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Provisional Peer-Reviewed Toxicity Values for

Soluble Tungsten Compounds
(Various CASRNs)

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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 SOLUBLE TUNGSTEN COMPOUNDS (Various CASRNs)

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. EPA policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

QUESTIONS REGARDING PPRTVs

Questions regarding the 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

Tungsten is a metallic element that increases the hardness, toughness, elasticity, and strength of steel and other metal alloys (HSDB, 2009b). Tungsten is used in electron lamps and filaments for incandescent lamps (HSDB, 2009b). It is also used in the preparation of green and blue pigments (HSDB, 2009b). The U.S. military has used tungsten as a replacement for lead and depleted uranium in munitions since the late 1990s (Osterburg et al., 2014). Tungsten has very low solubility in water and is not volatile (ECB, 2000). The chemical symbol for tungsten is W.

Sodium tungstate is a chemical intermediate for metallic tungsten and tungsten compounds (HSDB, 2009a). Sodium tungstate is used as a flame retardant textile treatment agent (HSDB, 2009a). In addition, sodium tungstate is a reagent for biological products (HSDB, 2009a). Sodium tungstate has high solubility in water and is not volatile (HSDB, 2009a). The empirical formula for sodium tungstate is Na_2O_4W (see Figure 1).

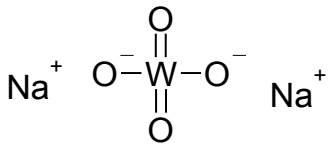

Figure 1. Sodium Tungstate Structure

Sodium tungsten dihydrate is used in the fireproofing and waterproofing of fabrics (O'Neil, 2006). The compound is also a catalyst in oxidation reactions and a corrosion inhibitor in steel (O'Neil, 2006). Sodium tungstate dihydrate has high solubility in water (O'Neil, 2006). The empirical formula for sodium tungstate dihydrate is $Na_2WO_4 \cdot 2H_2O$ (see Figure 2).

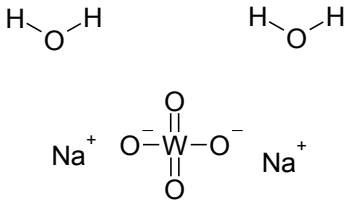

Figure 2. Sodium Tungstate Dihydrate Structure

Because soluble compounds of tungsten (i.e., sodium tungstate dihydrate and sodium tungstate) are expected to ionize in the blood (McInturf et al., 2011; McInturf et al., 2008), the systemic toxicities of the chemicals would be due to elemental tungsten (and not the particular salt), thus the toxicities of the soluble compounds of tungsten would be expected to be similar on a molar basis. Therefore, although the subchronic and chronic provisional oral reference doses (p-RfDs) presented below are derived based on doses for elemental tungsten, the values are applicable for soluble tungsten compounds (e.g., sodium tungstate dihydrate and sodium

tungstate). A table of physicochemical properties for sodium tungstate and sodium tungstate dihydrate are provided below (see Table 1). A summary of available toxicity values for soluble tungsten compounds from the U.S. EPA and other agencies/organizations is provided in Table 2.

Table 1. Physicochemical Properties of Sodium Tungstate (CASRN 13472-45-2) and Sodium Tungstate Dihydrate (CASRN 10213-10-2)

Property (unit)	13472-45-2[a]	10213-10-2[b]
Boiling point (°C at 760 mmHg)	ND	ND
Melting point (°C)	698	Decomposes at 100°C
Density (g/cm^3 at 20°C)	4.18	3.25[c]
Vapor pressure (mmHg at 2,327°C)	ND	ND
pH (unitless)	ND	ND
Solubility in water (g/L at 20°C)	742[c]	909
Relative vapor density (air = 1)	ND	ND
Molecular weight (g/mol)	293.82	329.85[d]

[a]HSDB (2009a).
[b]O'Neil (2006).
[c]Lide (2005a); Lide (2005b)
[d]Sigma-Aldrich (2014).

ND = no data.

Table 2. Summary of Available Toxicity Values for Soluble Tungsten Compounds (Various CASRNs)

Source/Parameter[a,b]	Value	Notes	Reference
Noncancer			
ACGIH (TLV-TWA)			
Soluble compounds of tungsten	1 mg W/m^3	Basis: central nervous system impairment; pulmonary fibrosis	ACGIH (2013); ACGIH (2001)
ACGIH (TLV-STEL)			
Soluble compounds of tungsten	3 mg W/m^3	Basis: central nervous system impairment; pulmonary fibrosis	ACGIH (2013); ACGIH (2001)
ATSDR (MRLs) Tungsten	NV	NA	ATSDR (2015)
Cal/EPA	NV	NA	Cal/EPA (2015b); Cal/EPA (2015a)
NIOSH (REL-TWA)			
Soluble compounds of tungsten	1 mg W/m^3	NA	NIOSH (2015)
NIOSH (REL-STEL)			
Soluble compounds of tungsten	3 mg W/m^3	NA	NIOSH (2015)
OSHA (PEL) Soluble compounds of tungsten	1 mg W/m^3	Construction industry	OSHA (2011); OSHA (2006)
IRIS	NV	NA	U.S. EPA (2015)
DWSHA	NV	NA	U.S. EPA (2012a)
HEAST	NV	NA	U.S. EPA (2011a)
CARA HEEP	NV	NA	U.S. EPA (1994)
WHO	NV	NA	WHO (2015)
Cancer			
IRIS	NV	NA	U.S. EPA (2015)
HEAST	NV	NA	U.S. EPA (2011a)
IARC	NV	NA	IARC (2015)
NTP	NV	NA	NTP (2014)
Cal/EPA	NV	NA	Cal/EPA (2015b); Cal/EPA (2015a)

Table 2. Summary of Available Toxicity Values for Soluble Tungsten Compounds (Various CASRNs)

Source/Parameter[a,b]	Value	Notes	Reference
DWSHA	NV	NA	U.S. EPA (2012a)
ACGIH (WOE) Tungsten	NV	Sufficient data were not available to recommend carcinogenicity notations	ACGIH (2013); ACGIH (2001)

[a]Sources: ACGIH = American Conference of Governmental Industrial Hygienists; ATSDR = Agency for Toxic Substances and Disease Registry; Cal/EPA = California Environmental Protection Agency; CARA = Chemical Assessments and Related Activities; DWSHA = Drinking Water Standards and Health Advisories; HEAST = Health Effects Assessment Summary Tables; HEEP = Health and Environmental Effects Profile; IARC = International Agency for Research on Cancer; IRIS = Integrated Risk Information System; NIOSH = The National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; WHO = World Health Organization.
[b]Parameters: MRL = minimum risk level; PEL = permissible exposure limit; REL-STEL = recommended exposure limit−short-term exposure limit; REL-TWA = recommended exposure limit−time weighted average; TLV-STEL = threshold limit value−short-term exposure limit; TLV-TWA = threshold limit value−time-weighted average; WOE = cancer weight of evidence.

NA = not applicable; NV = not available

Literature searches were conducted on sources published from 1900 through August 2015 for studies relevant to the derivation of provisional toxicity values for tungsten and sodium tungstate (CASRN 7440-33-7, 13472-45-2, and 10213-10-2). Searches were conducted using U.S. 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 Office of Water (OW), U.S. EPA TSCATS2/TSCATS8e, NIOSH, NTP, and OSHA.

REVIEW OF POTENTIALLY RELEVANT DATA (NONCANCER AND CANCER)

Tables 3A and 3B provide an overview of the relevant database for tungsten and sodium tungstate and include all potentially relevant repeated-dose short-term-, subchronic-, and chronic-duration studies. Principal studies are identified. 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 Soluble Tungsten Compounds (Various CASRNs)

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[a]								
ND								
2. Inhalation[a]								
ND								
Animal								
1. Oral[a]								
Short-term	22−24 M/22−24 F, C57BL6 mouse, sodium tungstate dihydrate in drinking water, 28 d	0, 1.3, 39, 78, 125 in drinking water ADD: 0, 1.3, 39, 78, 125	Reduced immune response to *Staphylococcal* enterotoxin B (decreased number of activated helper and cytotoxic T cells in spleen, decreased in situ production of interferon [IFN]-γ)	78	40 for decreased number of cytotoxic T cells in spleen	125 (immune suppression)	Osterburg et al. (2014)	PR
Short-term	4 M/0 F, Wistar rat, sodium tungstate dihydrate in diet, 28 d	0, 5, 50 ppm in diet ADD: 0, 0.65, 6.6	No effects observed.	6.6	NDr	NDr	Chatterjee et al. (1973)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for Soluble Tungsten Compounds (Various CASRNs)

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]
Subchronic	**10 M/10 F, S-D rat, sodium tungstate dihydrate in deionized water via gavage, 90 d**	**0, 6, 47, 78, 125** **ADD: 0, 6, 47, 78, 125**	**Goblet cell metaplasia and inflammation of glandular stomach (both sexes). Also, renal tubular lesions (both sexes) and decreased body weight (males only) in high-dose group.**	**47**	**2.3 for goblet cell metaplasia in males**	**78 (stomach lesions)**	**USACHPPM (2007a); USACHPPM (2007b)**	**NPR, PS The results from this study were published in a peer-reviewed report by McCain et al. (2015).**
Subchronic	5 M/0 F, C57BL/6J mouse, sodium tungstate dihydrate in drinking water, 16 wk	0, 15, 200, 1,000 mg/L in drinking water ADD: 0, 4, 49, 250	Decreased body weight and bone marrow cellularity in tibiae and femora.	49	78 for decreased bone marrow cell count	250 (decreased body weight, decreased bone marrow cellularity)	Kelly et al. (2013)	PR
Subchronic	5 M/6 F, unspecified strain rat, as sodium tungstate in diet, 70 d	0, 0.1, 0.5, 2.0% in diet ADD: 0, 91, 455, 1,820 (M) 0, 102, 510, 2,040 (F)	Decreased body weight and mortality.	NDr	NDr	91 (decreased body weight)	Kinard and Van de Erve (1941) Sodium tungstate produced deaths within 30 d at 0.5 or 2% W in diet.	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for Soluble Tungsten Compounds (Various CASRNs)

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]
Subchronic	5 M/5 F, mixed Wistar albino and Minnesota piebald rat, tungsten metal powder in diet, 70 d Note: This is the only available study on the repeated oral dose toxicity of tungsten metal.	0, 2, 5, 10% in diet ADD: 0, 1,325, 3,450, 6,550 (M) 0, 1,450, 4,000, 7,325 (F)	Decreased body-weight gain in females.	4,000	NDr	7,325 (decreased body-weight gain)	Kinard and Van de Erve (1943) Tungsten metal at up to 10% W in diet produced no deaths after 70 d.	PR
Subchronic	10 M/0 F, S-D rat, sodium tungstate in drinking water, 19 wk	0, 100, 200 ppm in drinking water ADD: 0, 13.9, 27.8	No effects observed.	27.8	NDr	NDr	Luo et al. (1983)	PR
Chronic	10 M/0 F, S-D rat, sodium tungstate in drinking water, 30 wk	0, 100 ppm in drinking water ADD: 0, 11.9	No effects observed.	11.9	NDr	NDr	Luo et al. (1983)	PR
Chronic	37 M/35 F (exposed) 52 M/52 F (control), Long-Evans rat, sodium tungstate in drinking water, lifetime	0, 5 ppm in drinking water ADD: 0, 0.6 (M) 0, 0.7 (F)	No effects observed.	0.7	NDr	NDr	Schroeder and Mitchener (1975b)	PR
Chronic	54 M/54 F, white Swiss mouse, sodium tungstate in drinking water, lifetime	0, 5 ppm in drinking water ADD: 0, 1 (M) 0, 1 (F)	No effects observed.	1	NDr	NDr	Schroeder and Mitchener (1975a)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for Soluble Tungsten Compounds (Various CASRNs)

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]
Reproductive/ developmental	12−16 M/12−16 F, C57BL6 mouse, sodium tungstate dihydrate in drinking water, 19 wk (12 wk premating, 1 wk mating, 3 wk gestation, and 3 wk post-parturition); F1 offspring exposed for an additional 90 d	0, 1.3, 39, 78, 125 in drinking water ADD: 0, 1.3, 39, 78, 125	Systemic: Reduced immune response to *Staphylococcal* enterotoxin B in F0 animals at PNW 3 (decreased number of activated helper and cytotoxic T cells in spleen, decreased in situ production of IFN-γ) and F1 animals at approximately PNWs 15−16 (decreased number of activated helper and cytotoxic T cells in spleen). Reproductive: No effects observed. Developmental: No standard developmental endpoints were reported. Impaired immune function was observed at PNWs 15−16; however, effects could have been due to the subchronic, postweaning exposure rather than developmental exposure.	78 125 (reproductive) NDr (developmental)	Data not amenable to BMD modeling NDr NDr	125 (immune suppression) NDr (reproductive) NDr (developmental)	Osterburg et al. (2014)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for Soluble Tungsten Compounds (Various CASRNs)

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]
Reproductive/ developmental	40 M/40 F, S-D rat, sodium tungstate dihydrate in deionized water via gavage, 70 d (14 d premating, 14 d mating, 22 d gestation, 20 d post-parturition)	0, 3, 39, 78 ADD: 0, 3, 39, 78	Systemic: Lack of clinical signs of toxicity, body-weight effects, clear dose-related histopathological changes, or clear dose-related changes in neurobehavioral tests of F0 dams (pup retrieval, open field behavior, acoustic startle/prepulse inhibition, Morris water maze). Reproductive: No effects observed. Developmental: No effects observed.	78 78 (reproductive) 78 (developmental)	NDr NDr NDr	NDr NDr (reproductive) NDr (developmental)	McInturf et al. (2011); McInturf et al. (2008)	PR
Reproductive/ developmental	15 M/24 F, Wistar rat, sodium tungstate in drinking water, 12 wk and mate with untreated animals	0, 1 mg/mL in drinking water ADD: 0, 147 (M) 0, 160 (F)	Systemic: Body weight decreased in females of the exposed group, compared with controls. Reproductive: No effects observed.	NDr 160 (reproductive)	NDr NDr	160 (decreased body weight) NDr (reproductive)	Ballester et al. (2007); Ballester et al. (2005)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for Soluble Tungsten Compounds (Various CASRNs)

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[a]								
ND								

[a]Dosimetry: Doses are equivalent tungsten doses in units of mg W/kg-day (doses for sodium tungstate × 62.6%). Tungsten accounts for 62.6% of total molecular weight of Na_2WO_4 (sodium tungstate). Presented BMDL/BMCL values were determined by the EPA for the purposes of this PPRTV assessment. Values are presented as adjusted daily dose (ADD, in mg/kg-day) for oral noncancer effects.
[b]Notes: PS = principal study; PR = peer reviewed; NPR = not peer reviewed.

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).

BMR = bench mark response; ER = extra risk; F = female; FEL = frank effect level; M = male; NA = not applicable; ND = no data; NDr = not determined; PNW = postnatal week; RD = relative deviation; S-D = Sprague-Dawley.

Table 3B. Summary of Potentially Relevant Cancer Data for Soluble Tungsten Compounds (Various CASRNs)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	BMDL/ BMCL[a]	Reference (comments)	Notes[b]
Human						
1. Oral (mg/kg-d)[a]						
ND						
2. Inhalation (mg/m^3)[a]						
ND						
Animal						
1. Oral (mg/kg-d)[a]						
Carcinogenicity (tumor promotion)	10 M/0 F, S-D rat, sodium tungstate in drinking water, 19 wk	0, 100, 200 ppm HED: 0, 3.33, 6.67 ± known carcinogen NSEE	No effects observed.	NDr	Luo et al. (1983) (No evidence for tumor promotion by tungstate was found.)	PR
Carcinogenicity (tumor promotion)	10 M/0 F, S-D rat, sodium tungstate in drinking water, 30 wk	0, 100 ppm HED: 0, 2.86 ± known carcinogen NSEE	No effects observed.	NDr	Luo et al. (1983) (No evidence for tumor promotion by tungstate was found.)	
Carcinogenicity	37 M/35 F (exposed) 52 M/52 F (control), Long-Evans rat, sodium tungstate in drinking water, lifetime	0, 5 ppm HED: 0, 0.1 (M), 0, 0.2 (F)	No effects observed.	NDr	Schroeder and Mitchener (1975b)	PR
Carcinogenicity	54 M/54 F, white Swiss mouse, sodium tungstate in drinking water, lifetime	0, 5 ppm HED: 0, 0.1 (M), 0, 0.1 (F)	No effects observed.	NDr	Schroeder and Mitchener (1975a)	PR

Table 3B. Summary of Potentially Relevant Cancer Data for Soluble Tungsten Compounds (Various CASRNs)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	BMDL/ BMCL[a]	Reference (comments)	Notes[b]
Carcinogenicity (tumor promotion)	0 M/10−24 F, S-D rat, sodium tungstate in drinking water, sacrificed 125 or 198 d after injection with known carcinogen, NMU	0, 0, 150 ppm sodium tungstate HED: 0, 0 + NMU, 4.8 + NMU	After 125 d, the group exposed to tungstate + NMU had statistically significantly increased incidence of mammary carcinomas, compared with the NMU-only group. After 198 d, both the NMU-only and tungstate + NMU groups had >90% incidence of mammary carcinomas. In both subgroups, the first palpable mass was observed on D 56 in the tungstate + NMU group. In the NMU-only group, the first palpable mass was observed on D 71 and D 85 in the 125- and 198-d subgroups, respectively. No mammary carcinomas were observed in the unexposed control group. Tungstate-only exposure not tested.	NDr	Wei et al. (1985) (Limited evidence for tumor promotion by tungstate was found.)	PR
2. Inhalation (mg/m^3)[a]						
ND						

[a]Dosimetry: Doses are equivalent tungsten doses in units of mg W/kg-day (doses for sodium tungstate × 62.6%). Tungsten accounts for 62.6% of total molecular weight of Na_2WO_4 (sodium tungstate). Presented BMDL/BMCL values were determined by the EPA for the purposes of this PPRTV assessment.
[b]Notes: PS = principal study; PR = peer reviewed; NPR = not peer reviewed.

ND = no data; NDr = not determined.

HUMAN STUDIES

Oral Exposures

No studies have been identified.

Inhalation Exposures

No studies have been identified.

A number of epidemiology studies have associated occupational exposure to dusts containing tungsten (and tungsten compounds such as tungsten carbide and tungsten oxides) and other metals in the hard metal alloy industry with pulmonary fibrosis, memory and sensory deficits, and increased mortality due to lung cancer (ATSDR, 2005). However, respiratory and neurological effects in hard metal alloy workers have been attributed to cobalt, rather than tungsten (ATSDR, 2005).

Cross-sectional surveys of the U.S. general population have examined possible associations between levels of metals (including tungsten) in urine or blood and serum thyroid levels (Yorita Christensen, 2012), cardiovascular and cerebrovascular disease (Agarwal et al., 2011), and various medical conditions (Mendy et al., 2012). In these studies, however, the subjects' route of exposure is unknown. The studies are described in Table C-2 in Appendix C.

ANIMAL STUDIES

Oral Exposures

Overview of Animal Oral Exposure Studies

Noncancer endpoints evaluated in animals repeatedly exposed orally to sodium tungstate include: (1) comprehensive endpoints as per U.S. EPA Health Effects Testing Guideline Office of Prevention, Pesticides and Toxic Substances (OPPTS) 870.3100 in Sprague-Dawley (S-D) rats exposed by gavage for 90 days (USACHPPM, 2007a, b); (2) neurological, systemic, and reproductive endpoints as per U.S. EPA Testing Guideline OPPTS 870.3650 in S-D rats exposed by gavage for 70 days before mating, during mating and gestation, and during early postnatal periods (McInturf et al., 2011; McInturf et al., 2008); (3) hematological and spleen cell endpoints in response to bacterial toxin injection in C57BL6 mice following 28-day exposure or about 19 weeks of exposure starting 90 days before mating and continuing through gestation and weaning (Osterburg et al., 2014); (4) body weight and bone marrow cellularity endpoints in C57BL/6J mice exposed via drinking water for 16 weeks (Kelly et al., 2013); (5) body weight and histology of esophagus and forestomach in S-D rats exposed via drinking water for 19 or 30 weeks (Luo et al., 1983); (6) body weight and reproductive endpoints in Wistar rats exposed via drinking water for 12 weeks (Ballester et al., 2007; Ballester et al., 2005); (7) body weight in Wistar rats exposed via the diet for 28 days (Chatterjee et al., 1973); and (8) body weight in rats and mice exposed via drinking water for life (Schroeder and Mitchener, 1975a, b). Comprehensive oral noncancer toxicity assays of sodium tungstate in chronically exposed animals are not available.

Decreased body weight, increased incidence of lesions in the kidney and glandular stomach, decreased bone marrow cellularity, and decreased immune responses are the most clearly identified effects noted in the subchronic-duration studies. In the 90-day gavage rat study, biologically significantly decreased body weight (12−15%) occurred in males, but not females, exposed to 125 mg W/kg-day (USACHPPM, 2007a, b). Increased incidences of kidney lesions (cortical tubule regeneration) were also found in male and female rats at

125 mg W/kg-day (USACHPPM, 2007a, b). Increased incidences of stomach lesions (inflammation and goblet cell metaplasia) occurred in male and female rats at 78 and 125 mg/kg-day, but not at 47 mg W/kg-day (USACHPPM, 2007a, b). The 70-day gavage rat study, in which 78 mg W/kg-day was the highest dose tested, found no exposure-related effects on reproductive performance or histology of various organ tissues in F0 males or females, no exposure-related histological tissue changes in F1 offspring at Postnatal Day (PND) 20 and 70, and no biologically significant changes in neurobehavioral endpoints in F1 offspring at PND 4 and 7 (McInturf et al., 2011; McInturf et al., 2008). No effects on reproductive endpoints were found in C57BL6 mice following 19 weeks of exposure at doses up to 125 mg W/kg-day (Osterburg et al., 2014). Similarly, no effects on reproductive endpoints were found in male or female Wistar rats following 12 weeks of exposure at 147 or 160 mg W/kg-day, respectively, although statistically significantly decreased body-weight gains were reported for both sexes (Ballester et al., 2007; Ballester et al., 2005). Decreased responses to bacterial infection (decreased numbers of activated (CD71+) helper and cytotoxic T cells in the spleen) were found in F0- and F1-generation mice at 125 mg W/kg-day, but not at 78 mg W/kg-day (Osterburg et al., 2014). The lowest-observed-adverse-effect level (LOAEL) and no-observed-adverse-effect level (NOAEL) for decreased splenic response to injection with a bacterial toxic in adult C57BL6 mice after 28 days of exposure are also 125 and 78 mg W/kg-day, respectively (Osterburg et al., 2014). In the 16-week drinking water mouse study, 250 and 49 mg W/kg-day are the LOAEL and NOAEL, respectively, for decreased body weight and decreased total bone marrow cellularity (Kelly et al., 2013).

Studies of similar noncancer endpoints in animals repeatedly exposed orally to tungsten metal are not available. However, an early series of studies found that sodium tungstate was more potent than tungsten metal in causing mortality and body-weight changes in rats (Kinard and Van de Erve, 1943, 1941). Deaths occurred in groups of rats within 30 or 7 days of exposure to 0.5 or 2% tungsten as sodium tungstate in the diet, respectively (Kinard and Van de Erve, 1941). In contrast, no deaths occurred in groups of rats exposed to 2, 5, or 10% tungsten as tungsten metal in the diet for 70 days (Kinard and Van de Erve, 1943).

In the only available chronic-duration/carcinogenicity animal studies (Schroeder and Mitchener, 1975a, b), the study authors reported no evidence for carcinogenic responses in rats or mice exposed to sodium tungstate in drinking water for life at a concentration of 5 ppm tungsten (estimated human equivalent doses [HEDs] of 0.1−0.2 mg W/kg-day). Limitations of these studies include inadequate reporting of histological findings for nonneoplastic lesions, inclusion of only one exposure level, and absence of an exposure level close to a maximum tolerated dose (MTD). Body weights of exposed mice and rats were within 10% of the mean for nonexposed control animals in these studies. No evidence of sodium tungstate's tumor promotion capability was found in one rat assay of tumors initiated by *N*-nitrososarcosine ethyl ester (NSEE) (Luo et al., 1983). In another rat assay, tumors appeared earlier in rats exposed to nitroso-*N*-methylurea (NMU) followed by sodium tungstate in drinking water, compared with rats exposed to NMU alone (Wei et al., 1985).

Short-Term-Duration Studies

Osterburg et al. (2014)

Groups of male and female C57BL6 mice (22−24/sex/group) were given water ad libitum containing sodium tungstate dihydrate for 28 days. Sodium tungstate dihydrate was added to the water bottles at levels calculated to administer ingested doses of approximately 2, 62.5, 125, or

200 mg/kg-day sodium tungstate based on an estimated water consumption of 4.5 mL/mouse-day. Water consumption was measured daily, and water bottles were changed 2−3 times weekly. Body weights were measured weekly, and quantities of tungstate were adjusted appropriately in water bottles. Control animals were allowed ad libitum access to filtered water. Equivalent tungsten doses are calculated to be 0, 1.3, 39, 78, or 125 mg W/kg-day (tungsten accounts for 62.6% of total molecular weight of Na_2WO_4). The mice were 8−12 weeks old when supplied by Charles River Laboratories, Wilmington, Massachusetts and acclimated for 7 days before the start of treatment. At the start of testing, the mice weighed between 19 and 22 g. A low-molybdenum diet was available to all mice ad libitum.

