–5.
- U.S. EPA (Environmental Protection Agency). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at <http://www.epa.gov/iris/>. Accessed on 10-17-2013.
- U.S. EPA (Environmental Protection Agency). (1985) Health and environmental effects profile (HEEP) for guanidine nitrate. Environmental Criteria and Assessment Office, Cincinnati, OH. ECAO-CIN-P147. September.
- U.S. EPA (Environmental Protection Agency). (1990) High Production Volume Challenge Program Chemical Listing. Available online at <http://www.epa.gov/hpv/>.
- U.S. EPA (Environmental Protection Agency). (1991) Nitrate Quickview (CASRN 14797-55-8). Integrated risk information system (IRIS), Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at [http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showQuickView&substance\\_nmbr=0076](http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showQuickView&substance_nmbr=0076).
- U.S. EPA (Environmental Protection Agency). (1993) Integrated Risk Information System (IRIS) Toxicological Review of Aldicarb. Available online at <http://www.epa.gov/IRIS>.

U.S. EPA (Environmental Protection Agency). (1994) Chemical assessments and related activities (CARA). Office of Health and Environmental Assessment, Washington, DC; EPA/600/R-94/904. Available online at <http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=60001G8L.txt>.

U.S. EPA (Environmental Protection Agency). (2011) Health effects assessment summary tables (HEAST). Prepared by the Office of Research and Development, National Center for Environmental Assessment, Cincinnati OH for the Office of Emergency and Remedial Response, Washington, DC. Available online at <http://epa-heat.ornl.gov/>.

U.S. EPA (Environmental Protection Agency). (2012) 2012 Edition of the drinking water standards and health advisories. Office of Water, Washington, DC; EPA/822/S-12/001. Available online at <http://water.epa.gov/action/advisories/drinking/upload/dwstandards2012.pdf>.

Vroom, F.Q. and Engel, W.K. (1969) Nonneoplastic steroid responsive Lambert-Eatonmyasthenic syndrome. *Neurol* 19:281.

Wang, N.C.Y., Zhao, Q.J., Wesselkamper, S.C., Lambert, J.C., Petersen, D., and Hess-Wilson, J.K. (2012) Application of computational toxicological approaches in human health risk assessment. I. A tiered surrogate approach. *Regul Toxicol Pharmacol* 63(1):10–19.

WHO (World Health Organization). Online catalogs for the Environmental Health Criteria Series. Geneva Switzerland: WHO. Available online at <http://www.who.int/ipcs/publications/ehc/en/>. Accessed on 10-17-2013.