Following the 28-day exposure, mice were injected intraperitoneally (i.p.) with either sterile saline or 20 μg *Staphylococcal* enterotoxin B (SEB) to evaluate the adaptive immune response (11−12/sex/group per treatment). Twenty-four hours later, the mice were sacrificed. Blood and spleen tissue samples were collected and stained with lymphocyte and/or myeloid immunophenotyping panels and analyzed by flow cytometry. In situ cytokine production by splenic T cells was also measured.

No exposure-related effects on survival or clinical signs of toxicity were reported. No exposure-related changes were observed in body weight or standard hematological endpoints (e.g., counts of white blood cell [WBC] and red blood cell [RBC], hemoglobin concentration [Hb], hematocrit [Hct]). No alterations in immunophenotypes of blood cells were reported. In the spleen, there was no exposure-related difference in the number of helper or cytotoxic T cells following SEB infection. However, the number of activated (CD71+) helper and cytotoxic T cells in response to SEB infection were significantly decreased by 43 and 66%, respectively, in mice exposed to 125 mg W/kg-day, compared with controls (see Table B-2). Levels of CD71− and CY71+ helper and cytotoxic T cells did not differ among exposure groups in mice injected with saline (instead of SEB). Additionally, in situ interferon gamma (IFN-γ) production was significantly reduced by about 55% in isolated spleen cells harvested from mice exposed to 125 mg W/kg-day following the SEB challenge, compared with the control (see Table B-2). Again, no exposure-related effects were observed in mice injected with saline. Taken together, these data indicate NOAEL and LOAEL values of 78 and 125 mg W/kg-day, respectively, for immune suppression.

A second group of male and female C57BL6 mice (number not specified) were given water ad libitum containing 0, 20, or 200 mg tungstate/kg-day (0, 12.5, or 125 mg W/kg-day) for 28 days. After the 28-day exposure, mice were challenged in a delayed-type hypersensitivity Type IV (Type IV DTH) experiment using 4-hydroxy-3-nitrophenylacetic acid active acid (NP-O-Su) as a secondary antigen. During the sensitization phase, tungstate exposure continued. Ten days later, the mice were challenged with an NP-O-Su injection into the right hind foot pad; the left hind foot pad received a saline injection for comparison. The extent of footpad swelling was measured with a dial gauge 24 hours postinjection. The Type IV DTH experiment was repeated using doses of 0, 0.2, 2, or 200 mg tungstate/kg-day (0, 0.1, 1.3, or 125 mg W/kg-day).

In the first DTH experiment, foot-pad swelling was significantly decreased by ~30% in mice exposed to 12.5 or 125 mg W/kg-day, compared with controls. In the second DTH experiment, mice exposed to 125 mg W/kg-day also showed a significant decrease in swelling (~70% compared with controls). No significant, exposure-related changes were observed in foot-pad swelling in mice exposed to 0.1 or 1.3 mg W/kg-day. The adversity of the diminished

response is uncertain, and could be viewed as a positive action of tungstate (potentially therapeutic) in allergically sensitized individuals. Therefore, a NOAEL/LOAEL determination is not made for the Type IV DTH studies.

Chatterjee et al. (1973)

Groups of male Wistar rats (4/group) were fed ad libitum with basal diet containing 0, 5, or 50 ppm tungsten (W) as sodium tungstate dihydrate for 28 days. Rats weighed 60−80 g when they were obtained from CIBA Pharmaceuticals (Bombay, India). Treatment began after a one-week observation period (age of rats unspecified). Rats were weighed biweekly. No exposure-related effects on body-weight gain were reported (no other toxicity endpoints were evaluated). Using the reported starting body-weight range and body-weight-gain data and a calculated food consumption rate of 0.01 kg/day (based on the allometric equation for laboratory mammals reported by U.S. EPA (1988): food consumption = 0.056 × ($BW^{0.6611}$) where body weight was 0.084 kg and 0.081 kg for the 5 and 50 ppm groups, respectively), daily tungsten intakes were calculated to be 0, 0.65, or 6.6 mg W/kg-day. A NOAEL of 6.6 mg W/kg-day is determined for lack of body-weight effects in male rats.

Subchronic-Duration Studies

USACHPPM (2007a); USACHPPM (2007b)

The U.S. Army Center for Health Promotion and Preventative Medicine (USCHPPM) conducted a 90-day oral toxicity study in rats in accordance with EPA Health Effects Testing Guidelines OPPTS 870.3100. The results of this study were later published in a peer-reviewed paper by McCain et al. (2015). Groups of 5-week-old S-D rats (10/sex/group) were administered 10, 75, 125, or 200 mg/kg-day sodium tungstate (Na_2WO_4) as sodium tungstate dihydrate in water via gavage 7 days/week. Equivalent tungsten doses are calculated to be 6, 47, 78, or 125 mg W/kg-day (tungsten accounts for 62.6% of total molecular weight of Na_2WO_4). Control animals were given deionized water via gavage at the same volume per body weight as all other dose groups (1 mL/kg). At the start of testing, the rats weighed between 199 and 230 g and were randomly allocated to treatment groups based on body weight. Gavage doses were adjusted weekly in accordance with body-weight changes.

Clinical examinations were performed prior to treatment and once weekly during treatment, and the animals were observed daily for clinical signs of toxicity. Body weights and feeder weights were measured on Days −3, −1, 0 (day of first dose), and 7, and weekly thereafter. Ophthalmic examinations were performed before treatment and within a week of scheduled necropsy. Urinalysis was performed in eight rats/sex/group within 2 weeks of necropsy (volume, color, appearance, pH, specific gravity, glucose, bilirubin, urobilinogen, ketone, blood, protein, nitrite, and leukocytes). Following the 90-day exposure period, blood samples were collected for hematology, blood coagulation, and clinical chemistry. Hematological endpoints included WBC count and differential, RBC count, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, RBC distribution width, platelets, and mean platelet volume. Blood coagulation was evaluated using average prothrombin time and average activated prothrombin time. Clinical chemistry endpoints included alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium, cholesterol, creatinine kinase, creatinine, glucose (nonfasting), lactate dehydrogenase, total bilirubin, total protein, triglycerides, sodium, potassium, and chlorine.

At sacrifice, a complete necropsy to detect gross pathological lesions was conducted on all animals. Terminal body weight and organ weights for the brain, heart, liver, kidneys, spleen, adrenals, thymus, epididymides/uterus, and testes/ovaries were recorded. The following tissues were harvested and embedded in paraffin for histopathological evaluation in the 0, 78-, and 125-mg W/kg-day groups: brain, pituitary, thyroid w/parathyroid, thymus, lungs, trachea, heart, bone marrow, salivary gland, liver, spleen, kidney, adrenal, pancreas, gonads, uterus, aorta, esophagus, stomach, duodenum, jejunum, ileum, caecum, colon, urinary bladder, lymph node, peripheral nerve, thigh musculature, eye, spinal cord (three levels), exorbital lachrymal gland, and all gross lesions. Sections from select target tissues (kidney, stomach, and epididymides) were also examined in the 6- and 47-mg W/kg-day groups.

No exposure-related clinical signs of toxicity were observed. Body weight was biologically significantly decreased by 12−15% in males in the 125-mg W/kg-day group over the last 3 weeks of the study, compared with controls. Reduced body weight was accompanied by statistically significant reductions in food consumption in this group (quantitative data were not reported by the study authors). No exposure-related changes in body weight or food consumption were observed in lower-dose males and females. No ophthalmic abnormalities were observed in any group. No significant, dose-related changes were observed in urinalysis, clinical chemistry, or hematological endpoints. Decreased absolute liver, heart, testes, and epididymis weights in high-dose males and increased kidney and spleen weights in high-dose females were reported to have occurred, but no statistically significant dose-related changes in organ-to-body weight ratios were reported. Pairwise analysis was not conducted, because analysis of variance (ANOVA) did not indicate significant effects of dose on any organ:body weight ratio.

Exposure-related histological changes were observed in the stomach, kidneys, and epididymides. Statistically significantly increased incidences of goblet cell metaplasia and subacute inflammation were observed in the glandular stomach of males and females in the 78- and 125-mg W/kg-day groups (see Table B-1). Subacute inflammation was characterized by the presence of eosinophils and a few mononuclear cells throughout the submucosa. The study authors considered goblet cell metaplasia and subacute inflammation to be nonadverse and related to a physiologic effect of gavage administration. However, this explanation is not consistent with the observed absence of lesions in control groups and the dose-related increases in treated groups. The glandular stomach lesions are considered biologically significant, dose-related, and compound-related effects for this assessment (see Table B-1). Supporting the biological significance of these lesions is the understanding that goblet cells are not part of the normal mammalian gastric epithelium, and that metaplasia (replacement of normal cells with columnar absorptive cells and goblet cells of intestinal morphology) is a common histological change associated with repeated inflammation of the gastric mucosa (Liu and Crawford, 2005). Increased incidences of mild-to-severe regeneration of renal cortical tubules were observed in males and females in the 125-mg W/kg-day group (see Table B-1). Affected tubules exhibited abundant pale basophilic cytoplasm and closely packed, typically basally located, nuclei with a moderate degree of anisokaryosis and scattered karyomegaly. Tubular dilation was observed in the more severely affected rats. Minimal regeneration of renal cortical tubules was observed in all dose groups (incidences were not reported by the study authors). Group mean and individual animal severity scores were not provided. Chronic progressive nephropathy (basophilic tubules with thickened basement membranes) was observed in all dose groups, but with no apparent dose-response relationship (incidence data were not reported by the study authors). Luminal

cellular debris was observed in the epididymides of 3/10 male rats in the 125-mg W/kg-day group, an incidence that is not significantly elevated over the control incidence of 0/10 (see Table B-1). There were no testicular lesions that explained the presence of degenerated cells in the lumen of the epididymis; therefore, the biological significance is uncertain. The remaining histological findings were considered by the study authors to be incidental or spontaneous, rather than exposure related (lesion type, location, and incidence and severity data were not provided in the available report).

A LOAEL of 78 mg W/kg-day and a NOAEL of 47 mg W/kg-day are identified in male and female S-D rats for significantly increased incidence of rats with goblet cell metaplasia and inflammation in the glandular stomach, compared with controls. Additional effects found in the highest dose group (125 mg W/kg-day) were decreased body weight in males and significantly increased incidences of male and female rats with mild to severe renal cortical tubule regeneration.

Kelly et al. (2013)

Groups of male C57BL/6J mice (5/dose/sacrifice) were given water ad libitum containing 0, 15, 200, or 1,000 mg/L tungsten as sodium tungstate dihydrate for 1, 4, 8, 12, or 16 weeks. Mice were 4-weeks-old when purchased from Jackson Labs (Bar Harbor, Maine). Treatment began after a 1-week acclimation period. Body weight was measured weekly, and daily water consumption was monitored, but quantitative data were not reported. Using reference male mouse body weight (0.0316 kg) and water consumption (0.00782 L/day) values for the subchronic-duration studies (U.S. EPA, 1988), the estimated tungsten intakes in the 15, 200, and 1,000 mg/L groups are 4, 49, and 250 mg W/kg-day, respectively. At all interim and terminal sacrifices, blood was collected for hematology (RBC count, WBC count and differential, hematocrit, hemoglobin, platelets), tibiae weight and length was measured, and bone marrow was harvested from both tibiae and femora. The total number of tibial and femoral bone marrow cells per animal was quantified, and two million bone marrow cells/animal were used to quantify B cell developmental fractions A–F using flow cytometry. Fractions A, B, C/C′, D, E, and F represent pre-pro-B cells, early pro-B, late pro- and large pre-B cells, small pre-B cells, immature B cells, and mature B cells, respectively. Clonogenicity (activity) of bone marrow precursor cells was assessed with the pre-B colony forming unit (CFU) assay. At the terminal sacrifice, serum AST and ALT levels were also measured.

Body weight was statistically significantly lower in animals in the 250-mg W/kg-day group by approximately 15%, compared with controls (data presented graphically). No exposure-related statistically significant changes were observed in tibia weight or length or serum AST or ALT enzyme levels.

After one week of exposure, peripheral WBC counts were statistically significantly decreased in the 4, 49, and 250-mg W/kg-day groups by approximately 10, 25, or 50%, compared with controls (data presented graphically). White cell differential counts showed that lymphocytes were significantly decreased in the 49 and 250-mg W/kg-day groups, and granulocytes and monocytes were significantly decreased in the 250-mg W/kg-day group. No statistically significant, dose-related findings were observed in WBC counts at any other time point. No exposure-related changes were observed in erythrocyte or platelet counts or hemoglobin or hematocrit levels at any time-point.

Total bone marrow cellularity was statistically significantly decreased by 20% in mice in the 250-mg W/kg-day group at 16 weeks, but not in lower dosage groups or in any dose groups at earlier time points (see Table B-3). After 16 weeks, all tungsten-exposed groups demonstrated a statistically significant 83−117% increase in the percentage of late pro- and large pre-B cells in the bone marrow, compared with control values (Fraction C/C′; see Table B-3). This effect was not observed at the earlier time points. When normalized to total bone marrow cellularity, the number of late pro- and large pre-B cells at 16 weeks was statistically significantly increased in the 4- and 49-mg W/kg-day groups by 88 and 75%, respectively, compared with controls. The 50% increase in the 250-mg W/kg group was not statistically significant (see Table B-3). There were also statistically significant increases (45−98% compared with control) in the percentages of mature B cells (Fraction F) in the 49- or 250-mg/kg-day groups at early time points (1, 4, or 8 weeks), but these changes were not observed at 12 or 16 weeks. When normalized to total bone marrow cellularity, the number of mature B cells was not statistically significantly altered in any dosage group at any time point, compared with control. No exposure-related changes were observed in the percentage or number of pre-pro-B, early pro-B, small pre-B, or immature B cells (Fractions A, B, D, or E) at any time point.

B cell developmental profiles were also examined in separate groups of mice exposed to 4 mg W/kg-day for 4 weeks, followed by 4- or 8-week unexposed recovery periods. However, the lack of consistent statistically significant effects of 4 weeks of exposure on any B cell fractions makes the presented results from these groups difficult to interpret.

No exposure-related changes were found in the number of B lymphoid precursors at any time point. Clonogenicity of bone marrow progenitor cells into lineage-specific precursors ex vivo was statistically significantly increased in the CFU-pre-B assay using cells from mice exposed for 16 weeks at 250 mg W/kg-day (113% increase), compared with controls (see Table B-3). The adversity of this effect is uncertain, especially because peripheral WBC counts were not elevated. Statistically significant effects on clonogenicity of bone marrow progenitor cells were not observed with cells from mice in the lower exposure groups.

In nonadherent bone marrow cells, deoxyribonucleic acid (DNA) damage assessed by the Comet assay was increased compared with control values, but findings across time points and dosages were not consistent with a monotonic response with increasing dose or duration (see Table B-4). Statistically significant increases were observed at 1, 4, 12, and 16 weeks in the 4-mg W/kg-day group; at 1, 4, and 8 weeks in the 49-mg W/kg-day group; and at 4 and 12 weeks in the 250-mg W/kg-day group. The amount of DNA damage did not increase with increasing dose; across time points, the magnitude of damage was higher in cells from 4-mg W/kg-day mice than cells from 250-mg/kg-day mice. In CD19+ B cells, DNA damage was statistically significantly increased in the 4-mg W/kg-day group at 1 and 4 weeks and the 49-mg W/kg-day group at 4 weeks, but decreased (not statistically significant) at 1 and 4 weeks in the 250-mg/kg-day group (see Table B-4; DNA damage in CD19+ B cells was not assessed at later time-points). Immunoblot staining for γH2AX (another assay for DNA damage) in CD19+ B cells from exposed mice after 1 week of exposure was not statistically significantly elevated compared with control values.

In summary, the results from this study indicate that 250 and 49 mg W/kg-day are the LOAEL and NOAEL, respectively, for decreased body weight and decreased total bone marrow cellularity in male C57BL/6J mice exposed by drinking water for 16 weeks. Persistent,

exposure-related changes in blood cell counts were not observed. Increased percentages of late pro- and large pre-B cells (Fraction C/C′) were observed in all exposed groups from this study [i.e., Kelly et al. (2013)], after 16 weeks of exposure, but the adversity of this change is uncertain. Kelly et al. (2013) noted that late pro- and large pre-B cells are particularly sensitive to DNA damaging agents and hypothesized that an increased percentage of B cells in this developmental stage may increase the probability of DNA damaging events in B cells and potentially increase the probability for leukemia. However, a clear concordance was not observed between DNA damage and percentages of late pro-and large pre-B cells. For example, in nonadherent bone marrow cells isolated from mice exposed to the highest dose, DNA damage was not increased, compared with control values at 1 or 16 weeks, but 16 weeks of exposure to each of the dosage levels caused increased percentages of late pro-and large pre-B cells.

Kinard and Van de Erve (1941)

Groups of rats (strain unspecified; 37-days-old; 5−6/sex/group) were caged separately and fed ad libitum with ground dog chow containing 0, 0.1, 0.5, or 2.0% tungsten as sodium tungstate for up to 70 days. Food consumption was determined from the food remaining in the feeders after each feeding, but the study authors noted that significant food wastage made this measurement unreliable. Body weight was measured at 9−12-day intervals. Sodium tungstate caused 100% mortality in the 2.0% group (within the first week of exposure) and 50 and 33% mortality of males and females, respectively, in the 0.5% group (deaths occurred between Days 17 and 29 of exposure). No mortalities were observed in the 0.1% group or control groups (see Table B-5). Body weight and body-weight gain were reduced 9−11 and 12−16%, respectively, in the 0.1% group compared with controls (see Table B-5). Absolute body-weight data were not reported for other groups; however, body-weight gain in surviving rats from the 0.5% group was decreased by 114−131%, compared with controls (see Table B-5). In surviving rats, apparent food consumption was decreased compared with controls (−17 and −4% in males and females, respectively, from the 0.1% group; −53 and −28% in males and females, respectively, from the 0.5% group, although again, the researchers did not consider these data to be reliable. Using reference rat body weight (0.235 kg for males and 0.173 kg for females) and food consumption (0.021 kg/kg BW-day for males and 0.017 kg/kg BW-day for females) values for the subchronic-duration studies (U.S. EPA, 1988), the estimated tungsten intakes in the 0, 0.1, 0.5, and 2% groups were calculated to be 0, 91, 455, and 1,820 mg W/kg-day for males and 0, 102, 510, and 2,040 mg W/kg-day for females. A LOAEL of 91 mg W/kg-day is identified for reduced body weight in males. A NOAEL was not identified.

Additional groups of rats were fed diets containing 0.1, 0.5, or 3.96% tungsten equivalents of tungsten trioxide or 0.5, 2.0, or 5.0% tungsten equivalents of ammonium-*p*-tungstate. This allowed a comparison of toxicity among three soluble compounds of tungsten. Mortalities for tungsten trioxide exposed groups of increasing dose were 0, 73% (average of 80 and 66% for males and females), and 100%. Mortalities for ammonium-*p*-tungstate exposed groups were 0, 80, and 100%. Tungsten trioxide at the lowest tested concentration (0.1% W) decreased body weight by 6.3 and 7.4% in surviving males and females after 70 days. Ammonium-*p*-tungstate at the lowest tested concentration (0.5% W) decreased body weight by 3.9 and 5.3% in surviving males and females.

Considering body-weight data, the toxicity of the three tungsten compounds increased in the order: ammonium-*p*-tungstate < tungsten trioxide < sodium tungstate, which follows the order of increasing solubility. At the lowest tested concentrations, body-weight decreases were

3.9−5% for ammonium-*p*-tungstate (0.5% W), 6.3−7.4% for tungsten trioxide (0.1% W), and 9−11% for sodium tungstate (0.1%W). At the 0.5% W concentration, a different order of potency to produce mortality was observed: ammonium-*p*-tungstate (no mortalities) < sodium tungstate (50% males, 33% females) < tungsten trioxide (80% males, 66% females). At the next tested concentration (2% W), ammonium-*p*-tungstate produced 80% mortalities, and sodium tungstate produced 100% mortalities. Tungsten trioxide was not tested at 2% W.

Kinard and Van de Erve (1943)

Groups of 10 rats (mixed Wistar albino and Minnesota piebald strains; 38-days-old; 5/sex/group) were caged separately and fed ad libitum with ground dog chow containing 0, 2, 5, or 10% tungsten metal powder for 70 days. Food consumption was determined from the food remaining in the feeders after each feeding. Weight gains were recorded at 10-day intervals. Animals were sacrificed after 70 days, and their gastrointestinal tracts were examined grossly. During the dosing period, each male rat fed 2, 5, or 10% tungsten consumed an average of 21, 54.5, or 104 g of tungsten, respectively. Female rats, in the same period, consumed an average of 14.8, 41, or 75 g of tungsten. During the 70-day period, weight gains of male rats fed 2, 5, or 10% tungsten were 94, 113, or 108% of those of male rats fed the control diet, while the weight gains of female rats were 104, 97, or 85% of control weight gains, respectively (see Table B-6). However, the absolute body-weight data is not available for evaluation. The study authors attributed the different weight gains by male and female rats fed diets containing 10% tungsten to reduced food consumption in the female rats. No exudation of blood into the mucous membranes of the small or large intestines was found in rats fed tungsten for 70 days. These results show that oral exposure to tungsten metal in the diet is markedly less toxic than water-soluble compounds of tungsten, such as sodium tungstate. No mortalities occurred in rats fed diets containing up to 10% tungsten as tungsten metal for up to 70 days, whereas deaths occurred within 30 days of exposure in rats fed diets containing 0.5 or 2% tungsten as sodium tungstate. Using tungsten consumption and body-weight data from Kinard and Van de Erve (1941), tungsten metal doses were calculated to be approximately 0, 1,325, 3,450, or 6,550 mg W/kg-day and 0, 1,450, 4,000, or 7,325 mg W/kg-day for males and females, respectively. A LOAEL of 7,325 mg W/kg-day and a NOAEL of 4,000 mg W/kg-day are identified in female rats for decreased body-weight gain compared with controls.

Luo et al. (1983)

Male weanling inbred S-D rats were given demineralized drinking water ad libitum containing 0, 100, or 200 ppm tungsten as sodium tungstate for 19 weeks (10/group). All rats were fed ad libitum a nutritionally adequate semipurified diet containing 0.064 ppm of tungsten. Body weights were monitored throughout the exposure period. At sacrifice, the esophagus and forestomach were removed and fixed in 10% formalin for histopathological examinations. The average body weight of 100 ppm tungsten-fed rats was similar to controls from Weeks 0−19; body-weight data for rats given 200 ppm for 19 weeks were not reported. Using reference male weanling S-D rat body weight (0.267 kg) and water consumption (0.037 L/day) values for the subchronic-duration studies (U.S. EPA, 1988), daily intake of tungsten in the 100- and 200-ppm groups was estimated to be 13.9 and 27.8 mg W/kg-day. No histopathological alterations were observed in any of control or tungsten-only exposed rats. NOAELs of 27.8 mg W/kg-day for a lack of observed effects in male rats are identified for 19-week exposures.

Chronic-Duration/Carcinogenicity Studies

Luo et al. (1983)

Male weanling inbred S-D rats were given demineralized drinking water ad libitum containing 0 or 100 ppm for 30 weeks (10/group). All rats were fed ad libitum a nutritionally adequate semipurified diet containing 0.064 ppm of tungsten. Body weights were monitored throughout the exposure period. At sacrifice, the esophagus and forestomach were removed and fixed in 10% formalin for histopathological examinations. Body-weight data for rats given 100 ppm for 30 weeks were not reported. Using reference male weanling S-D rat body weight (0.523 kg) and water consumption (0.062 L/day) values for the chronic-duration studies (U.S. EPA, 1988), daily intake of tungsten in the 100-ppm groups was estimated to be 11.9 mg W/kg-day. No histopathological alterations were observed in any of control or tungsten-only exposed rats. NOAELs of 11.9 mg W/kg-day for a lack of observed effects in male rats are identified for 30-week exposures, respectively.

Schroeder and Mitchener (1975b)

A group of 72 Long-Evans rats (37 males/35 females) was given 5 ppm of tungsten for life as sodium tungstate in deionized drinking water to which essential elements manganese, cobalt, copper, zinc, and molybdenum were added in amounts similar to those in commercial diets. A control group (52 males/52 females) received the same basal drinking water without tungsten. The diet fed was low in trace metals. The animals were weighed at weekly intervals for an unspecified number of weeks, at monthly intervals for the remaining weeks of the first year, and then at 3-month intervals. Urine was collected from 12 rats/sex/group for analysis of urinary protein, pH, and glucose when rats were 162-days-old. Blood samples were collected from 12 rats/sex/group for analysis of serum cholesterol, glucose, and uric acid levels at various time-points throughout the study, starting when rats were 90-days-old. At 20 months of age, a pneumonia epidemic led to the death of ~30% of the animals in both the control and tungsten-exposed groups (no significant difference in the number of deaths among groups). The remaining animals (26 control males, 24 control female, 25 exposed males, and 20 exposed females) were necropsied at natural death. At necropsy, the animals were weighed and examined for gross pathological changes. The heart, lung, kidney, liver, spleen, and all tumors were fixed for microscopic examination. The longevity of rats was calculated based on the mean lifespan of the last five surviving animals.

A statistically significant increase in body weight from controls was first observed at 180 days in males and 360 days in females. Body weight in males remained significantly higher than controls to 540 days; in females at 540 days, body weight was slightly, but not significantly, increased (see Table B-9). However, these findings are not considered biologically significant because observed increases in body weight were <10% different from controls. At necropsy following natural death, no significant differences in body weight or heart weight were reported in tungsten-fed rats compared with controls (it is unclear whether any other organs were weighed). The longevity of tungsten-fed females was comparable with female controls. However, the longevity of tungsten-fed males was statistically significantly decreased by 13% compared with male controls (see Table B-9). Urinary parameters of tungsten-fed animals were unremarkable. Intermittently during the study, fasting serum cholesterol and glucose levels were significantly different from controls; however, no trends were apparent. There were no exposure-related increases in total gross or malignant tumor incidences (see Table B-9). It is not clear whether the heart, lung, kidney, liver, and spleen were assessed for nonneoplastic histological changes.

Using reference Long-Evans rat body weight (0.472 kg for males and 0.344 kg for females) and water consumption (0.057 L/day for males and 0.046 L/day for females) values for the chronic-duration studies (U.S. EPA, 1988), estimated daily tungsten intakes were 0.6 and 0.7 mg W/kg-day for males and females, respectively. Based on these estimates, HEDs were calculated to be 0.1 and 0.2 mg W/kg-day for males and females, respectively, using a DAF of 0.24 for rats based on body weight to 3/4 power scaling (U.S. EPA, 2011b). Although a significant decrease in longevity was observed in male rats, based on the lifespan of the final five surviving rats; the occurrence of a pneumonia epidemic 20 months into the study decreases confidence that this was an exposure-related effect. A NOAEL of 0.7 mg W/kg-day is identified for female rats, respectively. No evidence for a carcinogenic response to tungsten was found.

Schroeder and Mitchener (1975a)

Using methods similar to those with rats (Schroeder and Mitchener, 1975b), white Swiss mice, 54 of each sex, were given 0 (control) or 5 ppm of tungsten for life as sodium tungstate in their drinking water. Body weight was monitored, and at natural death, gross and microscopic examinations were performed on major organs. The body weights of tungsten-fed mice at 540 days of exposure were comparable with controls (see Table B-9). The longevity of tungsten-fed male mice was decreased by 15%, compared with male controls; however, this finding was not statistically significant. The longevity of tungsten-fed females was comparable with female controls (see Table B-9). Exposure to tungsten was reported to be not "significantly tumorigenic"; however, tumor incidence data were not reported. Using reference mouse body weight (0.0317 kg for males and 0.0288 kg for females) and water consumption (0.0078 L/day for males and 0.0073 L/day for females) values for the chronic-duration studies (U.S. EPA, 1988), estimated daily tungsten intakes in males and females were 1 mg W/kg-day. Based on these estimates, HEDs were calculated to be 0.1 mg W/kg-day for males and females, using a DAF of 0.14 for mice based on body weight to 3/4 power scaling (U.S. EPA, 2011b). A NOAEL of 1 mg W/kg-day for a lack of observed effects is identified for male and female mice. No evidence for a carcinogenic response to tungsten was found.

Reproductive/Developmental Studies

Osterburg et al. (2014)

In a one-generation study, groups of male and female C57BL6 mice (12−16/sex/group) were given water ad libitum containing sodium tungstate dihydrate for 12 weeks prior to mating, through 1 week mating, 3 weeks gestation, and 3 weeks postparturition (19 weeks total). The F1 generation was exposed for an additional 90 days. Sodium tungstate dihydrate was added to the water bottles at levels calculated to administer ingested doses of approximately 2, 62.5, 125, or 200 mg sodium tungstate/kg-day based on an estimated water consumption of 4.5 mL/mouse-day. Water consumption was measured daily, and water bottles were changed 2−3 times weekly. Body weights were measured weekly, and quantities of tungstate were adjusted appropriately in water bottles. Control animals were allowed ad libitum access to filtered water. Equivalent tungsten doses are calculated to be 0, 1.3, 39, 78, or 125 mg W/kg-day (tungsten accounts for 62.6% of total molecular weight of Na_2WO_4). Animals were 8−12-weeks-old when supplied by Charles River Laboratories, Wilmington, Massachusetts. Mice were acclimated for 7 days prior to the start of treatment. At the start of testing, the mice weighed between 15 and 18 g. A low-molybdenum diet was available to all mice ad libitum. Mice were housed individually during the course of the study and pair mated for breeding. After confirmation of the pregnancy, males were removed.

The number of live births, litter size, and sex ratio were recorded for each litter. F0 animals were sacrificed at weaning (Study Week 19), while F1 offspring (12−16/group) were sacrificed after the additional 90-day exposure (approximately Study Weeks 31−32). Twenty-four hours prior to sacrifice, the mice were injected intraperitoneally with either sterile saline or 20 μg SEB to evaluate the adaptive immune response (6−8/sex/group per treatment). At sacrifice, blood and spleen tissue samples were collected and stained with lymphocyte and/or myeloid immunophenotyping panels and analyzed by flow cytometry. Complete blood counts and hematological parameters were evaluated. In situ cytokine production by splenic T cells was also measured.

No exposure-related changes were observed in body weight. No significant, dose-related changes in the number of live births, litter size, or sex ratio of the pups were observed; mating success was not reported. With two exceptions (monocyte percentage and RBC distribution width), there were no statistical differences in hematological endpoints between F0 and F1 animals within the same group; therefore, the hematological data for F0 and F1 animals were combined for analysis. The only statistically significant, dose-related hematological finding was a decrease in the percentage of monocytes in F0 and F1 animals (combined) exposed to "higher concentrations." A pair-wise analysis was not reported, and F0 and F1 animal data and statistics were not reported individually. No alterations in immunophenotypes of blood cells were reported. In the spleen, there was no exposure-related difference in the number of helper or cytotoxic T cells following SEB infection in either F0 or F1 animals. However, the number of activated (CD71+) helper and cytotoxic T cells in response to SEB infection in F0 and F1 mice exposed to 125 mg W/kg-day was significantly decreased by 60−80%, compared with respective controls (see Table B-2). Levels of CD71− and CY71+ helper and cytotoxic T cells did not differ among exposure groups in mice injected with saline (instead of SEB). Additionally, in situ IFN-γ production was significantly reduced by 47% in isolated spleen cells harvested from F0 mice exposed to 125 mg W/kg-day following the SEB challenge, compared with control (see Table B-2). Again, no exposure-related effects were observed in F0 mice injected with saline. Cytokine production was not significantly altered in F1 mice (see Table B-2).

In summary, a reproductive NOAEL of 125 mg W/kg-day is identified based on lack of litter effects. A developmental NOAEL/LOAEL determination cannot be made. Standard developmental endpoints were not assessed, and the observed immune suppression at Postnatal Weeks (PNWs) 15−16 may be attributable to the 90-day postweaning exposure to tungsten rather than the developmental exposure. A systemic NOAEL of 78 mg W/kg-day and a LOAEL of 125 mg W/kg-day are identified for immune suppression in F0 and F1 animals, consistent with the 28-day study reported above.

McInturf et al. (2011); McInturf et al. (2008)

Groups of S-D rats (40/sex/group) were administered 0, 5, 62.5, or 125 mg/kg-day sodium tungstate (Na_2WO_4) as sodium tungstate dihydrate in deionized water via gavage 7 days/week for 70 days (including 14 days premating, 14 days mating, 22 days gestation, and through PND 20). Rats were 8-weeks-old when they were obtained from Charles River Laboratories (Wilmington, MA), and dosing began after a 2-week quarantine period. The rats were single-housed except during the mating period, observed daily for clinical signs of toxicity, and weighed weekly. During gestation, pregnant dams were weighed on Gestation Days (GDs) 1, 10, 15, and 20. Gavage doses were adjusted weekly based on body weight. Equivalent

tungsten doses are calculated to be 3, 39, or 78 mg W/kg-day (tungsten accounts for 62.6% of total molecular weight of Na_2WO_4).

At parturition (PND 0), gestation length was recorded. On PND 1, the number of pups in each litter was recorded and all pups were inspected for external malformations. On PND 4, litters were culled to eight pups (four/sex when possible) and weighed. Individual pup weights were measured starting on PND 5 and weekly thereafter until PND 70. Forty F0 males and twenty F0 females per group were necropsied on the last day of treatment (PND 20), and two pups/sex/litter were sacrificed on PND 20 and 70. In 20 male F0 and 10 female F0 rats/group and one pup/sex/litter per sacrifice, various organs (reported as "heart, spleen, kidney, liver, lungs, brain, testes, ovaries, thymus, bone, gastrointestinal tract, etc.") were removed and postfixed in 4% paraformaldehyde for histological evaluation. In the remaining 20 male F0 and 10 female F0 rats/group and one pup/sex/litter per sacrifice, sodium tungstate concentrations were measured in the heart, spleen, kidney, lungs, liver, gastrointestinal tract, brain, femur, blood, and thymus. Tungsten levels were also quantified in mammary secretions of all dams once between PNDs 10 and 14 (see Table C-3 for toxicokinetic data).

Both pups (number not specified) and dams (20/group) were assessed for neurobehavior during the postnatal period; however, results were only reported for rats in the 0, 3, and 78-mg W/kg-day groups (it is unclear whether or not the rats in the 39-mg W/kg-day group were evaluated for neurobehavior). Pups (number not specified) were assessed for early, reflexive behaviors with the righting reflex on PND 4 and separation distress (as measured by ultrasonic vocalization frequency) on PND 7. Dams (20/group) were assessed for instinctual maternal responses using maternal retrieval of pups on PND 2 (latency to retrieve three pups moved to the opposite side of the home cage), open-field behavior on PND 27 (7 days posttreatment), acoustic startle/prepulse inhibition on PND 28 (8 days posttreatment), and the Morris water maze on PNDs 35−88 (15−18 days posttreatment).

No mating or fertility indices were provided in the study report; however, the study authors stated that sodium tungstate had "no apparent effect on mating success." No dose-related changes were observed in gestational weight gain or the number of pups per litter. Mean group gestational length was significantly increased by 2% in high-dose dams; however, this slight increase to 22.08 days from 21.55 days in controls is not considered to be biologically significant. A reproductive NOAEL of 78 mg W/kg-day is identified for the lack of effects on reproductive performance (mating success) in male and female S-D rats.

No dose-related effects were observed for incidence of external malformations or histological lesions in PND 20 or 70 pups. Pup weight and postnatal growth were not altered with gestational and lactational exposure to sodium tungstate. However, pups demonstrated a dose-related increase in the number of ultrasonic distress vocalizations when separated from their dams for 60 seconds on PND 7 (see Table B-7). This finding may suggest increased stress and anxiety in pups exposed to tungsten during gestation and lactation. The number of vocalizations was statistically significantly increased by 76% in the 78-mg W/kg-day group, compared with controls. The 18% increase observed in the 3-mg W/kg-day group was not statistically significant. Data for the 39-mg W/kg-day group were not reported; it is unclear whether or not this group was evaluated for pup neurobehavior. Tungsten exposure did not alter righting reflex in pups on PND 4 (quantitative data not reported by study authors). The highest dose, 78 mg W/kg-day, is a developmental NOAEL for offspring postnatal growth, external

malformations in offspring at birth, histological lesions in tissues of offspring at PNDs 20 and 70, and righting reflex on PND 4. Although a statistically significant (76%) increase in the number of distress vocalizations was observed when PND 7 pups were separated from their dams in high-dose pups, compared with control pups, the biological significance of this effect is not certain.

No dose-related changes in body weight or clinical signs of toxicity were reported in male or female F0 rats. The report stated that histiocytic inflammation from minimal to mild with cardiomyocyte degeneration and necrosis was observed in "several" F0 animals in the 78-mg W/kg-day group on PND 20. According to the method section, histopathological examinations were conducted in 10 F0 animals/sex/group; however, a data table in the report states that "two animals of five" in the high-dose group displayed minimal or mild myocarditis with cardiomyocyte degeneration and necrosis. Therefore, due to reporting inconsistencies and ambiguities, as well as the apparently small number of animals examined, it is unclear whether or not cardiac lesions were exposure related. No other significant exposure-related lesions were observed (incidence data were not provided by the study authors). In dams, no dose-related effects were observed for maternal retrieval, acoustic startle/prepulse inhibition, or water maze training and acquisition. Altered open-field behaviors were observed in low- and high-dose dams on PND 27; however, findings did not demonstrate a consistent dose-related pattern (see Table B-7). Compared with controls, low-dose females demonstrated a significant increase in distance traveled and time spent ambulating, while time spent engaged in stereotypic behaviors was significantly decreased. High-dose females spent significantly less time resting than controls; however, no significant differences were observed in distance travelled or time spent ambulating. Instead, significantly more time was spent engaged in stereotypic behaviors, compared with controls. Findings from the 39-mg W/kg-day group may provide insight into the seemingly contradictory results; however, it is unclear whether or not the dams from the mid-dose group were evaluated in neurobehavioral assays. A NOAEL of 78 mg W/kg-day is identified for systemic effects in F0 males and females exposed to sodium tungstate for 70 days for lack of clinical signs of toxicity, body-weight effects, clear dose-related histopathological changes, or clear dose-related changes in neurobehavioral tests of dams (maternal retrieval of pups on PND 2, open-field behavior on PND 27 [7 days postexposure], acoustic startle/prepulse inhibition on PND 28, and the Morris water maze 15−18 days postexposure).

Ballester et al. (2005)

Groups of male Wistar rats (15/group) were given drinking water containing 0 or 2 mg/mL of sodium tungstate for 12 weeks. Equivalent tungsten doses are calculated to be 1 mg W/mL (tungsten accounts for 62.6% of total molecular weight of Na_2WO_4). Body weight and blood sugar levels were measured regularly during the 12-week exposure period. Sexual function was measured after 10 weeks of exposure. Exposed and control animals were placed in a cage overnight with one untreated female animal, and removed the next morning. Nightly pairings continued for up to 9 nights until the presence of a vaginal tag and/or spermatozoa were observed. After assessment of mating success, all males were sacrificed. Serum was collected and glucose, insulin, FSH, LH, and testosterone levels were measured. Testes were fixed for histological and ultrastructural evaluation. Expression levels of testicular insulin receptors were evaluated using Western blot analysis.

Body-weight gain was significantly decreased by 17% in exposed males, compared with controls (see Table B-8). Before treatment began, the average body weight for control and

treated animals was 200 g. The body-weight gain for the control group was 234.1 g and 195.2 g for rats receiving 1 mg W/mL. Based on these data, the terminal body weight was 434.1 g for the control group and 395.2 g for rats receiving 1 mg W/mL. Thus, terminal body weight was decreased by 9% (not biologically significant) compared to controls.

No exposure-related changes were observed in serum glucose or insulin levels. No exposure-related changes were observed in the mating index, serum hormone levels, or testicular histology or ultrastructure. No changes in testicular insulin receptor expression were reported. Using reference male Wistar rat body weight (0.217 kg) and water consumption (0.032 L/day) values for the subchronic-duration studies (U.S. EPA, 1988), daily tungsten intakes were estimated to be 147 mg W/kg-day. A stand-alone reproductive NOAEL of 147 mg W/kg-day is identified for male rats. A systemic NOAEL of 147 mg W/kg-day is identified based on a lack of statistically and/or biologically significant effects.

Ballester et al. (2007)

Groups of female Wistar rats (24/group) were given drinking water containing 0 or 2 mg/mL of sodium tungstate for 12 weeks. Equivalent tungsten doses are calculated to be 1 mg W/mL (tungsten accounts for 62.6% of total molecular weight of Na_2WO_4). During the exposure period, body weight, food and water intake, and glycemia were measured regularly. Sexual function was measured after 10 weeks of exposure. Exposed and control animals were placed in a cage overnight with one untreated male animal, and removed the next morning. Nightly pairings continued for up to 9 nights until the presence of a vaginal plug and/or spermatozoa were observed. Females that successfully mated were allowed to deliver, and the fertility index (number of parturitions/number of matings) and the litter sizes were recorded. After assessment of reproductive function, all females were sacrificed. Serum was collected and glucose, insulin, ALT, follicle stimulating hormone (FSH), luteinizing hormone (LH), and progesterone levels were measured. Ovaries were fixed for histological evaluation. Expression levels of ovarian and uterine estrogen, progesterone, follicle-stimulating hormone, lutenizing hormone, prolactin, insulin, and GLUT 3 hexose transporters were evaluated using Western blot analysis and real-time polymerase chain reaction.

Body-weight gain was significantly decreased by 41% in exposed females, compared with controls (see Table B-8). Before treatment began, the average body weight for control and treated animals was 200 g. The body-weight gain for the control group was 77.2 g and 45.7 g for rats receiving 1 mg W/mL. Based on these data, the terminal body weight was 277.2 g for the control group and 245.7 g for rats receiving 1 mg W/mL. Thus, terminal body weight was decreased by 11% (biologically significant) compared to controls.

No exposure-related changes were observed in food or water intake in females. No exposure-related changes were observed in serum glucose, insulin, or ALT levels. No exposure-related changes were observed in the mating or fertility indices, litter size, serum hormone levels, or ovarian histology. Expression levels of ovarian and uterine estrogen, progesterone, FSH, LH, prolactin, insulin, and GLUT 3 hexose transporters were not altered with exposure. Using reference female Wistar rat body weight (0.156 kg) and water consumption (0.025 L/day) values for the subchronic-duration studies (U.S. EPA, 1988), daily tungsten intakes were estimated to be 160 mg W/kg-day. A reproductive NOAEL of 160 mg W/kg-day is identified for female rats. A systemic LOAEL of 160 mg W/kg-day is identified for decreased body weight.

Tumor-Promotion Studies

Luo et al. (1983)

The effect of tungsten on NSEE-induced esophageal and forestomach carcinogenesis was investigated in male S-D rats. Male weanling rats were given demineralized drinking water ad libitum containing 0 or 100 ppm tungsten as sodium tungstate for 19 weeks (10/group) or 30 weeks (10/group). Another group of 10 rats were exposed to 200 ppm tungsten for 19 weeks. Other groups (20−41/group) were exposed to NSEE alone via gavage twice weekly between Weeks 4 and 8 (8 doses of NSEE) or between Weeks 4 and 12 (16 doses of NSEE) (see Table B-10 for more details). Additional groups of rats (15−22/group) were exposed to 100 ppm tungsten for 19 weeks plus 16 doses of NSEE, 200 ppm tungsten for 19 weeks plus 8 doses of NSEE, or 100 ppm tungsten for 30 weeks plus 16 doses of NSEE (see Table B-10 for more details). All rats were fed ad libitum a nutritionally adequate semipurified diet containing 0.064 ppm of tungsten and 0.026 ppm molybdenum. Body weights were monitored throughout the exposure period. At sacrifice, the esophagus and forestomach were removed and fixed in 10% formalin for histopathological examinations. The changes of epithelial cells were divided into four stages: Stage I, hyperplastic lesions (hyperkeratosis, simple hyperplasia, and papillary hyperplasia); Stage II, precancerous lesions (marked endophytic growth of epithelium, marked dysplasia, papilloma, and papillomatosis); Stage III, early carcinoma (malignant changes of basal cells or papilloma, carcinoma in situ, and early infiltrative carcinoma); and Stage IV, advanced carcinoma (carcinoma with extensive invasions).

Body weights, reported only for control, 100 ppm tungsten-treated, and NSEE-treated groups up to 19 weeks, were comparable through 13 weeks. Reduced growth was observed in NSEE-treated rats from 13−20 weeks, while the average body weight of 100 ppm tungsten-treated rats was similar to controls during this period.

Using reference male S-D rat body weight (0.267 kg) and water consumption (0.037 L/day) values for the chronic-duration studies (U.S. EPA, 1988), daily intake of tungsten in the 100 and 200 ppm groups was estimated to be 13.9 and 27.8 mg W/kg-day, respectively. Using these estimates, HEDs were calculated to be 3.33 and 6.67 mg W/kg-day, respectively, using a DAF of 0.24 for rats based on body weight to 3/4 power scaling (U.S. EPA, 2011b).

No nonneoplastic or neoplastic histopathological lesions in the esophagus or forestomach were observed in any of the tungsten-only exposed rats (13.9 or 27.8 mg W/kg-day for 19 weeks or 11.9 mg W/kg-day for 30 weeks). In rats exposed to 16 doses of NSEE alone, increased incidences of hyperplastic and precancerous lesions in the esophagus and forestomach were found, as well as increased incidences of early or late carcinomas. Rats exposed to eight doses of NSEE alone also had increased incidences of esophageal hyperplastic or precancerous lesions (compared with nonexposed controls), but no esophageal carcinomas. Forestomach lesion incidence data were not reported for this group; however, they were reportedly "similar" to esophageal lesions. Incidences of rats with hyperplastic lesions, precancerous lesions, or carcinomas in esophagus or forestomach were not significantly different between groups exposed to 13.9 mg W/kg-day plus NSEE and comparable groups exposed to NSEE alone. Incidences for esophageal or forestomach carcinomas were not significantly different between groups exposed to 27.8 mg W/kg-day (for 19 weeks) plus eight doses of NSEE and groups exposed to eight doses of NSEE alone. The results suggest that tungsten did not display a tumor-promoting activity under the conditions of this experiment. However, the incidence of rats with precancerous esophageal lesions in the 27.8 mg W/kg-day plus eight doses of NSEE

group was significantly elevated, compared with incidence in the group exposed to eight doses of NSEE alone. Neoplastic and nonneoplastic tumor incidences for all groups are reported in Table B-10.

Luo et al. (1983) also reported that supplemental molybdenum (2 or 200 ppm in diet) significantly inhibited NSEE-induced carcinogenesis, and that exposure to supplemental tungsten alone (11.9 mg W/kg-day in diet for 30 weeks) produced markedly decreased liver concentrations of molybdenum (98% decreased compared with control). Based on these findings, Luo et al. (1983) hypothesized that exposure to tungsten at 23.8 mg W/kg-day increased the incidence of precancerous lesions induced by eight doses of NSEE (from 4/31 with NSEE alone to 22/22 with tungsten + NSEE; see Table B-10) by counteracting the cancer inhibiting action of molybdenum.

Wei et al. (1985)

The effect of tungsten on NNMU-induced mammary carcinogenesis was investigated in 35-day-old virgin S-D rats. Three groups of female rats (Groups 1−3), one of which was used as a control (Group 1), were given ad libitum a nutritionally adequate semipurified diet containing 0.026 ppm of molybdenum and 0.064 ppm toluene and deionized drinking water. In a 4th group, 150 ppm of sodium tungstate was added to the drinking water. After 15 days, all noncontrol animals (Groups 2−4) received a single intravenous injection of 5 mg/100 g body weight NMU, however, it is unclear from the study report the duration that the rats received tungsten in the diet. One week after administration of carcinogen NMU, 10 ppm of molybdenum was added to the drinking water in Group 3. Rats were palpated for mammary tumors twice weekly and weighed weekly. Animals were sacrificed on Day 125 or 198 after NMU administration (10−24/group/sacrifice), and all mammary tumors were removed for histological evaluation. There were no significant differences in body weight among the groups. None of the control rats developed mammary tumors. NMU alone produced mammary carcinomas, with 97.8% being adenocarcinomas or papillary carcinomas and 2.2% being fibroadenomas. After 125 days, the mammary cancer incidence in Group 4 (sodium tungstate + NMU; 79.2%) was statistically significantly higher than that in Group 2 (NMU alone, 50.0%) or Group 3 (NMU + molybdenum; 45.5%). After 198 days, the mammary cancer incidence in Group 3 (NMU+ molybdenum; 1.5 and 50.0%) was statistically significantly lower than in Group 2 (NMU alone; 2.0 and 90.5%) or Group 4 (NMU+ sodium tungstate; 2.6 and 95.7%). Only histologically confirmed carcinomas were included in the statistical evaluation (see Table B-11). The first palpable mammary tumor was found in the tungsten-supplemented rats (both 125- and 198-day subgroups) only 56 days after the injection of NMU, whereas in both unsupplemented and molybdenum-supplemented rats, the first palpable mammary tumors were observed 71 and 85 days after NMU treatment in the 125- and 198-day subgroups, respectively. Most carcinomas were highly aggressive, but nonmetastatic. This study shows an inhibitory effect of molybdenum on NMU-induced mammary carcinogenesis and a promoting effect of tungsten, an antagonist of molybdenum, on tumor growth.

Using reference female S-D rat body weight (0.338 kg) and water consumption (0.045 L/day) values for the chronic-duration studies (U.S. EPA, 1988), daily intake of tungsten was estimated to be 20 mg W/kg-day. Using this estimate and a DAF of 0.24 for rats based on body weight to 3/4 power scaling (U.S. EPA, 2011b), the HED was calculated to be 4.8 mg W/kg-day.

Inhalation Exposures

No studies have been identified.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Genotoxicity

Sodium tungstate has been tested in a number of short-term-duration genotoxicity tests (see Table C-1 in Appendix C for more details). The results were predominantly negative, although positive findings were noted in a few assays. Genotoxicity studies for tungsten metal were not identified. Sodium tungstate did not induce mutations in *Salmonella typhimurium* or *Escherichia coli* [Covance Laboratories (2004a) as cited in Jackson et al. (2013)], but was weakly positive for inducing reverse mutations at *trp5* and *ilv1* in *Saccharomyces cerevisiae* (Singh, 1983). Sodium tungstate induced mutations in *Plasmodium fischeri* (Ulitzur and Barak, 1988) and induced SOS DNA repair in *E. coli* (Rossman et al., 1991; Rossman et al., 1984). In mammalian cells, sodium tungstate did not induce mutations in mouse lymphoma cells (L5178Y TK±) [Covance Laboratories (2004b) as cited in Jackson et al. (2013)]; chromosomal aberrations (CAs) in Chinese hamster ovary (CHO) cells [Covance Laboratories (2003) as cited in Jackson et al. (2013)], Syrian hamster embryo cells (Larramendy et al., 1981), or human purified lymphocytes (Larramendy et al., 1981); or sister chromatid exchange (SCEs) in human whole-blood cultures (Larramendy et al., 1981). Sodium tungstate reportedly induced mutations in Chinese hamster V79 lung cells (Zelikoff et al., 1986), but only an abstract report of this study is available, precluding independent evaluation. In in vivo animal tests, sodium tungstate did not induce bone marrow micronuclei (MN) in mice [Covance Laboratories (2004c) as cited in Jackson et al. (2013)] and equivocal findings were reported for DNA damage (assayed by the Comet assay and γH2AX immunoblotting) in bone marrow of orally exposed mice (Kelly et al., 2013).

Supporting Human Studies

Cross-sectional surveys examining possible associations between several medical conditions and concentrations of tungsten and other metals in urine or blood collected from the general U.S. population reported the following findings (the route of exposure in these studies is unknown; see Table C-2 in Appendix C for more details):

- an association between urinary tungsten concentration and serum levels of thyroid stimulating hormone (TSH), but not with levels of other thyroid hormones (Yorita Christensen, 2012);
- an association between elevated urinary tungsten concentration and cardiovascular and cerebrovascular disease (Agarwal et al., 2011); and
- an association between elevated urinary tungsten concentration and asthma, but not with other medical conditions, including coronary heart disease, heart attack, congestive heart failure, stroke, or thyroid problems (Mendy et al., 2012).

Epidemiology studies of hard alloy workers exposed to dusts containing mixtures of tungsten and other metals reported increased risks for respiratory and neurologic effects, but the results are confounded by exposure to mixtures of metals and have been attributed to cobalt (ATSDR, 2005).

Other Animal Toxicity Studies

A number of other animal toxicity studies were identified that had designs of limited usefulness for PTV development, used exposure routes or durations not suitable for PTV development, or were inadequately reported. Reported findings in these studies include (see Table C-2 in Appendix C for more details):

- no carcinogenic response within 12 months of intratracheal instillation of tungsten into guinea pigs (Schepers, 1971);
- pre- and postimplantation losses and delayed fetal ossification following 8-month exposures of female rats to 0.005 mg/kg-day of unspecified tungsten compound for up to 8 months before and during pregnancy [Nadeenko and Lenchenko (1977), and Nadeenko et al. (1977, 1978) as cited in ATSDR (2005)];
- histological lesions in the gastrointestinal tract, liver, and kidney in rats exposed for a "subacute" duration to doses of 10 or 100 mg/kg sodium tungstate (Nadeenko, 1966); and
- impaired conditioned behavior responses without brain lesions in rats exposed to 0.05 and 0.5 mg/kg-day sodium tungstate for 7 months and decreased blood cholinesterase activity and unspecified brain lesions in rabbits exposed to 0.05 or 5 mg/kg for 8 months (Nadeenko, 1966).

Reported acute lethality values for sodium tungstate include: oral LD_{50} values ranging from 240 mg/kg for mice to 1,190 mg/kg for rats (Nadeenko, 1966) and 1,453 mg/kg for rats [Huntingdon Life Sciences (1999a) as cited in Jackson et al. (2013)]; a rat 4-hour median lethal concentration (LC_{50}) value >5.01 mg/L [Huntingdon Life Sciences (1999a) as cited in Jackson et al. (2013)]; and a rat dermal LD_{50} value >2,000 mg/kg [Huntingdon Life Sciences (1999c,d,e) as cited in Jackson et al. (2013)].

Other studies examining the respiratory tract in rats after intratracheal instillation of single doses of suspended dusts of tungsten metal, tungsten carbide + carbide dusts, or tungsten carbide + cobalt dusts have reported:

- pulmonary fibrosis in rats exposed to tungsten carbide + cobalt dusts, but not in rats exposed to tungsten metal or tungsten carbide + carbon dusts (Delahant, 1955; Schepers, 1955); and
- pulmonary fibrosis in rats exposed to intratracheal instillation of tungsten metal (Mezentseva, 1967).

Absorption, Distribution, Metabolism, and Elimination (ADME) Studies

Tungstate was widely distributed among tissues in the body of rats exposed to aqueous solutions of sodium tungstate by gavage for 7 months at doses of 5 or 125 mg/kg-day starting from 14 days before mating through PND 20 (McInturf et al., 2008). Tungstate was detected in all examined tissues in exposed animals at the end of exposure (including the brain in adults and offspring, the placenta, and dam milk secretions). By PND 70, tungstate concentrations in tissues were below detection limits (McInturf et al., 2008). In mice exposed to sodium tungstate in drinking water for 16 weeks, elemental tungsten accumulation in bone was shown to: (1) be rapid (peaking after 1 week of exposure); (2) increase with increasing exposure level; and (3) clear from bone less rapidly than it accumulated (Kelly et al., 2013). An early study showing no marked differences in tissue distribution in rats exposed to different forms of tungsten was likely due to lack of proper analytical methods available at that time (Kinard and Aull, 1945).

Mode-of-Action/Mechanism/Therapeutic Action Studies

In mechanistic studies, dietary exposure of rats to sodium tungstate altered ascorbic acid metabolism in rats exposed for 28 days (Chatterjee et al., 1973) and depleted xanthine oxidase activity in the lungs of rats exposed for 3 weeks (Rodell et al., 1987). A number of studies have reported that orally administered sodium tungstate corrected hyperglycemia in insulin- and noninsulin-dependent rats (Oliveira et al., 2014; Ballester et al., 2007; Ballester et al., 2005; Fernández-Alvarez et al., 2004; Muñoz et al., 2001; Le Lamer et al., 2000; Rodriguez-Gallardo et al., 2000; Barberà et al., 1997).

DERIVATION OF PROVISIONAL VALUES

Data are inadequate for derivation of reference values for tungsten metal. Early studies indicated that repeated oral exposure of tungsten metal (70 days at dietary concentrations up to 10%) is markedly less toxic (lethal) to rats than sodium tungstate or other compounds of tungsten (tungsten trioxide and ammonium tungstate) (Kinard and Van de Erve, 1943, 1941). However, these studies examined a limited number of endpoints and did not conduct histological examination of tissues, and thus, do not provide an adequate basis for deriving a subchronic p-RfD for tungsten metal. No other studies that examined pertinent endpoints in animals repeatedly exposed orally to tungsten metal were located.

Tables 4 and 5 present a summary of noncancer reference and cancer values, respectively, for sodium tungstate. IRIS data are indicated in the table, if available.

TABLE 4. Summary of Noncancer Reference Values for Soluble Tungsten Compounds (Various CASRNs)

Toxicity Type (units)	Species/Sex	Critical Effect	p-Reference Value	POD Method	POD	UF_C	Principal Study
Subchronic p-RfD	Rat/males	Glandular stomach goblet cell metaplasia	8×10^{-3} mg W/kg-d	BMD	$BMDL_{10}$ = 2.3 mg W/kg-d	300	USACHPPM (2007a); USACHPPM (2007b)
Chronic p-RfD	Rat/males	Glandular stomach goblet cell metaplasia	8×10^{-4} mg W/kg-d	BMD	$BMDL_{10}$ = 2.3 mg W/kg-d	3,000	USACHPPM (2007a); USACHPPM (2007b)
Subchronic and chronic p-RfC	Not derived due to inadequate data for soluble tungsten compounds.						

Table 5. Summary of Cancer Values for Soluble Tungsten Compounds (Various CASRNs)

Toxicity Type	Species/Sex	Tumor Type	Cancer Value	Principal Study
p-OSF or p-IUR	Not derived due to inadequate data to assess the carcinogenicity of tungsten and soluble compounds.			

DERIVATION OF ORAL REFERENCE DOSES

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

The 90-day gavage study of S-D rats is selected as the principal study for deriving a subchronic p-RfD for soluble tungsten compounds (USACHPPM, 2007a, b). The results of this study were later published in a peer-reviewed paper by McCain et al. (2015). Increased incidence of glandular stomach goblet cell metaplasia is selected as the critical effect.

Justification of the Critical Effect

Increased incidence of glandular stomach cell metaplasia is selected as the critical effect for deriving the subchronic p-RfD for soluble tungsten compounds. Several other endpoints associated with tungsten exposure were considered for identification of critical effect however the biological significance and/or relevance of these effects are questionable. For example, cortical tubule regeneration was observed in the kidneys of male and female rats following 90 days of gavage exposure (USACHPPM, 2007a, b), however this lesion is indicative of an adaptive repair response rather than an actual pathological effect. A potential immunosuppressive effect was noted in mice challenged with bacterial antigen following 28 days of tungsten exposure (Osterburg et al., 2014). The study authors reported decreased percentages of 'activated' splenic CD71+ helper and cytotoxic T cells, however the total number of T cells in the spleen did not differ from control mice bringing into question the functional relevance of the observed effect. Therefore, glandular stomach goblet cell metaplasia in male rats (USACHPPM, 2007a, b) was considered as a potential critical effect in lieu of these aforementioned effects.

In addition to the qualitative considerations for identification of a critical effect, glandular stomach goblet cell metaplasia represents the most sensitive LOAEL among all considered effects; the LOAEL (78 mg W/kg-day; NOAEL of 46 mg W/kg-day) for this effect in the 90-day gavage S-D rat study by (USACHPPM, 2007a, b) is lower than LOAELs for other effects observed in other studies of animals orally exposed to sodium tungstate for subchronic durations (see Table 3A). These LOAELs are:

- 125 mg W/kg-day (and NOAEL of 78 mg W/kg-day) for decreased body weight in male rats and increased incidence of male and female rats with mild to severe cortical tubule regeneration in the kidneys (USACHPPM, 2007a, b);
- 250 mg W/kg-day (and NOAEL of 49 mg W/kg-day) for decreased body weight and decreased bone marrow cellularity in male C57BL/6J mice exposed for 16 weeks (Kelly et al., 2013).
- 125 mg W/kg-day (and NOAEL of 78 mg W/kg-day) for decreased splenic response to bacterial toxin injection (decreased percentage splenic CD71+ helper and cytotoxic T cells 24 hours after injection with SEB) in C57BL/6J mice exposed for 28 days (adult mice) or 90 days before mating, followed by gestation, birth, and weaning (F0 and F1 mice) (Osterburg et al., 2014); and
- 160 mg W/kg-day for decreased body weight in female rats (Ballester et al., 2007; Ballester et al., 2005).

The authors of the principal study interpreted the glandular stomach lesions to be a "nonspecific response to some physiologic effect of gavage administration, as opposed to a manifestation of systemic toxicity," but for this assessment, these lesions are considered to be biologically significant, dose-related, and compound-related effects, as evidenced by their absence in the control and lower-dose groups, which also received gavage treatment, and their

dose-related pattern of occurrence. Supporting the biological significance of these lesions to humans is the understanding that goblet cells are not part of the normal mammalian gastric epithelium, and that metaplasia (replacement of normal cells with columnar absorptive cells and goblet cells of intestinal morphology) is a histological change associated with repeated inflammation of the gastric mucosa, which could occur due to chemical exposure (Liu and Crawford, 2005). Furthermore, because it is unclear if the pathology of the glandular stomach was examined in rats from the available drinking water studies for tungsten or in any other toxicity study (e.g., dietary studies in rats or mice), it is not possible to determine whether the observed lesions are due in part to bolus dosing (i.e., gavage) in the principal study (USACHPPM, 2007a, b). Whereas glandular stomach goblet cell metaplasia could be considered an irritant effect due to gavage treatment of sodium tungstate, the available data do not support this conclusion. Irritation of the gastrointestinal tract is often coupled with decreased food consumption and a subsequent reduction in body weight. Whereas body weight and food consumption were statistically and/or biologically significantly reduced in males at 125 mg W/kg-day in the gavage study (USACHPPM, 2007a, b), the incidence of glandular stomach goblet cell metaplasia was statistically significantly increased at 78 mg W/kg-day at which no significant alterations in body weight and food consumption were observed [see Table 6 and Table B-1 in this document and Tables 3 and 4 from(McCain et al., 2015)]. Also, body weight and food consumption were not reduced in females at ≥75 mg W/kg-day, where the incidence of glandular stomach goblet cell metaplasia was statistically significantly increased in females. These data suggest that although a tungsten-induced irritant effect (i.e., reduced body weight and food consumption) may be occurring in male rats at 125 mg W/kg-day, it is most likely unrelated to the development of glandular stomach goblet cell metaplasia at 75 mg W/kg-day (USACHPPM, 2007a, b). In the absence of more definitive information, glandular stomach goblet cell metaplasia is considered a valid endpoint for human health risk assessment.

Selection of stomach lesions in S-D rats as the critical effect has some uncertainty, because the occurrence of these lesions was not mentioned in the available reports of a reproductive study for a duration equivalent to subchronic study (70 days, including 14 days premating, 14 days mating, 22 days gestation, and 20 days postparturition)in S-D rats exposed by gavage to sodium tungstate in water (McInturf et al., 2011; McInturf et al., 2008), or in any other study. However, the histological examinations of tissues in the F0 rats in this study were inconsistently and ambiguously reported in the published reports. Only the 2011 report discussed the histology data, noting in the methods section that "various organ tissues (heart, spleen, kidney, liver, lungs, brain, testes ovaries, thymus, bone, gastrointestinal tract, etc.,)" from 10 F0 animals/sex/group were prepared for histological examination, but noting in a data table [see Table 2, p. 136 of McInturf et al. (2011)] that in the high-dose (75 mg W/kg-day) F0 group "two animals of five displayed myocarditis with cardiomyocyte degeneration and necrosis." In concluding statements, McInturf et al. (2011) noted that pathological examinations showed no treatment-related histopathological lesions in any organs except the heart, but incidence data that would allow independent review were not included. In the absence of more complete reporting of incidence data for the histological findings, it is uncertain whether or not the F0 animals exposed to 78 mg W/kg-day in this study showed glandular stomach lesions similar to those reported in the 90-day toxicity study reported by USACHPPM (2007a) and USACHPPM (2007b). Furthermore, the development of glandular stomach lesions in rats may require treatment with sodium tungstate for at least 90 days as was done in the studies by USACHPPM (2007a) and USACHPPM (2007c). In the reproductive study by McInturf et al. (2011) and

McInturf et al. (2008), F0 rats were treated for a total of only 70 days, which may not be sufficient time for the development of glandular stomach lesions. Kinard and Van de Erve (1943) grossly examined the gastrointestinal tract of rats treated for 70 days with tungsten metal. It is unclear if the glandular stomach was specifically examined in the study and the study authors only ambiguously reported that "No extravasations of blood were observed in the gastrointestinal track."

Support for deriving a subchronic p-RfD for soluble tungsten compounds based on glandular stomach lesions in rats comes from the identification of 78 mg W/kg-day as a NOAEL for reproductive effects in F0 S-D rats and systemic and neurobehavioral effects in F1 rat offspring following a total of 70 days of gavage exposure before mating, during mating and gestation, and during early postnatal periods (McInturf et al., 2011; McInturf et al., 2008). This study did not include an exposure level that identified LOAELs for evaluated endpoints in F0- or F1-generation rats. Similarly, a reproductive study of C57BL/6J mice exposed to doses as high as 125 mg W/kg-day in drinking water for a total of 90 days before mating, followed by exposure during gestation, birth, and weaning, found no statistically significant effects on F0 body weight or reproductive performance (the number of live births, litter size, and sex ratio of offspring) (Osterburg et al., 2014). These observations indicate that reproductive and developmental effects will not occur at doses lower than the rat LOAEL for glandular stomach lesions, 78 mg W/kg-day.

A number of other effects have been observed at dose levels below 78 mg W/kg-day in studies of animals subchronically exposed to sodium tungstate, but these effects are of uncertain biological significance and not suitable to serve as critical effects for the subchronic p-RfD.

- Mice exposed for 16 weeks to dose levels of 4, 49, or 250 mg W/kg-day had a statistically significantly greater percentage of bone marrow B cells in late pro-/large B cell developmental stages (C/C′), compared with control values (Kelly et al., 2013). The change did not steadily increase in magnitude with increasing dose level and was not apparent at earlier time points (Weeks 1, 4, 8, or 12). At 16 weeks, mean percentages of bone marrow B cells in the C/C′ fraction for the control, low-, medium-, and high-dose groups were 0.06, 0.11, 0.11, and 0.13, respectively (see Table B-3).
- Statistically significant changes in an index of DNA damage (increased tail moment in the Comet assay) in nonadherent bone marrow cells also was reported for mice in the 4- and 49-mg W/kg-day dose groups at most time points (Weeks 1, 4, 8, 12, and 16), but statistically significant changes were not observed at most time points in the 250-mg W/kg-day group (Kelly et al., 2013) (see Table B-4). The magnitude of the significant increases in tail moment (compared with control values) ranged from 26−151%, but the data do not provide clear evidence for increased damage with increasing dose level or duration of exposure (see Table B-4). Similar Comet assay results were found with CD19+ B cells isolated from bone marrow of mice in Weeks 1 and 4 (Kelly et al., 2013) (see Table B-4).
- In mice allergically sensitized to 4-hydroxy-3-nitrophenylacetic acid active ester (NP-O-Su) and exposed to sodium tungstate in drinking water for 28 days before sensitization, the response to a challenge injection of NP-O-SU was decreased in mice exposed to 12.5 or 125 mg W/kg-day (but not to 1.3 or 0.1 mg W/kg-day), compared with sensitized mice without exposure to sodium tungstate (Osterburg et al., 2014). Swelling at the site of challenge injection was decreased by about 30% in groups exposed to 12.5

or 125 mg W/kg-day, compared with swelling in sensitized mice without tungstate exposure. The biological significance of the diminished response is uncertain, and could be viewed as a positive action of tungstate (potentially therapeutic) in allergically sensitized individuals.

Justification of the Principal Study

The 90-day gavage study of rats was selected as the principal study, because:

1. it was an adequately designed, conducted, and reported study of subchronic toxicity in rodents, and
2. it identified a LOAEL for glandular stomach lesions in rats that was lower than LOAELs for other effects (described in the previous section) identified in other adequately designed and conducted studies of subchronic duration oral toxicity in rats (McInturf et al., 2011; McInturf et al., 2008) and mice (Osterburg et al., 2014; Kelly et al., 2013).

Approach for Deriving the Subchronic p-RfD

Data sets for the most sensitive endpoint from the principal study, glandular stomach lesions (goblet cell metaplasia in male and female rats), were selected to derive potential PODs via benchmark dose (BMD) modeling (see Table 6). For comparative purposes, other potential sensitive data sets were selected for BMD modeling, including kidney, body weight, and immune system endpoints (see Table 6). BMDs and benchmark dose lower confidence limits (BMDLs) from the best fitting models for the selected dichotomous-variables data sets are presented in Table 7. Also presented in Table 7 are HED BMDLs, converted from the animal BMDLs using U.S. EPA (2011b) recommended body weight$^{3/4}$ scaling factors (DAFs) for systemic effects. The body weight$^{3/4}$ scaling factor was not applied to the stomach lesion-based BMDLs, because allometric scaling has not been extensively evaluated with portal-of-entry effects and models to predict differences in deposited mass per glandular stomach surface area across species have not been developed for soluble tungsten compounds (U.S. EPA, 2011b).

Table 6. Data for Sensitive Endpoints in Rodents Exposed Orally to Sodium Tungstate for Subchronic Durations

Endpoint	Dose (mg W/kg-d)				
USACHPPM (2007a); USACHPPM (2007b)—Rat, gavage, 90 d					
Glandular stomach goblet cell metaplasia	**0**	**6**	**47**	**78**	**125**
Male	0/10	1/10	4/10	8/9	8/10
Female	0/10	0/10	4/10	8/10	10/10
Mild to severe renal cortical tubule regeneration	**0**	**6**	**47**	**78**	**125**
Male	0/10	0/10	0/10	1/9	10/10
Female	0/10	0/10	0/10	1/10	8/10
Male terminal BW (g) Mean ± SD (*n*)	553 ± 31 (10)	571 ± 49 (10)	548 ± 38 (10)	535 ± 67 (10)	486 ± 52 (9)
Kelly et al. (2013)—Mouse, drinking water, 16 wk					
Total bone marrow cell count (10^6)	**0**	**4**	**49**	**250**	
Mean ± SD (*n*)	118.89 ± 9.51 (5)	121.02 ± 10. 44 (5)	122.12 ± 12.20 (5)	95.55 ± 11.97 (5)	
Osterburg et al. (2014)—Mouse, drinking water, 28 d, 19 wk (F0), or 32 wk (F1)					
% CD71+ helper T cells (mean ± SD)	**0**	**1.3**	**39**	**78**	**125**
28 d (*n* = 12/group)	4.85 ± 4.26	NR	3.61 ± 2.29	3.54 ± 1.39	2.76 ± 1.77
19 wk (F0, 6/group)	6.21 ± 0.96	6.41 ± 1.52	4.71 ± 4.51	4.61 ± 3.18	2.28 ± 1.00
32 wk (F1, 6/group)	7.20 ± 1.86	4.13 ± 1.06	3.14 ± 0.93	4.96 ± 1.20	2.85 ± 1.30
% CD71+ cytotoxic T cells (mean ± SD)	**0**	**1.3**	**39**	**78**	**125**
28 d (12/group)	12.87 ± 7.10	NR	8.60 ± 6.79	5.75 ± 2.88	4.44 ± 4.92
19 wk (F0, 6/group)	7.98 ± 1.20	6.81 ± 2.82	5.75 ± 4.14	2.31 ± 5.14	1.58 ± 0.56
32 wk (F1, 6/group)	6.33 ± 1.20	5.37 ± 0.71	3.29 ± 0.61	5.77 ± 1.20	2.52 ± 0.61

NR = not reported; SD = standard deviation.

Table 7. BMD and BMDL Values from Best Fitting Models for Sensitive, Biologically Relevant Endpoints in Rodents Exposed Orally to Sodium Tungstate for Subchronic Durations[a]

Endpoint	Best Fitting Model	BMD (mg W/kg-d)	BMDL (mg W/kg-d)	HED[b] BMDL (mg W/kg-d)
USACHPPM (2007a); USACHPPM (2007b)—Data sets: rat, 90 d, gavage				
Glandular stomach goblet cell metaplasia	Male—LogLogistic Female—Multistage 2-degree	BMD_{10} = 7.7 BMD_{10} = 19.9	$BMDL_{10}$ = 2.3 $BMDL_{10}$ = 8.1	NDr; portal-of-entry effect
Glandular stomach subacute inflammation	Male—not amenable to modeling Female—LogLogistic	NDr BMD_{10} = 43	NDr $BMDL_{10}$ = 26	NDr; portal-of-entry effect
Renal cortical tubule regeneration	Male—Weibull Female—Gamma	BMD_{10} = 77.5 BMD_{10} = 75.9	$BMDL_{10}$ = 61.6 $BMDL_{10}$ = 58.6	$BMDL_{10}$ = 14.8 $BMDL_{10}$ = 14.1
Terminal body weight, male rat	Male—Polynomial 2-degree, constant variance	BMD_{10} = 108.2	$BMDL_{10}$ = 91.9	$BMDL_{10}$ = 22.1
Kelly et al. (2013)—Data set: mouse, 16 wk, drinking water				
Total bone marrow cell count	Male—Polynomial 3-degree, constant variance	BMD_{1SD} = 183.7	$BMDL_{1SD}$ = 77.8	$BMDL_{1SD}$ = 10.9
Osterburg et al. (2014)—Data sets: mouse, 28 d, 19 wk (F0), or 32 wk (F1)				
% CD71+ cytotoxic T cells	28 d—Hill, nonconstant variance 19 wk (F0)—not amenable to modeling 32 wk (F1)—not amenable to modeling	BMD_{1SD} = 49.1 NDr NDr	$BMDL_{1SD}$ = 39.9 NDr NDr	$BMDL_{1SD}$ = 5.6 NDr NDr
% CD71+ helper T cells	28 d—Exponential (2), nonconstant variance 19 wk (F0)—not amenable to modeling 32 wk (F1)—not amenable to modeling	BMD_{1SD} = 333.6 NDr NDr	$BMDL_{1SD}$ = 118.4 NDr NDr	$BMDL_{1SD}$ = 16.6 NDr NDr

[a]Modeling results are described in more detail in Appendix D.
[b]HEDs were calculated using species-specific DAFs based on the animal:human $BW^{1/4}$ ratio recommended by U.S. EPA (2011b): mouse:human ratio = 0.14; rat:human ratio = 0.24.

NDr = not determined.

The $BMDL_{10}$ value of 2.3 mg W/kg-day for glandular stomach goblet cell metaplasia in male rats was selected as the POD. This selection is consistent with the selection of stomach lesions as the critical effect based on a comparison of animal LOAELs, and with the BMDL for male stomach lesions being lower than HED BMDLs for other sensitive endpoints (see Table 7). For systemic effects, because soluble compounds of tungsten (i.e., sodium tungstate dihydrate and sodium tungstate) are expected to ionize in the blood (McInturf et al., 2011; McInturf et al., 2008), the toxicities of the chemicals would be due to tungsten (and not the particular salt), thus the toxicities of the soluble compounds of tungsten would be expected to be similar on a molar basis. Therefore, although the subchronic p-RfD presented below is derived based on doses for

tungsten, the value is applicable for sodium tungstate dihydrate and sodium tungstate (i.e., soluble tungsten compounds).

The subchronic p-RfD for soluble tungsten compounds, based on the $BMDL_{10}$ of 2.3 mg W/kg-day for goblet cell metaplasia in the glandular stomach of male rats, is derived as follows:

Subchronic p-RfD = $BMDL_{10} \div UF_C$
= 2.3 mg W/kg-day ÷ 300
= **8×10^{-3} mg W/kg-day**

Table 8 summarizes the UFs for the subchronic p-RfD for soluble tungsten compounds.

Table 8. Uncertainty Factors for the Subchronic p-RfD for Soluble Tungsten Compounds

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 soluble tungsten compounds, 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 soluble tungsten compounds in humans.
UF_D	3	A UF_D of 3 is applied because the database contains several adequately designed subchronic-duration animal toxicity studies of sodium tungstate that collectively identify glandular stomach lesions as a critical effect occurring at dose levels that do not cause reproductive, developmental, or immune system effects (Osterburg et al., 2014; Kelly et al., 2013; McInturf et al., 2011; McInturf et al., 2008; USACHPPM, 2007a, b). However, there is indication of neurotoxicity effects (Nadeenko, 1966), which was not comprehensively investigated.
UF_L	1	A UF_L of 1 is applied because the POD is a BMDL.
UF_S	1	A UF_S of 1 is applied because the POD comes from a subchronic-duration study of rats.
UF_C	300	Composite Uncertainty Factor = $UF_A \times UF_H \times UF_D \times UF_L \times UF_S$.

The confidence in the subchronic p-RfD for soluble tungsten compounds is medium as explained in Table 9 below.

Table 9. Confidence Descriptors for the Subchronic p-RfD for Soluble Tungsten Compounds

Confidence Categories	Designation	Discussion
Confidence in study	H	Confidence in the principal study (USACHPPM, 2007a, b) is high because the study design and conduct was in accordance with EPA Health Effects Testing Guidelines for a study of 90-d oral toxicity in rodents. In addition, the results were published in a peer-reviewed paper (McCain et al., 2015).
Confidence in database	M	Confidence in the subchronic-duration oral exposure database is medium, because the database contains one adequately designed subchronic-duration animal toxicity study that identifies glandular stomach lesions as a critical effect and other subchronic-duration studies indicating that reproductive, developmental, or immune system effects occur at doses higher than those causing stomach lesions (Osterburg et al., 2014; Kelly et al., 2013; McInturf et al., 2011; McInturf et al., 2008; USACHPPM, 2007a, b). However, there is limited information regarding neurotoxicity (Nadeenko, 1966), which was not comprehensively investigated.
Confidence in subchronic p-RfD[a]	M	The overall confidence in the subchronic p-RfD is medium.

[a]The overall confidence cannot be greater than the lowest entry in the table.

H = high; M = medium.

Derivation of Chronic Provisional RfD (Chronic p-RfD)

There are four studies in which rodents were chronically treated with sodium tungstate via drinking water (Osterburg et al., 2014; Luo et al., 1983; Schroeder and Mitchener, 1975a, b). In a reproductive study, Osterburg et al. (2014) treated F0 mice for 19 weeks, and in a chronic study, Luo et al. (1983) treated male rats for 19 or 30 weeks. However, these studies did not perform comprehensive evaluations. Osterburg et al. (2014) only focused on hematological and immunological endpoints and Luo et al. (1983) on esophageal and forestomach carcinogenesis. Schroeder and Mitchener (1975b) and Schroeder and Mitchener (1975a) treated rats and mice with sodium tungstate for lifetime durations, but these studies only tested one dose in drinking water. Thus, in the absence of comprehensive studies of toxicity endpoints that tested multiple doses in humans or animals chronically exposed to sodium tungstate by the oral route, a chronic p-RfD for soluble tungsten compounds is derived from the same POD for the subchronic p-RfD. Justification for selecting the critical effect and principal study is described in the previous section of this document.

The chronic p-RfD for soluble tungsten compounds is derived as follows:

$$\textbf{Chronic p-RfD} = \text{subchronic } BMDL_{10} \div UF_C$$
$$= 2.3 \text{ mg W/kg-day} \div 3{,}000$$
$$= \mathbf{8 \times 10^{-4} \text{ mg W/kg-day}}$$

Table 10 summarizes the UFs for the chronic p-RfD for soluble tungsten compounds.

Table 10. Uncertainty Factors for the Chronic p-RfD for Soluble Tungsten Compounds

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 soluble tungsten compounds, 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 soluble tungsten compounds in humans.
UF_D	3	A UF_D of 1 is applied because the database contains several adequately designed subchronic-duration animal toxicity studies of sodium tungstate that collectively identify glandular stomach lesions as a critical effect occurring at dose levels that do not cause reproductive, developmental or immune system effects (Osterburg et al., 2014; Kelly et al., 2013; McInturf et al., 2011; McInturf et al., 2008; USACHPPM, 2007a, b). Uncertainty associated with the absence of chronic-duration toxicity data is accounted for in the UF_S. However, there is indication of neurotoxicity effects (Nadeenko, 1966), which was not comprehensively investigated.
UF_L	1	A UF_L of 1 is applied because the POD is a BMDL.
UF_S	10	A UF_S of 10 is applied to account for uncertainty in deriving the chronic p-RfD from the subchronic p-RfD.
UF_C	3,000	Composite Uncertainty Factor = $UF_A \times UF_H \times UF_D \times UF_L \times UF_S$.

The confidence in the chronic p-RfD for soluble tungsten compounds is low as explained in Table 11 below.

Table 11. Confidence Descriptors for the Chronic p-RfD for Soluble Tungsten Compounds

Confidence Categories	Designation	Discussion
Confidence in study	H	Confidence in the principal study is high because the study design and conduct was in accordance with EPA Health Effects Testing Guidelines for a study of 90-d oral toxicity in rodents. In addition, the results were published in a peer-reviewed paper McCain et al. (2015).
Confidence in database	L	Confidence in the database is low, because it contains no comprehensive studies of toxic endpoints in humans or animals orally exposed to tungstate for chronic durations, and there is limited information regarding neurotoxicity (Nadeenko, 1966).
Confidence in chronic p-RfD[a]	L	The overall confidence in the chronic p-RfD is low.

[a]The overall confidence cannot be greater than the lowest entry in the table.

H = high; L = low.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

Human and animal data are inadequate to derive subchronic or chronic p-RfCs for soluble tungsten compounds.

Epidemiology studies of hard alloy workers exposed to dusts containing mixtures of tungsten and other metals reported increased risks for respiratory and neurologic effects, but the results are confounded by exposure to mixtures of metals and have been attributed to cobalt (ATSDR, 2005).

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Table 12 identifies the cancer weight-of-evidence (WOE) descriptor for soluble tungsten compounds.

Table 12. Cancer WOE Descriptor for Soluble Tungsten Compounds (Various CASRNs)

Possible WOE Descriptor	Designation	Route of Entry (oral, inhalation, or both)	Comments
"Carcinogenic to Humans"	NS	NA	No human data are available to support this designation.
"Likely to Be Carcinogenic to Humans"	NS	NA	No human data or adequate animal cancer bioassays are available to support this designation.
"Suggestive Evidence of Carcinogenic Potential"	NS	NA	No human data or adequate animal cancer bioassays are available to support this designation.
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	NA	**No human data or adequate animal cancer bioassays are available.**
"Not Likely to Be Carcinogenic to Humans"	NS	NA	No human data or adequate animal cancer bioassays are available to support this designation.

NA = not applicable; NS = not selected.

No studies were located examining possible associations between exposure to tungsten, sodium tungstate, or sodium tungstate dihydrate and increased risk of cancer in humans. Studies in animals are inadequate to assess the carcinogenicity of tungsten or sodium tungstate. Schroeder and Mitchener (1975a) and Schroeder and Mitchener (1975b) reported that no evidence for carcinogenic responses was found in rats or mice exposed to sodium tungstate in drinking water for life at a concentration of 5 ppm tungsten, but the studies are limited by inclusion of only one exposure level and absence of an exposure level close to a maximum tolerated dose. No evidence of sodium tungstate's tumor promotion capability was found in one rat assay of tumors initiated by NSEE (Luo et al., 1983). In another rat assay, tumors appeared slightly earlier in rats exposed to NMU followed by sodium tungstate in drinking water, compared with rats exposed to NMU alone (Wei et al., 1985). Results from a number of short-term-duration genotoxicity tests with sodium tungstate were predominately negative

(see Table C-1 in Appendix C for more details). Genotoxicity studies for tungsten metal were not identified.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Provisional Oral Slope Factor (p-OSF)

Not derived due to inadequate data.

Derivation of Provisional Inhalation Unit Risk (p-IUR)

Not derived due to inadequate data.

APPENDIX A. SCREENING PROVISIONAL VALUES

No screening values are presented.

APPENDIX B. DATA TABLES

Table B-1. Histological Lesions Observed in Male and Female Sprague-Dawley Rats after Exposure to Sodium Tungstate Dihydrate in Water for 90 Days by Gavage[a]

Parameter	Exposure Group, mg Sodium Tungstate/kg-d (mg W/kg-d)[b]				
Male	**0**	**10 (6)**	**75 (47)**	**125 (78)**	**200 (125)**
Kidney					
Mild to severe cortical tubule regeneration	0/10	0/10	0/10	1/9	10/10*
Glandular stomach					
Subacute inflammation	0/10	2/10	1/10	5/9*	4/10
Goblet cell metaplasia	0/10	1/10	4/10	8/9*	8/10*
Epididymides					
Luminal cellular debris	0/10	1/10	0/10	0/10	3/10
Female	**0**	**10 (6)**	**75 (47)**	**125 (78)**	**200 (125)**
Kidney					
Mild to severe cortical tubule regeneration	0/10	0/10	0/10	1/10	8/10*
Glandular stomach					
Subacute inflammation	0/10	0/10	1/10	8/10*	9/10*
Goblet cell metaplasia	0/10	0/10	4/10	8/10*	10/10*

[a]USACHPPM (2007a); USACHPPM (2007b).
[b]Equivalent tungsten doses were calculated based on molecular weights (tungsten accounts for 62.6% of total molecular weight of Na_2WO_4).
[c]Minimal renal cortical tubule regeneration was observed in all dose groups (incidence not reported). Group mean and individual animal severity scores were not reported.
*Statistically significantly different from controls at $p < 0.05$, as calculated for this review (Fisher's Exact test, two-tailed).

Table B-2. Splenic Immune Responses in Male and Female C57BL6 Mice after Exposure to Sodium Tungstate Dihydrate in Drinking Water for 28 Days or 1 Generation[a]

Parameter[b]	Exposure Group, mg Sodium Tungstate/kg-d (mg W/kg-d)[c]				
28-d study					
	0	**2 (1.3)**	**62.5 (39)**	**125 (78)**	**200 (125)**
% CD71+ helper T cells[d]	4.85 ± 1.23	NR	3.61 ± 0.66 (−26%)	3.54 ± 0.40 (−27%)	2.76 ± 0.51* (−43%)
% CD71+ cytotoxic T cells[d]	12.87 ± 2.05	NR	8.60 ± 1.96 (−33%)	5.75 ± 0.83 (−55%)	4.44 ± 1.42* (−66%)
IFN-γ (pg/mL)[e]	6.98 ± 0.77	NR	5.04 ± 1.10 (−28%)	3.16 ± 0.62 (−55%)	2.44 ± 0.73* (−65%)
1-Generation study—F0 mice (12 wk premating + 7 wk mating, gestation, lactation)					
	0	**2 (1.3)**	**62.5 (39)**	**125 (78)**	**200 (125)**
% CD71+ helper T cells[d]	6.21 ± 0.39	6.41 ± 0.62 (+3%)	4.71 ± 1.84 (−24%)	4.61 ± 1.30 (−26%)	2.28 ± 0.41* (−63%)
% CD71+ cytotoxic T cells[d]	7.98 ± 0.49	6.81 ± 1.15 (−15%)	5.75 ± 1.69 (−28%)	2.31 ± 2.10 (−71%)	1.58 ± 0.23* (−80%)
IFN-γ (pg/mL)[f]	7.11 ± 1.7	10.15 ± 2.66 (+43%)	3.89 ± 1.51 (−45%)	2.52 ± 1.22 (−65%)	3.74 ± 2.9* (−47%)
1-Generation study—F1 mice (exposure via F0 dam + direct exposure for 90 d postweaning)					
	0	**2 (1.3)**	**62.5 (39)**	**125 (78)**	**200 (125)**
% CD71+ helper T cells[d]	7.20 ± 0.76	4.13 ± 0.43 (−43%)	3.14 ± 0.38 (−56%)	4.96 ± 0.49 (−31%)	2.85 ± 0.53* (−60%)
% CD71+ cytotoxic T cells[d]	6.33 ± 0.49	5.37 ± 0.29 (−15%)	3.29 ± 0.25 (−48%)	5.77 ± 0.45 (−9%)	2.52 ± 0.25* (−60%)
IFN-γ (pg/mL)[g]	4.54 ± 1.22	4.46 ± 1.30 (−2%)	11.23 ± 5.18 (+147%)	2.16 ± 0.14 (−52%)	3.10 ± 1.15 (−32%)

[a]Osterburg et al. (2014).
[b]Values are expressed as mean ± standard error of the mean (SEM) (% change compared with control); % change control = [(treatment mean − control mean) ÷ control mean] × 100; n = 12 mice/group for the 28-day study and n = 6 mice/group for F0 and F1 mice in the 1-generation study. Distribution of sexes within each group was not reported.
[c]Equivalent tungsten doses were calculated based on molecular weights (tungsten accounts for 62.6% of total molecular weight of Na_2WO_4).
[d]Only control and high-dose values were reported in the text; data for other dose groups were extracted from Figures 4 and 5 in the primary report using GrabIt! software.
[e]Only control value was reported in the text; data for other dose groups were extracted from Figure 6 in the primary report using GrabIt! software.
[f]Only control and high-dose values were reported in the text; data for other dose groups were extracted from Figure 6 in the primary report using GrabIt! software.
[g]Data for all dose groups were extracted from Figure 6 in the primary report using GrabIt! software.
*Statistically significantly different from controls at $p < 0.05$, as reported by the study authors.

NR = not reported.

Table B-3. Total Bone Marrow Cellularity, Specific Cell Fraction Percentages and Counts, and Clonogenicity of Bone Marrow Precursors in Male C57BL/6J Mice after Exposure to Sodium Tungstate Dihydrate for 16 Weeks in Drinking Water[a]

Parameter[b]		Exposure Group, mg/L W in Water (average daily dose, mg W/kg-d)[c]			
		0	**15 (4)**	**200 (49)**	**1,000 (250)**
Number of animals		5	5	5	5
Total bone marrow cell counts at 16 wk (10^6)		118.89 ± 9.51	121.02 ± 10.44 (+2%)	122.12 ± 12.20 (+3%)	95.55 ± 11.97* (−20%)
% of cells per fraction (determined in 2 million bone marrow cells per time point)[d]	**1 wk**				
	C/C′ Fraction[e]	0.5 ± 0.09	0.45 ± 0.13 (−10%)	0.59 ± 0.15 (+18%)	0.43 ± 0.08 (−14%)
	F Fraction[f]	6.08 ± 0.84	7.4 ± 1.41 (+22%)	7.4 ± 1.83 (+22%)	12.06 ± 2.11* (+98%)
	4 wk				
	C/C′ Fraction	0.25 ± 0.06	0.26 ± 0.06 (+4%)	0.36 ± 0.12 (+44%)	0.36 ± 0.08 (+44%)
	F Fraction	4.57 ± 0.63	5.83 ± 1.50 (+28%)	6.63 ± 1.40* (+45%)	4.9 ± 0.79 (+7%)
	8 wk				
	C/C′ Fraction	0.08 ± 0.03	0.12 ± 0.05 (+50%)	0.11 ± 0.04 (+38%)	0.12 ± 0.05 (+50%)
	F Fraction	5.24 ± 1.16	7.32 ± 1.64 (+40%)	8.6 ± 1.95* (+64%)	8.62 ± 1.96* (+65%)
	12 wk				
	C/C′ Fraction	0.09 ± 0.02	0.07 ± 0.01 (−22%)	0.05 ± 0.02 (−44%)	0.06 ± 0.02 (−33%)
	F Fraction	7.35 ± 1.20	8.86 ± 1.18 (+21%)	7.05 ± 1.15 (−4%)	9.01 ± 1.58 (+23%)
	16 wk				
	C/C′ Fraction	0.06 ± 0.02	0.11 ± 0.01* (+83%)	0.11 ± 0.02* (+83%)	0.13 ± 0.02* (+117%)
	F Fraction	9.19 ± 1.62	9.04 ± 0.43 (−2%)	9.64 ± 1.48 (+5%)	9.57 ± 1.31 (+4%)

Table B-3. Total Bone Marrow Cellularity, Specific Cell Fraction Percentages and Counts, and Clonogenicity of Bone Marrow Precursors in Male C57BL/6J Mice after Exposure to Sodium Tungstate Dihydrate for 16 Weeks in Drinking Water[a]

Parameter[b]	Exposure Group, mg/L W in Water (average daily dose, mg W/kg-d)[c]			
	0	15 (4)	200 (49)	1,000 (250)
C/C′ Fraction cell number normalized to total bone marrow count at 16 wk (10^6)[g]	0.08 ± 0.02	0.15 ± 0.03* (+88%)	0.14 ± 0.04* (+75%)	0.12 ± 0.03 (+50%)
Clonogenicity of precursors at 16 wk (number of colonies)[h]	94.9 ± 23.2	146.1 ± 33.5 (+54%)	154.5 ± 32.5 (+63%)	202.0 ± 32.5* (+113%)

[a]Kelly et al. (2013).
[b]Values are expressed as mean ± SD (% change compared with control); % change control = [(treatment mean − control mean) ÷ control mean] × 100.
[c]Average daily doses (mg/kg-day) were calculated for this review using reference male mouse body weight (0.0316 kg) and water consumption (0.00782 L/day) values for subchronic-duration studies (U.S. EPA, 1988).
[d]No statistically significant differences were observed in the A (pre-pro B cells), B (early pro-B cells), D (small pre-B cells), or E (immature B cells) fractions at any time point.
[e]C/C′ fraction contains late pro-B cells and large pre-B cells.
[f]F fraction contains mature B cells.
[g]No statistically significant differences were observed in C/C′ fraction at earlier time points or the A, B, D, or E fractions at any time point.
[h]Colony number means and standard deviation values were extracted from Figure 6 in the primary report using GrabIt! software.
*Statistically significantly different from controls at $p < 0.05$, as reported by the study authors (ANOVA followed by Newman-Keuls post hoc test).

Table B-4. DNA Damage in Male C57BL/6J Mice after Exposure to Sodium Tungstate Dihydrate for 16 Weeks in Drinking Water[a]

		Exposure Group, mg/L W in Water (average daily dose, mg W/kg-d)[c]			
		0	**15 (4)**	**200 (49)**	**1,000 (250)**
Number of animals		5	5	6	5
Cell type	Exposure duration	Tail moment (Comet assay)[b]			
Nonadherent bone marrow cells	1 wk	62.6 ± 5.8	78.6 ± 4.9* (+26%)	78.0 ± 5.5* (+26%)	53.0 ± 4.9 (−15%)
	4 wk	21.1 ± 1.9	36.4 ± 2.6* (+73%)	42.6 ± 2.3* (+102%)	35.1 ± 1.9* (+66%)
	8 wk	10.7 ± 1.0	20.8 ± 1.6* (+94%)	24.0 ± 1.3* (+124%)	10.4 ± 1.0 (−3%)
	12 wk	18.8 ± 2.3	47.1 ± 2.6* (+151%)	25.3 ± 1.6 (+35%)	31.2 ± 1.3* (+66%)
	16 wk	17.2 ± 1.0	30.9 ± 2.3* (+80%)	26.6 ± 1.6 (+55%)	22.7 ± 1.0 (+32%)
CD19+ B cells (isolated from bone marrow)[d]	1 wk	62.6 ± 0.9	78.2 ± 0.9* (+25%)	62.5 ± 0.5 (0%)	44.0 ± 1.4 (−30%)
	4 wk	66.2 ± 0.9	84.2 ± 1.4* (+27%)	114.5 ± 1.4* (+73%)	59.5 ± 0.9 (−10%)

[a]Kelly et al. (2013).
[b]Values are expressed as mean ± SD (% change compared with control); % change control = [(treatment mean − control mean) ÷ control mean] × 100. Means and standard deviation values were extracted from Figure 7 in the primary report using GrabIt! software.
[c]Average daily doses (mg/kg-day) were calculated for this review using reference male mouse body weight (0.0316 kg) and water consumption (0.00782 L/day) values for subchronic-duration studies (U.S. EPA, 1988).
[d]Isolated B cells were not evaluated for DNA damage at Weeks 8, 12, or 16.
*Statistically significantly increased from controls at $p < 0.05$, as reported by the study authors (ANOVA followed by Newman-Keuls post hoc test).

Table B-5. Mean Body Weight and Survival of Male and Female Rats after Exposure to Sodium Tungstate for up to 70 Days in Diet[a]

Parameter[b]	Exposure Group, % W Equivalent in Diet (average daily dose, mg W/kg-d)[c]			
Male	**0**	**0.1% (91)**	**0.5% (455)**	**2.0% (1,820)**
Number of animals	5−6	5−6	6	5
Terminal body weight (g)	285	260 (−9%)	NR	NA
Body-weight-gain (g)[d]	189	166 (−12%)	−26 (−114%)	NA
Survival[e]	100% (NR)	100% (NR)	50% (3/6)	0% (0/5)
Female	**0**	**0.1% (102)**	**0.5% (510)**	**2.0% (2,040)**
Number of animals	5−6	5−6	6	5
Terminal body weight (g)	188	168 (−11%)	NR	NA
Body-weight-gain (g)[d]	105	88 (−16%)	−33 (−131%)	NA
Survival[e]	100% (NR)	100% (NR)	33% (2/6)	0% (0/5)

[a]Kinard and Van de Erve (1941).
[b]Body weight and weight gain expressed as mean (% change compared with control); % change control = [(treatment mean − control mean) ÷ control mean] × 100
[c]Average daily doses were calculated for this review using reference rat body weight (0.235 kg for males and 0.173 kg for females) and food consumption (0.021 kg/kg bw-day for males and 0.017 kg/kg bw-day for females) values for the subchronic duration studies (U.S. EPA, 1988).
[d]Body-weight gain (Days 0−70) data were extracted from Chart 1 of the primary report using GrabIt! software.
[e]Survival expressed as % survival (number surviving/total number). All deaths in the 2.0% occurred within the first week. All deaths in the 0.5% group between Day 17 and 29 of exposure.

NR = not reported by study authors; NA = not applicable; all high-concentration rats died within 1 week.

Note: Statistics were not reported by study authors; data reporting is not adequate for statistical analysis.

Table B-6. Mean Body-Weight Gain of Male and Female Rats after Exposure to Powdered Tungsten Metal for 70 Days in Diet[a]

Parameter[b]	Exposure Group, % Powdered Tungsten Metal in Diet (average daily dose, mg W/kg-d)[c]			
Male	**0**	**2.0% (1,325)**	**5% (3,450)**	**10% (6,550)**
Number of animals	5	5	5	5
Body-weight gain (g)	183	172 (−6%)	207 (13%)	198 (8%)
Female	**0**	**2.0% (1,450)**	**5% (4,000)**	**10% (7,325)**
Number of animals	5	5	5	5
Body-weight gain (g)	110	114 (4%)	107 (−3%)	93 (−15%)

[a]Kinard and Van de Erve (1943).
[b]Body-weight gain expressed as mean (% change compared with control); % change control = [(treatment mean − control mean) ÷ control mean] × 100.
[c]Average daily doses (mg W/kg-day) were calculated for this review using tungsten consumption and body-weight data reported by the study authors.

Note: Statistics were not reported by study authors; data reporting is not adequate for statistical analysis.

Table B-7. Neurobehavior of Sprague-Dawley Rat Dams and Pups after Exposure to Sodium Tungstate Dihydrate for 70 Days in Drinking Water (14 Days Premating, 14 Days Mating, 22 Days Gestation, and through PND 20)[a]

Parameter[b]	Exposure Group, mg Sodium Tungstate/kg-d (mg W/kg-d)[c]			
Dams	**0**	**5 (3)**	**62.5 (39)**	**125 (78)**
Number of animals	20	20	20	20
Open-field behavior (7 d post exposure)				
Distance travelled (cm)	5,521 ± 689	8,640 ± 421* (+56%)	NR	5,532 ± 293 (0%)
Resting time (s)	933 ± 24	926 ± 27 (−1%)	NR	829 ± 29* (−11%)
Ambulatory time (s)	131 ± 30	306 ± 13* (+134%)	NR	82 ± 4 (−37%)
Time in stereotypic movements (s)	735 ± 26	568 ± 20* (−23%)	NR	889 ± 26* (+21%)
Pups	**0**	**5 (3)**	**62.5 (39)**	**125 (78)**
Number of animals/litters	NR	NR	NR	NR
Separation distress at PND 7				
Number of distress vocalizations during 60-s removal of dam	19.5 ± 3.2	23.1 ± 3.8 (+18%)	NR	34.4 ± 4.1* (+76%)

[a]McInturf et al. (2011); McInturf et al. (2008).
[b]Values expressed as mean ± SD (% change compared with control); % change control = [(treatment mean − control mean) ÷ control mean] × 100.
[c]Equivalent tungsten doses were calculated based on molecular weights (tungsten accounts for 62.6% of total molecular weight of Na_2WO_4).
*Statistically significantly different from controls at $p < 0.05$, as calculated by study authors (ANOVA followed by Tukey's post hoc comparisons).

NR = not reported by study authors; it is unclear whether or not neurobehavior was assessed in the mid-dose group.

Table B-8. Mean Body-Weight Gain of Male and Female Rats after Exposure to Sodium Tungstate for 12 Weeks in Drinking Water[a]

Parameter[b]	Exposure Group, mg/L W in Water (average daily dose, mg W/kg-d)[c]	
Male	**0**	**1,248 (147)**
Number of animals	15	15
Body-weight gain (g)	234.1 ± 7.5	195.2 ± 8.6* (−17%)
Female	**0**	**1,248 (160)**
Number of animals	24	24
Body-weight gain (g)	77.2 ± 3.0	45.7 ± 3.5* (−41%)

[a]Ballester et al. (2007); Ballester et al. (2005).
[b]Body-weight gain expressed as mean ± SEM (% change compared with control); % change control = [(treatment mean − control mean) ÷ control mean] × 100.
[c]Equivalent tungsten doses (mg/L) were calculated based on molecular weights (tungsten accounts for 62.6% of total molecular weight of Na_2WO_4). Average daily dose were calculated for this review using reference Wistar rat body weight and water consumption values for the subchronic duration studies (U.S. EPA, 1988).

Table B-9. Mean Body Weight, Longevity, and Tumor Incidence of Male and Female Long-Evans Rats and Swiss Mice after Lifetime Exposure to Sodium Tungstate in Drinking Water[a]

Parameter		Exposure Group, ppm Tungsten Equivalent in Drinking Water (average daily dose, mg W/kg-d) [HED, mg W/kg-d][b]	
Male rats		**0**	**5 (0.6) [0.1]**
Initial number of animals		52	37
Body weight (g)[c]	Day 180	409.6 ± 5.17	433.4 ± 7.1* (+6%)
	Day 360	484.5 ± 6.3	529.1 ± 8.2* (+9%)
	Day 540	501.3 ± 11.8	539.3 ± 9.1* (+8%)
Longevity[c,d]		1,126 ± 18.2	983 ± 7.3* (−13%)
Number of rats with tumors at necropsy[e]		4/26	4/25
Number of rats with malignant tumors at necropsy[e]		2/26	2/25
Female rats		**0**	**5 (0.7) [0.2]**
Initial number of animals		52	35
Body weight (g)[c]	Day 180	250.3 ± 4.8	265.5 ± 3.9 (+6%)
	Day 360	277.9 ± 5.52	297 ± 6.3* (+7%)
	Day 540	290.8 ± 5.52	315.2 ± 6.5 (+8%)
Longevity[c,d]		1,139 ± 29.6	1,063 ± 22.8 (−7%)
Number of rats with tumors at necropsy[e]		17/24	13/20
Number of rats with malignant tumors at necropsy[e]		8/24	5/20
Male mice		**0**	**5 (1) [0.1]**
Initial Number of animals		54	54
Body weight (g)[c]	Day 540	46.8 ± 1.33	43.5 ± 1.80 (−7%)
Longevity[c,d]		939 ± 44.25	797 ± 7.5 (−15%)
Number of mice with tumors at necropsy		NR	NR

Table B-9. Mean Body Weight, Longevity, and Tumor Incidence of Male and Female Long-Evans Rats and Swiss Mice after Lifetime Exposure to Sodium Tungstate in Drinking Water[a]

Parameter		Exposure Group, ppm Tungsten Equivalent in Drinking Water (average daily dose, mg W/kg-d) [HED, mg W/kg-d][b]	
Female mice		**0**	**5 (1) [0.1]**
Initial number of animals		54	54
Body weight (g)[c]	Day 540	42.6 ± 1.32	40.0 ± 1.25 (−6%)
Longevity[c,d]		922 ± 28.44	945 ± 22.99 (+2%)
Number of mice with tumors at necropsy		NR	NR

[a]Schroeder and Mitchener (1975a); Schroeder and Mitchener (1975b).
[b]Estimated daily tungsten intakes were calculated using reference Long Evans rat body weight (0.472 kg for males and 0.344 kg for females) and water consumption (0.057 L/day for males and 0.046 L/day for females) values for the chronic duration studies (U.S. EPA, 1988) and reference mouse body weight (0.0317 kg for males and 0.0288 kg for females) and water consumption (0.0078 L/day for males and 0.0073 L/day for females) values for the chronic duration studies (U.S. EPA, 1988). HEDs were calculated using species-specific DAFs based on the animal:human $BW^{1/4}$ ratio recommended by U.S. EPA (2011b): mouse:human ratio = 0.14; rat:human ratio = 0.24.
[c]Values are expressed as mean ± SEM (% change compared with control); % change control = [(treatment mean − control mean) ÷ control mean] × 100.
[d]Longevity was defined as the mean life span of the last five surviving animals per group.
[e]Rats that died during a pneumonia epidemic (Month 20) were not included in necropsy data.
*Statistically significantly different from controls at $p < 0.05$, as calculated by study authors (Student's *t* test).

NR = not reported by study authors.

Table B-10. Esophageal and Forestomach Lesions in Male Sprague-Dawley Rats after Exposure to Sodium Tungstate for 19−30 Weeks in Drinking Water, with or without *N*-Nitrososarcosine Ethyl Ether (NSEE)[a]

Parameter	Exposure Group, ppm Tungsten (average daily dose, mg W/kg-d) [HED, mg W/kg-d][b]						
19 wk	**0**	**0 + 8 doses NSEE[c]**	**0 + 16 doses NSEE[d]**	**100 (13.9) [3.33]**	**100 (13.9) [3.33] + 16 doses NSEE[d]**	**200 (27.8) [6.67]**	**200 (27.8) [6.67] + 8 doses NSEE[c]**
Esophagus							
Hyperplasic lesions[e]	0/10	26/31	20/20	0/10	15/15	0/10	22/22
Precancerous lesions[f]	0/10	4/31	20/20	0/10	15/15	0/10	22/22[i]
Early carcinoma[g]	0/10	0/41	12/20	0/10	9/15	0/10	1/22
Late carcinoma[h]	0/10	0/41	4/20	0/10	3/15	0/10	0/22
Forestomach							
Hyperplasic lesions	0/10	NR	20/20	0/10	15/15	NR	NR
Precancerous lesions	0/10	NR	20/20	0/10	15/15	NR	NR
Early carcinoma	0/10	NR	11/20	0/10	9/15	NR	NR
Late carcinoma	0/10	NR	3/20	0/10	3/15	NR	NR
30 wk	**0**	**0 + 16 doses NSEE[d]**		**100 (11.9) [2.86]**		**100 (11.9) [2.86] + 16 doses NSEE[d]**	
Esophagus							
Hyperplasic lesions	0/10	21/21		0/10		14/14	
Precancerous lesions	0/10	21/21		0/10		14/14	
Early carcinoma	0/10	6/21		0/10		2/14	
Late carcinoma	0/10	13/21		0/10		10/14	
Forestomach							
Hyperplasic lesions	0/10	21/21		0/10		14/14	
Precancerous lesions	0/10	21/21		0/10		14/14	
Early carcinoma	0/10	4/21		0/10		3/14	
Late carcinoma	0/10	17/21		0/10		11/14	

[a]Luo et al. (1983).
[b]Average daily doses (mg/kg-day) were calculated for this review using reference male weanling S-D rat body weight and water consumption values for the subchronic and chronic duration studies (U.S. EPA, 1988). HEDs were calculated using a DAF of 0.24 for rats based on the animal:human $BW^{1/4}$ ratio recommended by U.S. EPA (2011b).
[c]1 mL/kg NSEE given by gavage twice weekly between Week 4 and 8.
[d]1 mL/kg NSEE given by gavage twice weekly between Week 4 and 12.
[e]Hyperplastic lesions defined by study authors as hyperkeratosis, simple hyperplasia, and papillary hyperplasia.
[f]Precancerous lesions defined by study authors as marked endophytic growth of epithelium, marked dysplasia, papilloma, and papillomatosis.
[g]Early carcinoma defined by study authors as malignant changes of basal cells or papilloma, carcinoma in situ, and early infiltrative carcinoma.
[h]Advanced carcinoma defined by study authors as carcinoma with extensive invasions.
[i]Statistically significantly greater than rats treated with 8 doses of NSEE only at $p < 0.05$, as calculated for this review (Fisher's Exact test).

NR = not reported by study authors.

Table B-11. Mammary Carcinomas in Female Sprague-Dawley Rats after Exposure to Tungsten for 125 or 198 Days in Drinking Water, with NMU[a]

Parameter	Exposure Group, ppm Tungsten (average daily dose, mg W/kg-d) [HED, mg W/kg-d][b]			
	0	**0 + NMU[c]**	**0 + NMU[c] + 10 ppm Molybdenum**	**0 + NMU[c] + 150 ppm W (20 mg W/kg-d) [4.8 mg W/kg-d]**
125 d				
Number of animals	10	22	22	24
% animals with mammary carcinomas	0	50	45.5	79.2[d]
# of carcinomas/carcinoma-bearing rat	0	2	1.3	1.7
Day of 1st palpable mass	NA	71	71	56
198 d				
Number of animals	10	21	20	23
% animals with mammary carcinomas	0	90.5	50.0[c]	95.7
# of carcinomas/carcinoma-bearing rat	0	2	1.5	2.6
Day of 1st palpable mass	NA	85	85	56

[a]Wei et al. (1985)
[b]Average daily doses (mg/kg-day) were calculated for this review using reference female S-D rat body weight (0.338 kg) and water consumption (0.045 L/day) values for the chronic duration studies (U.S. EPA, 1988). HED was calculated using a DAF of 0.24 for rats based on the animal:human $BW^{1/4}$ ratio recommended by U.S. EPA (2011b).
[c]5 mg/kg NMU was administered intravenously on Day 15.
[d]Statistically significantly different than rats treated with NMU-only at $p < 0.05$, as calculated by the study authors.

NA = not applicable.

APPENDIX C. SUMMARIES OF SUPPORTING DATA

Table C-1. Summary of Soluble Tungsten Compounds Genotoxicity

Endpoint	Test System	Dose/ Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
Genotoxicity studies in prokaryotic organisms						
Mutation	*S. typhimurium*, *E. coli*	NR	–	ND	OECD 471 guidelines followed using sodium tungstate as test material.	Covance Laboratories (2004a) as cited in Jackson et al. (2013)
Reverse mutation	*S. cerevisiae*	0.1 M sodium tungstate	(+)	ND	Study authors reported weak positive results for conversion at *trp 5* and reversion at *ilv 1*.	Singh (1983)
Mutation (bacterial bioluminescence test)	*P. fischeri*	25 mmol/L sodium tungstate	+	ND		Ulitzur and Barak (1988)
SOS repair induction	*E. coli* $WP2_s$ (λ)	0.005 M sodium tungstate	+	ND	λ prophage induction was increased 5 times over control.	Rossman et al. (1991); Rossman et al. (1984)
Genotoxicity studies in nonmammalian eukaryotic organisms						
No data						
Genotoxicity studies in mammalian cells—in vitro						
Mutation	Mouse lymphoma cells (L5178Y TK ±)	NR	–	ND	OECD 476 guidelines followed using sodium tungstate as test material.	Covance Laboratories (2004b) as cited in Jackson et al. (2013)
Mutation	Chinese hamster lung cells (V79)	NR	+	ND	>three-fold increase above background.	Zelikoff et al. (1986) (abstract only)
CAs	CHO	NR	–	ND	OECD 473 guidelines followed using sodium tungstate as test material.	Covance Laboratories (2003) as cited in Jackson et al. (2013)
CAs	Syrian hamster embryo cells	0.03 M sodium tungstate dihydrate	–	ND		Larramendy et al. (1981)

Table C-1. Summary of Soluble Tungsten Compounds Genotoxicity

Endpoint	Test System	Dose/ Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
CAs	Human purified lymphocytes	0.03 M sodium tungstate dihydrate	–	ND		Larramendy et al. (1981)
SCE	Human whole blood cultures	0.03 M sodium tungstate dihydrate	–	ND		Larramendy et al. (1981)
Genotoxicity studies in mammals—in vivo						
Mouse bone marrow MN test	Mouse	NR	–		OECD 474 guidelines followed using sodium tungstate as test material.	Covance Laboratories (2004c) as cited in Jackson et al. (2013)

Table C-1. Summary of Soluble Tungsten Compounds Genotoxicity

Endpoint	Test System	Dose/ Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
DNA damage (Comet assay; γH2AX immunoblot)	4-wk-old mouse (C57BL/6J, 5 males/group); sodium tungstate dihydrate in drinking water for 1, 4, 8, 12, or 16 wk	0, 4, 49, 250 mg W/kg-d as sodium tungstate dihydrate	± In nonadherent bone marrow cells, DNA damage assessed by the Comet assay was increased compared with control, but findings across time points and dosages were not consistent with a monotonic response with increasing dose and duration. In CD19+ B cells, DNA damage was significantly increased in the 4-mg W/kg-d group at 1 and 4 wk and the 49-mg W/kg-d group at 4 wk, but significantly decreased at 1 and 4 wk in the 250-mg/kg-d group.		Significant increases in DNA damage in nonadherent bone marrow cells were observed at 1, 4, 12, and 16 wk in the 4-mg W/kg-d group; at 1, 4, and 8 wk in the 49-mg W/kg-d group; and 4 and 12 wk in the 250-mg W/kg-d group. The amount of DNA damage did not increase with increasing dose; across time points, the magnitude of damage was higher in cells from 4-mg W/kg-d mice than 250-mg/kg-d mice. DNA damage in CD19+ B cells was not assessed at later time-points (8 or 12 wk). Immunoblot staining for γH2AX (another assay for DNA damage) in CD19+ B cells from exposed mice after 1 wk of exposure was not significantly elevated, compared with control values.	Kelly et al. (2013)
Genotoxicity studies in subcellular systems						
ND						

[a]Lowest effective dose for positive results, highest dose tested for negative results.
[b]+ = positive; (+) = weak positive; – = negative; ± = equivocal.

ND = no data; NR = not reported.

Table C-2. Other Studies				
Test	**Materials and Methods**	**Results**	**Conclusions**	**References**
Supporting evidence—cancer effects in humans				
ND				
Supporting evidence—noncancer effects in humans				
Cross-sectional survey of U.S. population examining possible associations between serum thyroid levels and levels of metals in blood and urine	Multiple linear regression analysis of 2007−2008 NHANES data for urinary levels of tungsten (and lead, cadmium, mercury, barium, cobalt, antimony, thallium, and uranium) and serum levels of T3, FT3, T4, FT4, and TSH. Analysis also included serum levels of cadmium, lead, and mercury. Models were adjusted for age, sex, race-ethnicity, total serum lipids, serum cotinine, pregnancy and menopausal status, and use of medications thought to affect thyroid function.	Significant adjusted betas (SE) for change in natural log (ln)-transformed serum thyroid hormone per unit of tungsten in urine (µg/L urine): *Model with urinary tungsten only:* ln T3: −0.20 (p = 0.010) ln T4: −0.027 (p = 0.008). *Model with all metals measured in urine and blood:* ln TSH: +0.042 (p = 0.016). Analysis included data for 1,587 adults with no history of thyroid disease or use of thyroid medications.	An association between increasing urinary tungsten levels and increasing TSH levels was indicated. Levels of other thyroid hormones were not associated with urinary tungsten levels when models included all metals measured in urine and blood. Route of exposure of subjects is unknown.	Yorita Christensen (2012)

Table C-2. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Cross-sectional survey of U.S. population examining possible associations between incidence for CCVD and levels of metals in urine	Logistic regression analysis of 1999−2006 NHANES data for urinary levels of tungsten (and antimony, arsenic, barium, beryllium, cadmium, cesium, cobalt, lead, molybdenum, platinum, thallium, and uranium) and incidence of CCVD as determined from questionnaire asking about history of stroke, angina, heart attack, coronary heart disease, and congestive heart failure.	Adjusted OR (95% CI) for CCVD by log-transformed urinary levels of tungsten (µg/mg creatinine): OR = 1.78 (CI: 1.28−2.48). (*n* = 573, CCVD; *n* = 4,462 non-CCVD) Model was adjusted for age, sex, race, education, hypertension, diabetes, hyper-cholesterolemia, chronic kidney disease, body mass index, C-reactive protein, smoking status, and serum cotinine.	An association between elevated urinary concentrations of tungsten and cardiovascular and cerebrovascular disease was indicated. Associations were also indicated with elevated urinary concentrations of antimony, cobalt, and cadmium, but not arsenic, barium, beryllium, cesium, lead, molybdenum, platinum, thallium, or uranium. Route of exposure of subjects is unknown.	Agarwal et al. (2011)
Cross-sectional survey of U.S. population examining possible associations between various medical conditions and levels of metals in urine	Logistic regression analysis of 2007−2008 NHANES data for urinary levels of tungsten (and antimony, barium, beryllium, cadmium, cesium, cobalt, lead, molybdenum, thallium, platinum, and uranium) and the following medical conditions (determined by yes or no answers to questions): asthma, overweight, blood transfusions, vision problems, arthritis, gout, congestive heart failure, coronary heart disease, angina, heart attack, stroke, emphysema, thyroid problem, chronic bronchitis, liver condition, and cancer.	Adjusted OR (95% CI) for asthma by log-transformed urinary levels of tungsten (µg/g creatinine): OR = 1.72 (CI: 1.15−2.59) Study population included 922 male and 935 female subjects. Model was adjusted for age, sex, race/ethnicity, education, family income status, alcohol consumption, smoking status, serum cotinine, and other metals. No significant associations were found between urinary tungsten levels and other medical conditions assessed.	An association between elevated urinary levels of tungsten and asthma was indicated, but not for any of the other medical conditions assessed, including coronary heart disease, heart attack, congestive heart failure, stroke, or thyroid problems. Route of exposure of subjects is unknown.	Mendy et al. (2012)

Table C-2. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Supporting evidence—cancer in animals				
Carcinogenicity other than regular oral/inhalation exposure	Guinea pigs (unspecified sex and number) were injected intratracheally with either a suspension or a solution of tungsten. Animals were examined after 12 mo for pulmonary lesions.	Tungsten-induced pulmonary lesions and proliferation of epithelial cells were reported; however, no tumors were induced.	No evidence for carcinogenicity using this protocol (study design details were limited).	Schepers (1971)
Supporting evidence—noncancer effects in animals				
Reproductive/ developmental studies	12−15 female rats were orally administered 0 or 0.005 mg/kg-d of an unspecified tungsten compound for up to 8 mo before and during pregnancy.	Pre- and postimplantation losses and delayed fetal ossification	Due to inadequate reporting of study design and results, findings are difficult to interpret. As such, reliable NOAEL/LOAEL determinations cannot be made.	Nadeenko and Lenchenko (1977); Nadeenko et al. (1977, 1978) as cited in ATSDR (2005)
Neurotoxicity	Albino rats were orally exposed to 0, 0.005, 0.05, or 0.5 mg/kg-d of sodium tungstate for 7 mo. Strain, sex, and animal number were not reported. Neurobehavioral testing (conditioned reflexes to a light and bell) was conducted at an unspecified time point during the study. Details on the neurobehavioral protocol and endpoints examined are limited. Brains were examined microscopically at 8 mo.	Impaired conditioned responses were reported in rats from the 0.05- and 0.5-mg/kg-d groups. No brain lesions were reported.	Due to inadequate reporting of study design and results, findings are difficult to interpret. As such, reliable NOAEL/LOAEL determinations cannot be made.	Nadeenko (1966)

Table C-2. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Neurotoxicity	Albino rabbits (7/group) were exposed to 0, 0.005, 0.05, 0.5, or 5 mg/kg-d of sodium tungstate for 8 mo. Strain and sex and animal number were not reported. Blood cholinesterase activity was measured monthly and brains were examined microscopically at 8 mo.	Blood cholinesterase activity in rabbits was significantly decreased in the 5-mg/kg-d group from 4 to 8 mo and in the 0.5-mg/kg-d group at 8 mo. Unspecified lesions were observed in the cerebral cortex of rabbits in the 0.5 and 5-mg/kg-d groups. No neurotoxic effects were reported for lower dose groups.	Due to inadequate reporting of study design and results, findings are difficult to interpret. As such, reliable NOAEL/LOAEL determinations cannot be made.	Nadeenko (1966)
Short-term-duration studies	Acute oral and inhalation toxicities of sodium tungstate were determined in rats (OECD 401 and 403 guideline studies, respectively).	Oral LD_{50} = 1,453 mg/kg Inhalation LC_{50} (4-hr) >5.01 mg/L		Huntingdon Life Sciences (1998, 1999a) as cited in Jackson et al. (2013)
Short-term-duration studies	Acute lethality of tungsten trioxide, sodium tungstate, and sodium phosphotungstate was assessed in rats and mice. Acute lethality of sodium tungstate was also assessed in rabbits and guinea pigs.	LD_{50} values for sodium tungstate: Rat—1,190 mg/kg Mouse—240 mg/kg Rabbit—875 mg/kg Guinea pig—1,152 mg/kg LD_{50} values for tungsten trioxide: Rat—NR Mouse—840 mg/kg LD_{50} values for sodium phosphotungstate: Rat—1,600 mg/kg Mouse—700 mg/kg Clinical signs of toxicity in rats and mice following acute exposure to all compounds included hunching, decreased muscle tone, and paresis in hind limbs.	Acute toxicity of sodium tungstate in different species showed the following ranking: rat < guinea pig < rabbit < mouse. Acute toxicity of the 3 tungsten compounds ranked tungsten trioxide < sodium phosphotungstate < sodium tungstate in correlation with their relative solubilities.	Nadeenko (1966)

Table C-2. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Short-term-duration studies	Subacute toxicity was assessed in albino rats and rabbits (sex, species, animal number, and duration were not reported). Animals were orally exposed to 10, 25, 50, or 100 mg sodium tungstate/kg-d. General condition, behavior, weight, hematology, and blood cholinesterase activity were measured. Internal organs were examined microscopically in the 10- and 100-mg/kg-d groups only. It is unclear if a control group was used.	All doses of sodium tungstate retarded growth of rats and increased blood cholinesterase activity. The following histopathological changes were observed in the gastrointestinal tract, liver, and kidneys of animals in the 10- and 100-mg/kg-d groups: increase in the vascular permeability with hemorrhages, appearance of degenerative-dystrophic changes, and a moderate proliferative-cellular reaction.	Due to inadequate reporting of study design and results, findings are difficult to interpret. As such, reliable NOAEL/LOAEL determinations cannot be made.	Nadeenko (1966)
Short-term-duration studies other than oral/inhalation	Acute dermal toxicity of sodium tungstate was determined in rats. Dermal and eye irritation were determined in rabbits and skin sensitization was determined in guinea pigs (OECD 402, 404, 405, and 406 guideline studies, respectively).	Dermal LD_{50} > 2,000 mg/kg Skin irritation and sensitization studies were negative, but sodium tungstate was classified as slightly irritating to eyes.		Huntingdon Life Sciences (1999b, c, d, e) as cited in Jackson et al. (2013)

Table C-2. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Short-term-duration studies other than oral/inhalation	A 10% suspension of dusts of unknown particle size of tungsten metal dust, tungsten carbide and carbon (94:6 ratio), or tungsten carbide and cobalt (91:9 ratio) in isotonic saline was injected intratracheally into groups of six guinea pigs (age, sex, and strain not reported). A total of 150 mg of each dust was injected in three equal doses at weekly intervals. Animals were sacrificed at 1, 4, 8, and 12 mo and lungs were examined microscopically. (n = 1−3 animals/time point per group).	No compound-related deaths were reported. Large, circumscribed pigmented lesions were observed during gross examination of the lungs of all animals exposed to tungsten carbide and carbon. Beneath the visceral pleura, widely distributed, small, discrete foci of pigmentation were occasionally observed. These lesions were only observed at 1 and 4 mo following exposure to tungsten metal dust. Exposure to tungsten carbide and cobalt resulted in several histopathological changes at 1 mo (proliferation of interstitial cells with thickening of alveolar walls around massed tungsten particles, inflamed mucosa of bronci and bronchioles, and partial or complete closure of some bronchioles). After 1 yr, residue lesions were observed (persistent, focal, interstitial, cellular infiltration in relation to retained particles). Various degrees of peribronchial, peribronchiolar, and perivascular fibrocellular reactions were observed. Slight atrophic vesicular emphysema was also present.	The results suggest that inhalation of tungsten carbide particles together with cobalt may lead to pulmonary fibrosis and other histopathological lung changes; however, fibrosis was not observed in animals exposed only to tungsten metal dust or tungsten carbide and carbon dust.	Delahant (1955); Schepers (1955)

Table C-2. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Short-term-duration studies other than oral/inhalation	White rats were administered single doses of metallic tungsten via intratracheal injections of 50 mg of material suspended in 0.5 mL of physiologic saline. Age, weight, sex, strain, and animal numbers were not reported. Rats were sacrificed at 4, 6, or 8 mo after injection and lungs were examined microscopically. Other organs were evaluated macroscopically.	Lungs showed infiltration by macrophages, chiefly around pulmonary blood vessels, and thickening of the walls between the alveoli at 4 mo. At 6 mo, large numbers of round cells were seen surrounding the tungsten particles around the bronchi. Collagen fibers had overgrown these foci by 8 mo. At 8 mo, the endothelium was swollen and the walls of the vessels were thickened. Macroscopic changes were not observed in other organs at 4, 6, or 8 mo.	The results suggest that inhalation exposure to tungsten may result in pulmonary fibrosis.	Mezentseva (1967)
ADME				
ADME	Male C57BL/6J mice (5/group) were given water ad libitum containing 0, 15, 200, or 1,000 mg/L as sodium tungstate dihydrate for 16 wk (0, 4, 49, or 250 mg W/kg-d). Elemental tungsten concentration in the tibia bones was quantified at 1, 4, 8, 12, and 16 wk, using inductively coupled plasma mass spectrometry. An additional group was given 15 mg/L (4 mg W/kg-d) for 4 wk, and tungsten bone deposition was measured following 4- and 8-wk recovery periods.	Tungsten levels were significantly elevated in tibiae by Wk 1 in all exposure groups. Tungsten concentration was elevated in a dose-dependent manner, increased by approximately 5-, 80-, and 450-fold in the 4-, 49-, and 250-mg/kg-d groups (data presented graphically). Tungsten accumulation rates were significantly lower during subsequent weeks ($p < 0.001$), and levels at subsequent time-points were similar to levels at Wk 1. Following the 4- and 8-wk recovery periods, tungsten levels in bone were still significantly elevated compared with controls; however, levels were ~50% less than in mice exposed for 8 or 12 wk.	Tungsten accumulation in bone increased with increasing dose level. Accumulation in bone was rapid, peaking after 1 wk of exposure. Tungsten was not cleared from bone as rapidly as it accumulated.	Kelly et al. (2013)

Table C-2. Other Studies

Test	Materials and Methods	Results	Conclusions	References
ADME	Male and female Sprague-Dawley rats (40/group) were administered 0, 5, or 125 mg/kg-d sodium tungstate as sodium tungstate dihydrate (0, 3, or 78 mg W/kg-d) in deionized water via gavage 7 d/wk for 70 d (including 14 d premating, 14 d mating, 22 d gestation, and through PND 20). Ten F0 rats/sex were sacrificed on the last day of treatment (PND 20) and 1 pup/sex/litter was sacrificed on PND 20 and 70 for determination of tungstate concentrations in various tissues and organs by inductively coupled plasma mass spectrometry. Tungstate levels were also quantified in the mammary secretions of all dams once between PND 10 and 14.	At PND 20, significantly increased tungstate levels were observed in the kidney, lungs, liver, gastrointestinal tract, brain, and femur of pups and the spleen, kidney, lung, liver, brain, femur, blood, thymus, and testes of adults. Tungstate levels were below detection levels by PND 70 (50 d post exposure). Tungstate concentrations in dam milk secretions measured PND 10−14 were 0.005, 0.021, or 0.45 ppm in the 0-, 3-, or 78-mg W/kg-d groups, respectively. The measured concentration in the high-dose group was statistically significantly ($p < 0.05$) higher than controls, but the low-dose group did not differ significantly from controls.	Tungstate distributed widely in the body. It crossed the blood brain barrier and the placenta. Tungstate accumulation in milk increased with increasing dose level.	McInturf et al. (2011); McInturf et al. (2008)
ADME	Male and female rats (unspecified strain; 2/sex/group) were fed ground dog chow containing 2 or 10% W as tungsten metal; 10% W as purified tungsten metal; 0.1% W as tungsten oxide, 0.1% W as sodium tungstate, or 0.5% W as ammonium-*p*-tungstate for 100 d. Control groups were fed only ground dog chow.	Tungsten was generally observed (in trace amounts) in the blood, kidney, and liver, regardless of administered compound. In about 50% of the animals, trace amounts of tungsten were observed in the lung, muscles, and testes; again, findings were not related to administered compound. Tungsten was generally not observed in the brain, heart, or uterus (a single exception in each case).	There were no marked differences among the tissue distribution patterns of the various tungsten compounds tested. The later tissue distribution studies by Kelly et al. (2013) and McInturf et al. (2008), using inductively coupled plasma mass spectrometry, provide more accurate descriptions of tissue distribution than this early study.	Kinard and Aull (1945)

Table C-2. Other Studies				
Test	**Materials and Methods**	**Results**	**Conclusions**	**References**
Studies of Mode of Action/ Mechanism/ Therapeutic Action				
Mode of action/ mechanistic	Male Wistar rats (4/group) were fed basal diets containing 0, 5, or 50 ppm tungsten as sodium tungstate dihydrate for 28 d (0, 0.65, or 6.6 mg W/kg-d). L-ascorbic acid metabolism was evaluated.	Concentration of ascorbic acid was significantly increased in the liver and spleen of the 0.65-mg W/kg-d group, but not the 6.6-mg W/kg-d group, compared with control. Significantly decreased urinary excretion of ascorbic acid and glucuronic acid was observed in both exposure groups, compared with controls. Liver extracts from rats in both exposure groups synthesized significantly greater amounts of L-ascorbic acid from L-gluconolactone, 2,3-dioxobulonic acid from dehydroascorbic acid, and L-Xylulose from sodium L-gulonate, compared with livers extracts from control rats. Conversion of D-glucuronolactone to D-glucuronic acid by liver extracts was not altered with dietary tungsten exposure.	Dietary exposure to tungsten altered ascorbic acid metabolism.	Chatterjee et al. (1973)
Mode of action/ mechanistic	Rats were fed basal or tungsten supplemented diet (0.7 g sodium tungstate/kg) for 3 wk. Following treatment, lungs were removed for quantification of xanthine oxidase (XO) activity.	Tungsten-exposed rats had negligible XO activities in isolated lungs. When isolated lungs were exposed to hyperoxia, XO-depleted lungs from tungsten-exposed rats developed less acute edematous injury during perfusion with buffer or purified neutrophil elastase than XO-replete lungs from control rats.	The results indicate that supplemental sodium tungstate in the diet depleted XO activity in the lungs of rats.	Rodell et al. (1987)

Table C-2. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Mode of action/ mechanistic/ therapeutic action	Multiple studies reported that sodium tungstate corrected hyperglycemia in insulin- and noninsulin-dependent rat models of diabetes when administered orally. Prevention and/or regeneration of pancreatic beta-cell populations in a diabetic animals models have also been demonstrated (Oliveira et al., 2014; Fernández-Alvarez et al., 2004). Improved sexual and/or reproductive function in diabetic rat models was also reported following sodium tungstate administration (Ballester et al., 2007; Ballester et al., 2005).			Oliveira et al. (2014); Ballester et al. (2007); Ballester et al. (2005); Fernández-Alvarez et al. (2004); Muñoz et al. (2001); Le Lamer et al. (2000); Rodriguez-Gallardo et al. (2000); Barberà et al. (1997)

CCDV = cardiovascular and cerebrovascular disease; CI = confidence interval; FT3 = free triiodothyronine; FT4 = free thyroxine; ND = no data; NHANES = National Health and Nutrition Examination Survey; NR = not reported; OR = odds ratio; T3 = triiodothyronine; T4 = thyroxine; XO = xanthine oxidase.

APPENDIX D. BENCHMARK DOSE MODELING RESULTS

MODELING PROCEDURE FOR DICHOTOMOUS DATA

The benchmark dose (BMD) modeling of dichotomous data was conducted with the U.S. EPA's Benchmark Dose Software (BMDS) (Version 2.2.2). For these 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 default benchmark response (BMR) of 10% extra risk based on the U.S. 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 three-fold; 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).

In addition, data from exposures much higher than the study lowest-observed-adverse-effect level (LOAEL) do not provide reliable information regarding the shape of the response curve at low doses. However, such exposures can have a strong effect on the shape of the fitted model in the low-dose region of the dose-response curve in some cases. Thus, if lack of fit is due to characteristics associated with dose-response data for high doses, then the U.S. EPA's *Benchmark Dose Technical Guidance Document* allows for data to be adjusted by eliminating high-dose groups (U.S. EPA, 2012b).

MODELING PROCEDURE FOR CONTINUOUS DATA

The BMD modeling of continuous data was conducted with the U.S. EPA's BMDS (Version 2.2.2). For these data, all continuous models available within the software were fit using a default BMR of 1 standard deviation (SD) relative risk. For changes in body weight, a BMR of 10% change relative to the control mean was also used. An adequate fit was judged based on the goodness-of-fit *p*-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination was made as to whether the variance across dose groups was constant. If a constant variance model was deemed appropriate based on the statistical test provided in BMDS (i.e., Test 2), the final BMD results were estimated from a constant variance model. If the test for homogeneity of variance was rejected ($p < 0.1$), the model was run again while modeling the variance as a power function of the mean to account for this nonconstant variance. If this nonconstant variance model did not adequately fit the variance data (i.e., Test 3; $p < 0.1$), the data set was considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest BMDL was selected if the BMDLs estimated from different models varied greater than three-fold; otherwise, the BMDL from the model with the lowest AIC was selected as a potential POD from which to derive a p-RfD.

The following data sets were selected for BMD modeling:

- incidence data for goblet cell metaplasia and mild to severe renal cortical tubule regeneration, and body-weight data for male and female rats exposed by gavage for 90 days (USACHPPM, 2007a, b),
- total bone marrow cell counts in male C57BL/6J mice exposed to sodium tungstate in drinking water for 16 weeks (Kelly et al., 2013), and
- percentages of CD71+ helper and cytotoxic T cells in spleens of adult C57BL/6J mice exposed for 28 days, for 19 weeks (F0 mice) and for 32 weeks (F1 mice) following challenge with Staphylococcal enterotoxin B (Osterburg et al., 2014).

For the male rat stomach lesion incidence data, all models provided adequate fit by the χ^2 goodness-of-fit criteria (see Table D-1). The LogLogistic model was selected as the model with the lowest BMDL, because BMDLs from models with adequate fit ranged widely by about seven-fold (see Table D-1).

Table D-1. Modeling Results for Incidence Data for Goblet Cell Metaplasia in Glandular Stomach in Male Sprague-Dawley Rats Exposed to Sodium Tungstate in Water by Gavage for 90 Days[a]

Model	DF	χ^2	χ^2 Goodness-of-Fit *p*-Value[b]	Scaled Residuals[c]	AIC	BMD_{10} (mg W/kg-d)	$BMDL_{10}$ (mg W/kg-d)
Gamma[d]	4	2.34	0.67	0.12	40.86	6.76	4.65
Logistic	3	5.49	0.14	−0.05	46.14	21.10	14.01
LogLogistic[e,]	**3**	**2.51**	**0.47**	**0.32**	**43.00**	**7.72**	**2.34**
LogProbit[e]	3	3.1	0.38	0.78	43.68	12.30	7.70
Multistage (1-degree)[f]	4	2.34	0.67	0.12	40.86	6.76	4.65
Multistage (2-degree)[f]	4	2.34	0.67	0.12	40.86	6.76	4.65
Multistage (3-degree)[f]	4	2.34	0.67	0.12	40.86	6.76	4.65
Multistage (4-degree)[f]	4	2.34	0.67	0.12	40.86	6.76	4.65
Probit	3	5.4	0.14	−0.04	46.38	20.22	13.94
Weibull[d]	4	2.34	0.67	0.12	40.86	6.76	4.65

[a]USACHPPM (2007a); USACHPPM (2007b)
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals for dose group near BMD.
[d]Power restricted to ≥1.
[e]Slope restricted to ≥1.
[f]Betas restricted to ≥0.

DF = degree(s) of freedom.

The BMDS output for the selected model (LogLogistic) follows.

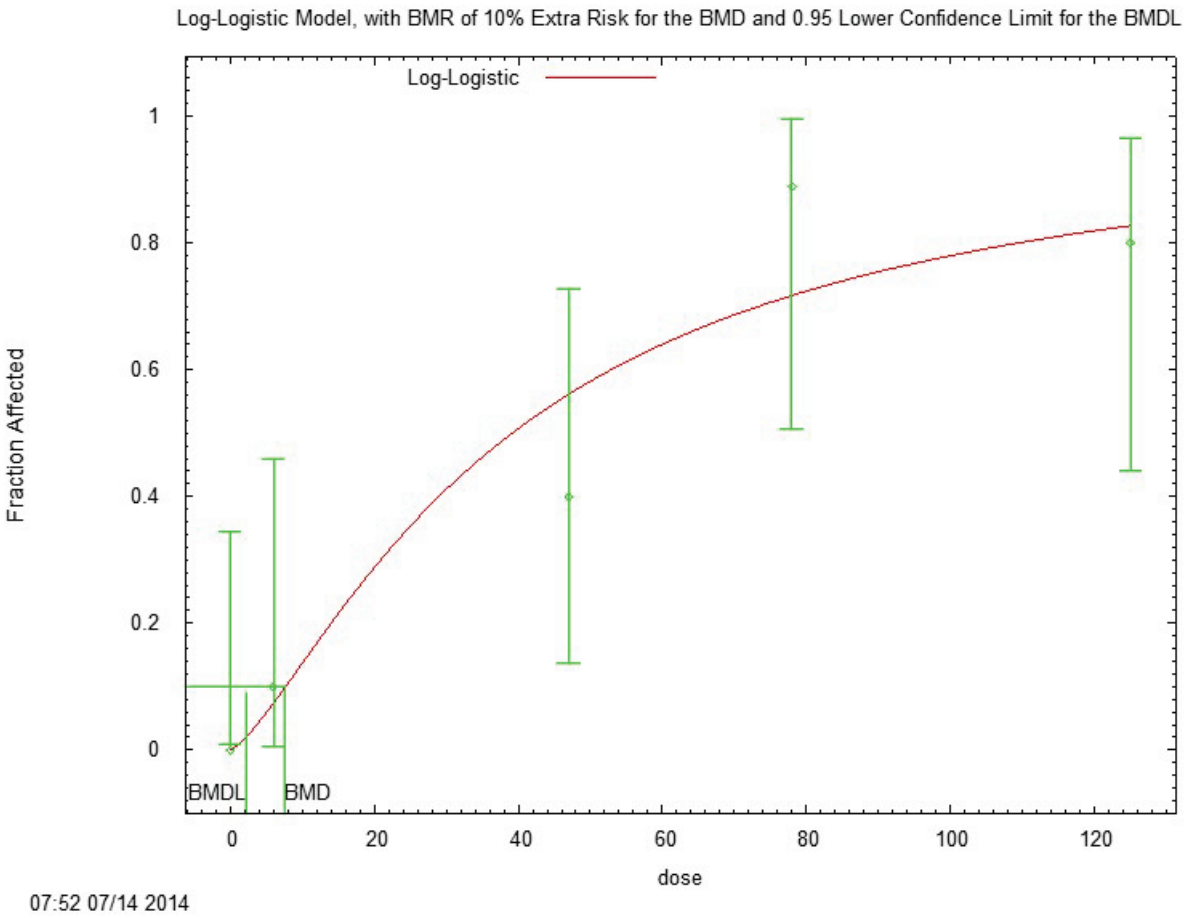

Figure D-1. LogLogistic (Glandular Stomach Goblet Cell Metaplasia, Male Rat)

```
 ================================================================
        Logistic Model. (Version: 2.14; Date: 2/28/2013)
        Input Data File:
C:/USEPA/PTV/NaTungstate/gobletcell/male/lnl_gobletcellmeta_male_Lnl-BMR10-Restrict.(d
)
        Gnuplot Plotting File:
C:/USEPA/PTV/NaTungstate/gobletcell/male/lnl_gobletcellmeta_male_Lnl-BMR10-Restrict.pl
t
                                          Mon Jul 14 07:52:07 2014
 ================================================================
 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 = 5
   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 = -4.65642
slope = 1.31441

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	-0.98
slope	-0.98	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	*	*	*
intercept	-4.94902	*	*	*
slope	1.34644	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-18.1245	5			
Fitted model	-19.5021	2	2.75535	3	0.4309
Reduced model	-33.4625	1	30.6761	4	<.0001

AIC: 43.0043

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	10	0.000
6.0000	0.0733	0.733	1.000	10	0.323
47.0000	0.5585	5.585	4.000	10	-1.009
78.0000	0.7144	6.430	8.000	9	1.159
125.0000	0.8252	8.252	8.000	10	-0.210

Chi^2 = 2.51 d.f. = 3 P-value = 0.4735

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 7.71959

BMDL = 2.34372

For the female rat stomach lesion incidence data, all models provided adequate fit to the data. BMDLs ranged from 4−18 mg W/kg-day. The 1-degree multistage model was considered an outlier based on poorer fit criteria than the other models, which all have sigmoidal shape capabilities. The range of BMDLs from the other models (7−18 mg W/mg/kg-day) was considered sufficiently close (within a three-fold range), so the model with the lowest AIC was selected (Multistage 2-degree).

Table D-2. Modeling Results for Incidence Data for Goblet Cell Metaplasia in Glandular Stomach of Female Sprague-Dawley Rats Exposed to Sodium Tungstate in Water by Gavage for 90 Days[a]

Model	DF	χ^2	χ^2 Goodness of Fit *p*-Value[b]	Scaled Residuals[c]	AIC	BMD_{10} (mg W/kg-d)	$BMDL_{10}$ (mg W/kg-d)
Gamma[d]	3	0.18	0.98	0.12	27.74	29.59	11.29
Logistic	3	0.8	0.85	0.51	28.57	30.01	17.97
LogLogistic[e]	3	0.41	0.94	0.14	28.10	31.75	14.50
LogProbit[e]	3	0.27	0.97	0.14	27.87	32.13	14.27
Multistage (1-degree)[f]	4	3.61	0.46	−1.06	31.15	5.89	4.02
Multistage (2-degree)[f]	**4**	**0.33**	**0.99**	**−0.31**	**26.06**	**19.94**	**8.14**
Multistage (3-degree)[f]	3	0.12	0.99	−0.21	27.66	25.22	7.42
Multistage (4-degree)[f]	2	0.11	0.95	−0.24	29.65	24.32	6.76
Probit	3	0.47	0.93	0.44	28.09	28.87	16.78
Weibull[d]	3	0.11	0.99	−0.16	27.64	26.13	10.57

[a]USACHPPM (2007a); USACHPPM (2007b)
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals for dose group near BMD.
[d]Power restricted to ≥1.
[e]Slope restricted to ≥1.
[f]Betas restricted to ≥0.

The BMDS output for the selected model (Multistage 2-degree) follows.

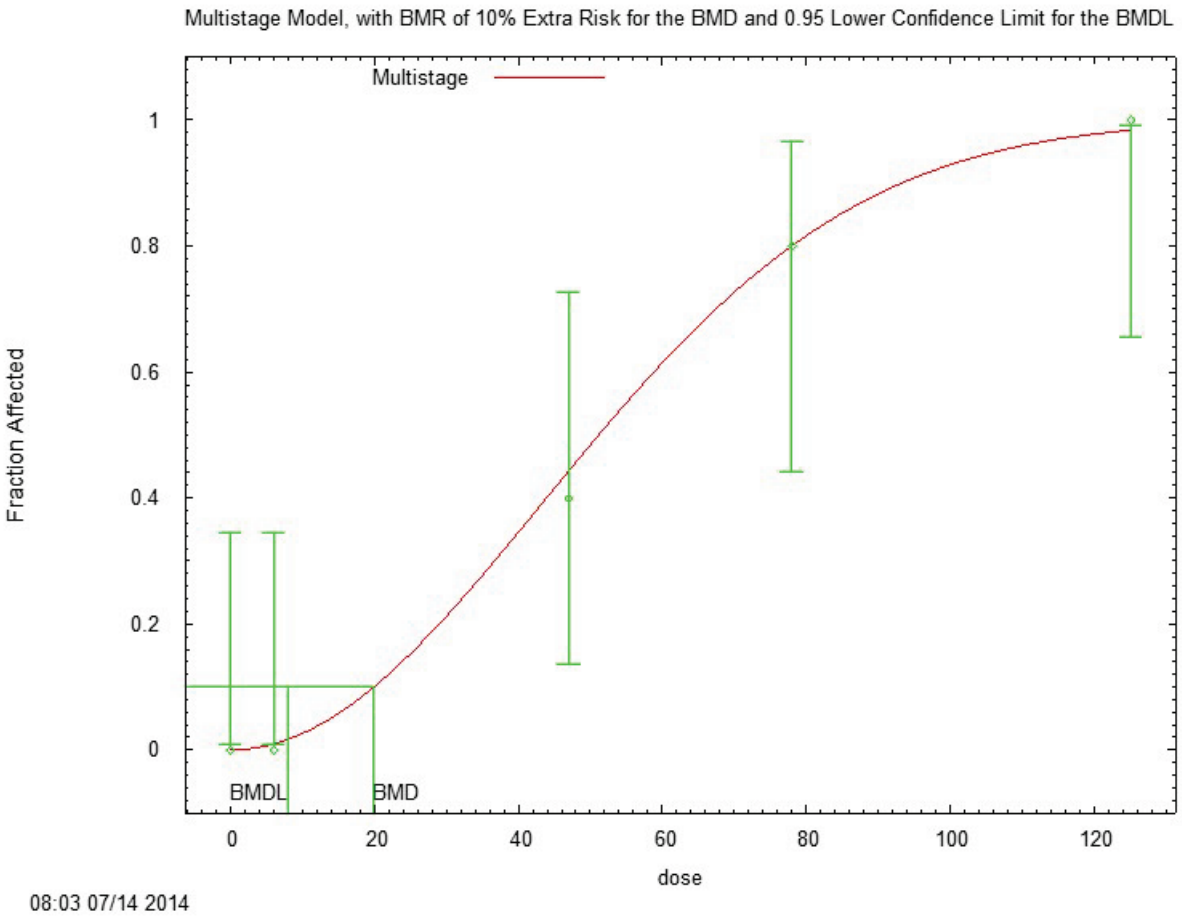

Figure D-2. Multistage (2-Degree) (Glandular Stomach Goblet Cell Metaplasia, Female Rat)

```
================================================================
        Multistage Model. (Version: 3.4;  Date: 05/02/2014)
        Input Data File:
C:/USEPA/PTV/NaTungstate/gobletcell/female/mst_gobletcellmeta_female_multi2.(d)
        Gnuplot Plotting File:
C:/USEPA/PTV/NaTungstate/gobletcell/female/mst_gobletcellmeta_female_multi2.plt
                                          Mon Jul 14 08:03:43 2014
 ================================================================
 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   The form of the probability function is:

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

   The parameter betas are restricted to be positive

   Dependent variable = Effect
   Independent variable = Dose

 Total number of observations = 5
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2
```

```
 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
                        Beta(1) =           0
                        Beta(2) = 6.32551e+015

           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -Background    -Beta(1)
                 have been estimated at a boundary point, or have been specified by
the user, and do not appear in the correlation matrix )

                Beta(2)

   Beta(2)           1

                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0           *               *                   *
        Beta(1)                0           *               *                   *
        Beta(2)      0.000264865           *               *                   *

* - Indicates that this value is not calculated.

                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -11.7341         5
   Fitted model         -12.0279         1      0.587544      4          0.9644
  Reduced model         -34.2965         1       45.1247      4         <.0001

           AIC:          26.0558

                                Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000      10.000        0.000
    6.0000     0.0095         0.095     0.000      10.000       -0.310
   47.0000     0.4429         4.429     4.000      10.000       -0.273
   78.0000     0.8004         8.004     8.000      10.000       -0.003
  125.0000     0.9841         9.841    10.000      10.000        0.403

 Chi^2 = 0.33      d.f. = 4        P-value = 0.9876

   Benchmark Dose Computation

Specified effect =           0.1
Risk Type        =      Extra risk
Confidence level =          0.95

             BMD =        19.9447

            BMDL =        8.14146

            BMDU =        25.1421
Taken together, (8.14146, 25.1421) is a 90     % two-sided confidence
interval for the BMD
```

For the male rat renal cortical regeneration incidence data, all models, except for the multistage 1- and 2-degree models, provided adequate fit to the data. BMDLs for models providing adequate fit were sufficiently close (differed by less than two- to three-fold), so the model with the lowest AIC was selected (Weibull).

Table D-3. Modeling Results for Incidence Data for Mild to Severe Cortical Tubule Regeneration in Kidneys of Male Sprague-Dawley Rats Exposed to Sodium Tungstate in Water by Gavage for 90 Days[a]

Model	DF	χ^2	χ^2 Goodness of Fit *p*-Value[b]	Scaled Residuals[c]	AIC	BMD_{10} (mg W/kg-d)	$BMDL_{10}$ (mg W/kg-d)
Gamma[d]	4	1.9	0.75	−0.91	11.29	67.19	57.00
Logistic	3	0	1	0	10.28	77.80	64.20
LogLogistic[e]	4	0.02	1	−0.02	8.31	77.41	66.67
LogProbit[e]	3	0	1	0	10.28	77.68	66.53
Multistage (1-degree)[f]	4	15.07	0.005	−0.62	30.57	16.60	10.35
Multistage (2-degree)[f]	4	8.84	0.07	−1.40	22.04	36.09	25.26
Multistage (3-degree)[f]	4	5.3	0.26	−1.00	16.60	48.71	36.48
Multistage (4-degree)[f]	4	3.06	0.55	−0.69	13.05	57.54	44.38
Probit	3	0	1	0	10.28	77.60	63.48
Weibull[d]	**4**	**0**	**1**	**0**	**8.28**	**77.52**	**61.55**

[a]USACHPPM (2007a); USACHPPM (2007b)
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals for dose group near BMD.
[d]Power restricted to ≥1.
[e]Slope restricted to ≥1.
[f]Betas restricted to ≥0.

The BMDS output for the selected model (Weibull) follows.

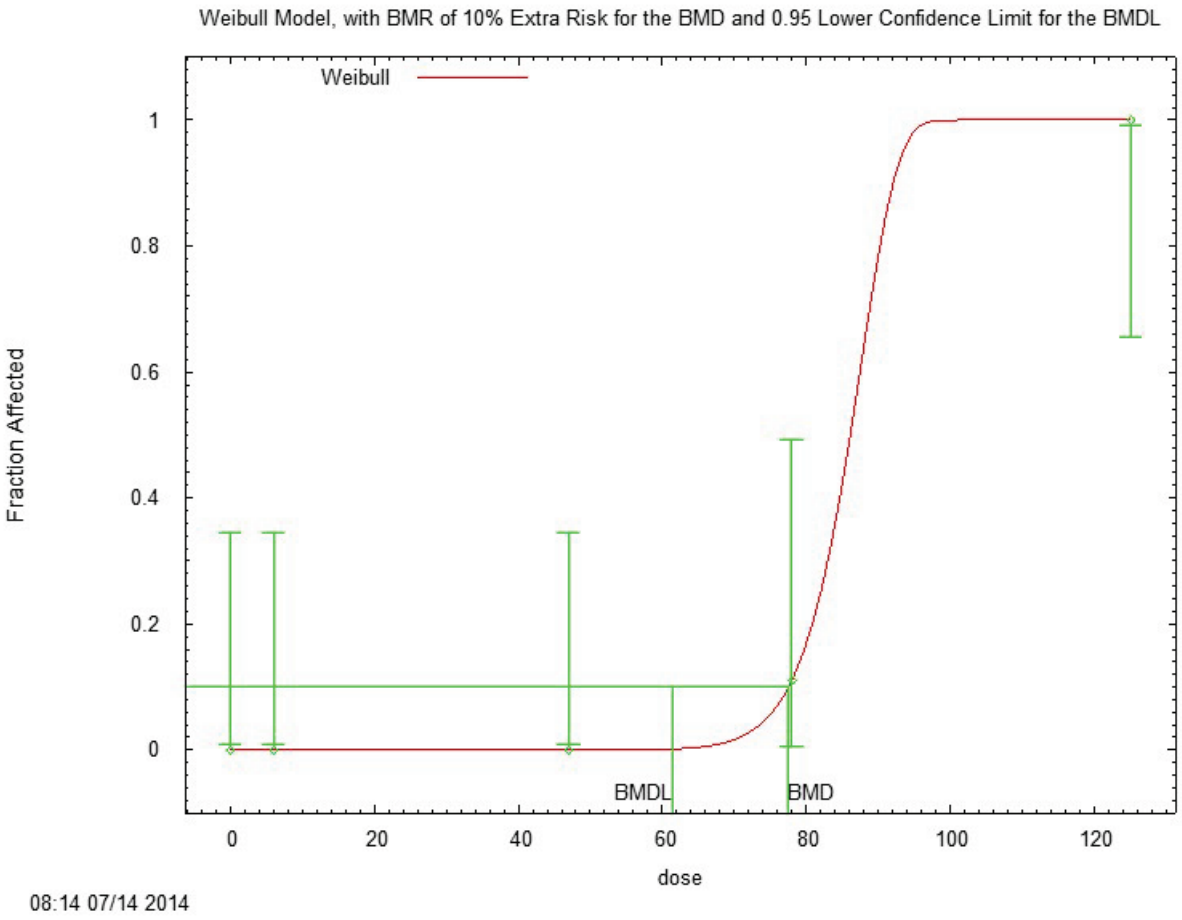

Figure D-3. Weibull (Renal Cortical Tubule Regeneration, Male Rat)

```
 ======================================================================
        Weibull Model using Weibull Model (Version: 2.16;  Date: 2/28/2013)
        Input Data File:
C:/USEPA/PTV/NaTungstate/corttubregen/male/wei_corttubregen_male_Wei-BMR10-Restrict.(d
)
        Gnuplot Plotting File:
C:/USEPA/PTV/NaTungstate/corttubregen/male/wei_corttubregen_male_Wei-BMR10-Restrict.pl
t
                                                Mon Jul 14 08:14:53 2014
 ======================================================================
 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   Dependent variable = Effect
   Independent variable = Dose
   Power parameter is restricted as power >= 1.000000

   Total number of observations = 5
   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 =    0.0833333
                          Slope = 2.25391e-012
                          Power =      5.73553

           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

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0              NA
          Slope     1.03115e-035          1.#QNAN             1.#QNAN
1.#QNAN
          Power               18              NA

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        -3.13949         5
   Fitted model        -3.13962         1   0.000258311      4              1
  Reduced model        -26.0941         1       45.9092      4         <.0001

           AIC:         8.27924

                                  Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          10        0.000
    6.0000     0.0000         0.000     0.000          10        0.000
   47.0000     0.0000         0.000     0.000          10       -0.011
   78.0000     0.1111         1.000     1.000           9        0.000
  125.0000     1.0000        10.000    10.000          10        0.000

 Chi^2 = 0.00      d.f. = 4        P-value = 1.0000

   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =        77.5191

            BMDL =       61.5537
```

For the female kidney lesion incidence data, all models, except for the multistage 1-degree model, provided adequate fit to the data. BMDLs for models providing adequate fit were sufficiently close (differed by less than two- to three-fold), so the model with the lowest AIC was selected (Gamma).

Table D-4. Modeling Results for Incidence Data for Mild to Severe Cortical Tubule Regeneration in Kidneys of Female Sprague-Dawley Rats Exposed to Sodium Tungstate in Water by Gavage for 90 Days[a]

Model	DF	χ^2	χ^2 Goodness of Fit *p*-Value[b]	Scaled Residuals[c]	AIC	BMD_{10} (mg W/kg-d)	$BMDL_{10}$ (mg W/kg-d)
Gamma[d]	**4**	**0.07**	**1.00**	**−0.19**	**18.60**	**75.93**	**58.56**
Logistic	3	0.11	0.99	0.16	20.70	80.08	58.45
LogLogistic[e]	3	0.02	1.00	0.05	20.55	78.50	59.22
LogProbit[e]	3	0	1.00	0.01	20.51	78.07	59.72
Multistage (1-degree)[f]	4	9.99	0.04	−0.53	31.27	23.08	13.85
Multistage (2-degree)[f]	4	5.04	0.28	−1.13	25.25	43.96	29.41
Multistage (3-degree)[f]	4	2.4	0.66	−0.78	21.76	57.03	41.00
Multistage (4-degree)[f]	4	0.94	0.92	−0.67	19.80	66.34	48.55
Probit	3	0.04	1.00	0.08	20.57	78.94	57.61
Weibull[d]	3	0.06	1.00	0.10	20.61	79.30	56.87

[a]USACHPPM (2007a); USACHPPM (2007b)
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals for dose group near BMD.
[d]Power restricted to ≥1.
[e]Slope restricted to ≥1.
[f]Betas restricted to ≥0.

The BMDS output for the selected model (Gamma) follows.

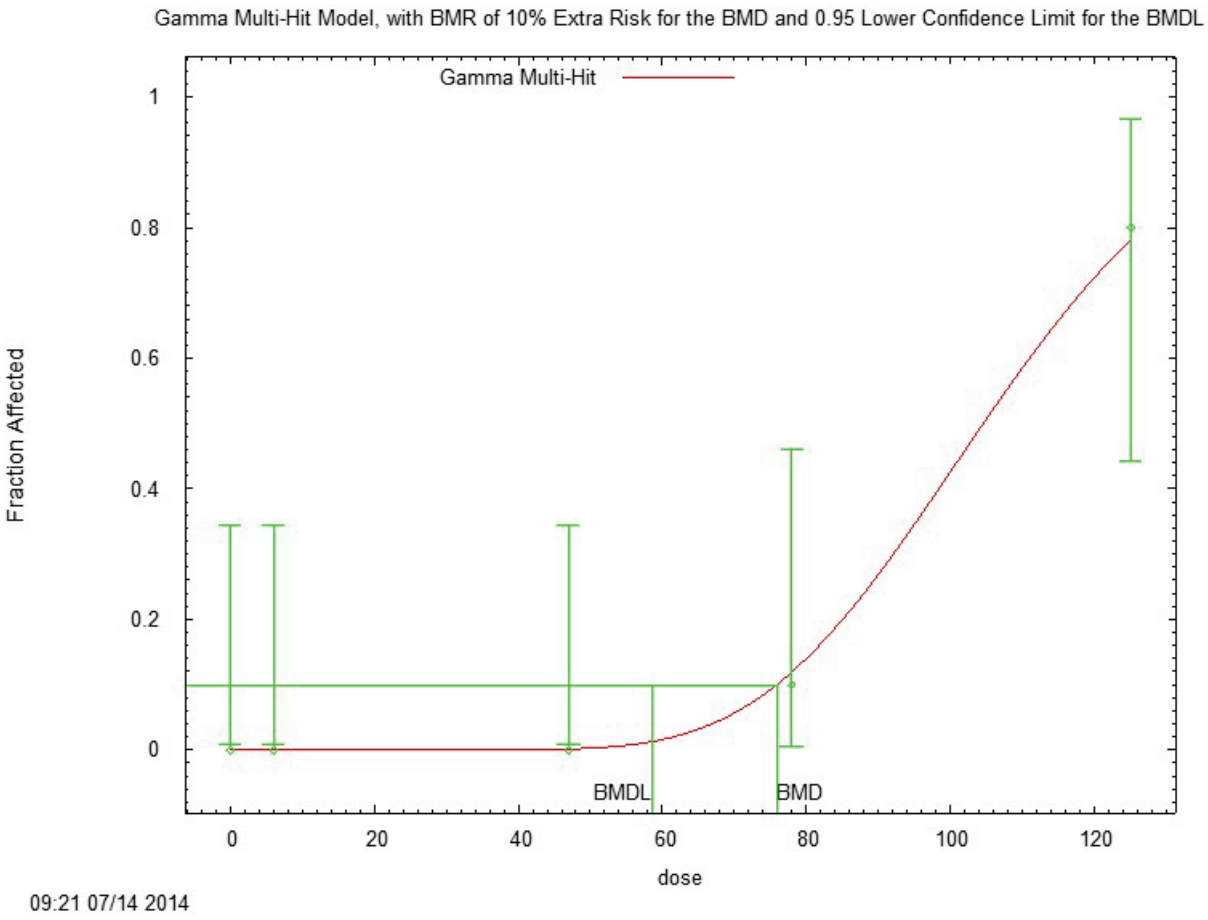

Figure D-4. Gamma (Renal Cortical Tubule Regeneration, Female Rat)

```
 ================================================================
        Gamma Model. (Version: 2.16;  Date: 2/28/2013)
        Input Data File:
C:/USEPA/PTV/NaTungstate/corttubregen/female/gam_corttubregen_female_Gam-BMR10-Restric
t.(d)
        Gnuplot Plotting File:
C:/USEPA/PTV/NaTungstate/corttubregen/female/gam_corttubregen_female_Gam-BMR10-Restric
t.plt
                                          Mon Jul 14 09:21:29 2014
 ================================================================
 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cummulative Gamma distribution function

   Dependent variable = Effect
   Independent variable = Dose
   Power parameter is restricted as power >=1

   Total number of observations = 5
   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 =    0.0833333
                         Slope =     0.141027
                         Power =      15.4879


           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

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0              NA
          Slope         0.168864        0.0135125             0.14238
0.195348
          Power               18              NA

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        -8.25485         5
   Fitted model        -8.30002         1     0.0903344      4           0.999
  Reduced model        -23.5697         1       30.6296      4         <.0001

           AIC:            18.6

                                Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          10        0.000
    6.0000     0.0000         0.000     0.000          10       -0.000
   47.0000     0.0015         0.015     0.000          10       -0.121
   78.0000     0.1192         1.192     1.000          10       -0.187
  125.0000     0.7799         7.799     8.000          10        0.154

 Chi^2 = 0.07      d.f. = 4        P-value = 0.9993

   Benchmark Dose Computation

Specified effect =           0.1
Risk Type        =      Extra risk
Confidence level =          0.95

             BMD =        75.9286

            BMDL =       58.5642
```

For the male rat body-weight data, all constant variance models provided adequate fits to the variance and means (see Table D-5). BMDLs for models providing adequate fit were sufficiently close (differed by less than two- to three-fold), so the model with the lowest AIC was selected (2-degree Polynomial).

Table D-5. Modeling Results for Terminal Body-Weight Data in Male Sprague Dawley Rats Exposed to Sodium Tungstate in Water by Gavage for 90 Days[a]

Model	Test for Significant Difference *p*-Value[b]	Variance *p*-Value[c]	Means *p*-Value[c]	Scaled Residuals[d]	AIC	BMD_{10} (mg W/kg-d)	$BMDL_{10}$ (mg W/kg-d)
Constant variance							
Exponential (Model 2)[e]	0.005	0.15	0.44	0.77	433.63	100.78	69.57
Exponential (Model 3)[e]	0.005	0.15	0.66	−0.03	433.80	108.40	79.57
Exponential (Model 4)[e]	0.005	0.15	0.44	0.77	433.63	100.78	68.37
Exponential (Model 5)[e]	0.005	0.15	0.36	−0.03	435.80	108.40	79.57
Hill[e]	0.005	0.15	0.36	−0.03	435.80	108.57	79.71
Linear[f]	0.005	0.15	0.48	0.76	433.44	100.34	71.30
Polynomial (2-degree)[f]	**0.005**	**0.15**	**0.84**	**−0.04**	**431.80**	**108.23**	**91.92**
Polynomial (3-degree)[f]	0.005	0.15	0.66	−0.02	433.79	108.91	91.93
Polynomial (4-degree)[f]	0.005	0.15	0.66	−0.01	433.79	109.23	91.94
Power[e]	0.005	0.15	0.66	−0.03	433.79	108.67	79.80

[a]USACHPPM (2007a); USACHPPM (2007b)
[b]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[d]Scaled residuals for dose group near the BMD.
[e]Power restricted to ≥1.
[f]Coefficients restricted to be negative.

The BMDS output for the selected model (2-degree polynomial) follows.

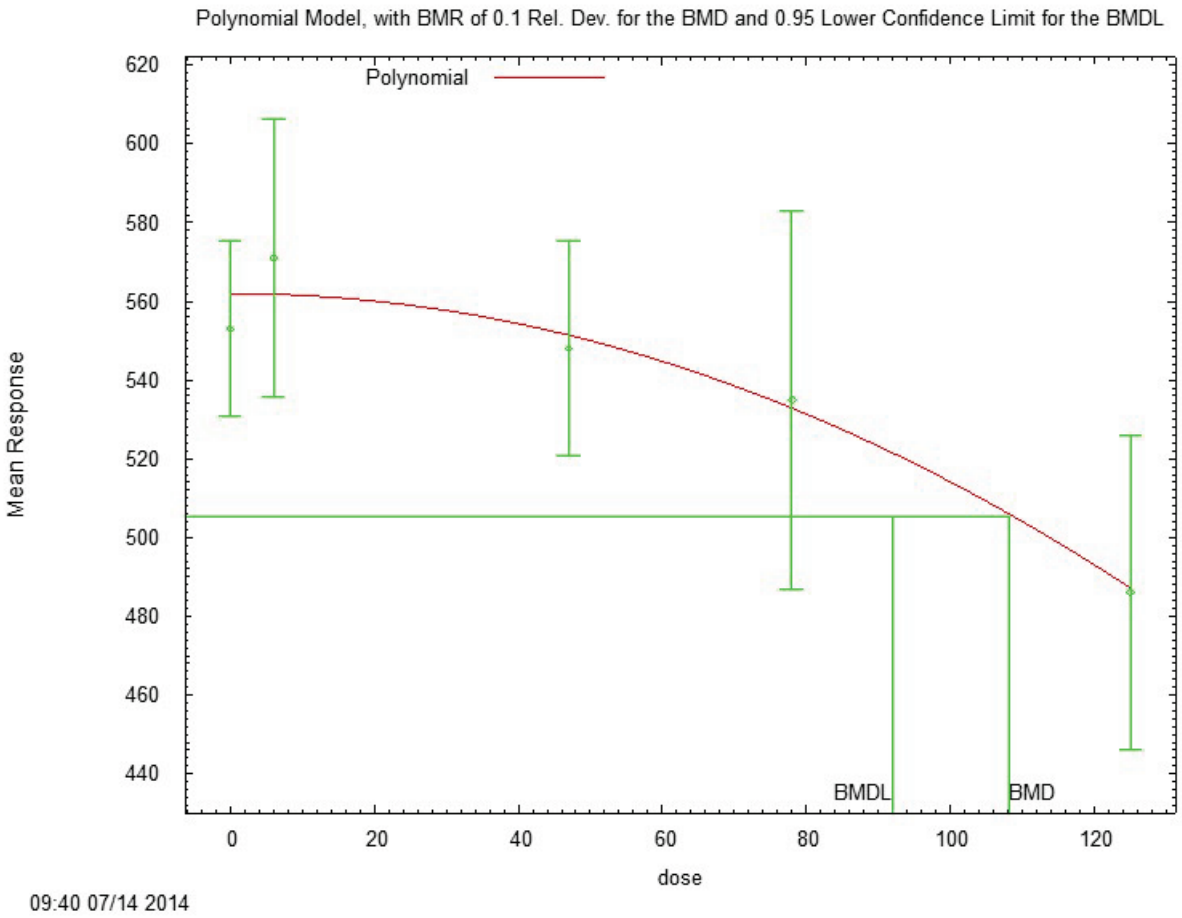

Figure D-5. Constant Variance Polynomial (2-Degree) (Body Weight, Male Rat)

```
 ======================================================================
        Polynomial Model. (Version: 2.18;  Date: 05/19/2014)
        Input Data File:
C:/USEPA/PTV/NaTungstate/terminalbdwt/ply_termbdwt_male_Ply2-ConstantVariance-BMR1Std-
RestrictUp.(d)
        Gnuplot Plotting File:
C:/USEPA/PTV/NaTungstate/terminalbdwt/ply_termbdwt_male_Ply2-ConstantVariance-BMR1Std-
RestrictUp.plt
                                              Mon Jul 14 09:40:08 2014
 ======================================================================

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

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   The polynomial coefficients are restricted to be negative
   A constant variance model is fit

   Total number of dose groups = 5
   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 Parameter Values
                          alpha =            1
                            rho =            0   Specified
                         beta_0 =      560.877
                         beta_1 =            0
                         beta_2 =  -0.00516693

           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho    -beta_1
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                   alpha       beta_0       beta_2

     alpha             1      -3e-008    -5.1e-009

    beta_0      -3e-008            1        -0.63

    beta_2    -5.1e-009        -0.63            1

                                Parameter Estimates

                                                                  95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
          alpha          2185.79          441.596             1320.28
3051.3
         beta_0          561.581          8.55925             544.805
578.357
         beta_1    -2.78654e-026               NA
         beta_2      -0.00479406
0.00117138         -0.00708991         -0.00249821

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

     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   -----------

    0    10        553          562           31         46.8          -0.58
    6    10        571          561           49         46.8          0.649
   47    10        548          551           38         46.8         -0.202
   78    10        535          532           67         46.8          0.175
  125     9        486          487           52         46.8        -0.0432

 Model Descriptions for likelihoods calculated


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

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2
```

```
 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

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

                     Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1         -212.479311            6     436.958622
             A2         -209.104822           10     438.209645
             A3         -212.479311            6     436.958622
          fitted        -212.898456            3     431.796911
              R         -220.102427            2     444.204855

                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              21.9952          8        0.004925
   Test 2              6.74898          4          0.1498
   Test 3              6.74898          4          0.1498
   Test 4             0.838289          3          0.8403

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

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

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

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

             Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =     Relative deviation

Confidence level =          0.95

             BMD =        108.232


            BMDL =        91.9183
```

For the mouse total bone marrow cell count data, with constant variance model applied, all of the models provided an adequate fit to the variance and all of the models except for the Exponential (Model 5) provided an adequate fit to the means. BMDLs for models providing adequate fit were sufficiently close (differed by less than two- to three-fold), so the model with the lowest AIC was selected (3-degree Polynomial).

Table D-6. Modeling Results for Total Bone Marrow Cell Counts in Male C57BL/6J Mice Exposed to Sodium Tungstate in Drinking Water for 16 Weeks[a]

Model	Test for Significant Difference *p*-Value[b]	Variance *p*-Value[c]	Means *p*-Value[c]	Scaled Residuals[d]	AIC	BMD_{1SD} (mg W/kg-d)	$BMDL_{1SD}$ (mg W/kg-d)
Constant variance							
Exponential (Model 2)[e]	0.01	0.94	0.36	1.15	119.79	95.54	63.22
Exponential (Model 3)[e]	0.01	0.94	0.60	2.16×10^{-08}	120.04	226.89	73.53
Exponential (Model 4)[e]	0.01	0.94	0.36	1.15	119.79	95.54	49.41
Exponential (Model 5)[e]	0.01	0.94	NA	-2.3×10^{-08}	122.04	228.73	50.14
Hill[e]	0.01	0.94	0.60	-7.15×10^{-06}	120.04	228.80	50.36
Linear[f]	0.01	0.94	0.41	1.07	119.56	101.07	69.69
Polynomial (2-degree)[f]	0.01	0.94	0.80	−0.02	118.21	157.24	76.72
Polynomial (3-degree)[f]	**0.01**	**0.94**	**0.86**	**−0.003**	**118.06**	**183.72**	**77.75**
Power[e]	0.01	0.94	0.60	-5.08×10^{-08}	120.04	229.80	77.97

[a]Kelly et al. (2013)
[b]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[d]Scaled residuals for dose group near the BMD.
[e]Power restricted to ≥1.
[f]Coefficients restricted to be negative.

NA = not applicable (BMDL computation failed or the BMD was higher than the highest dose tested).

The BMDS output for the selected model (3-degree polynomial) follows.

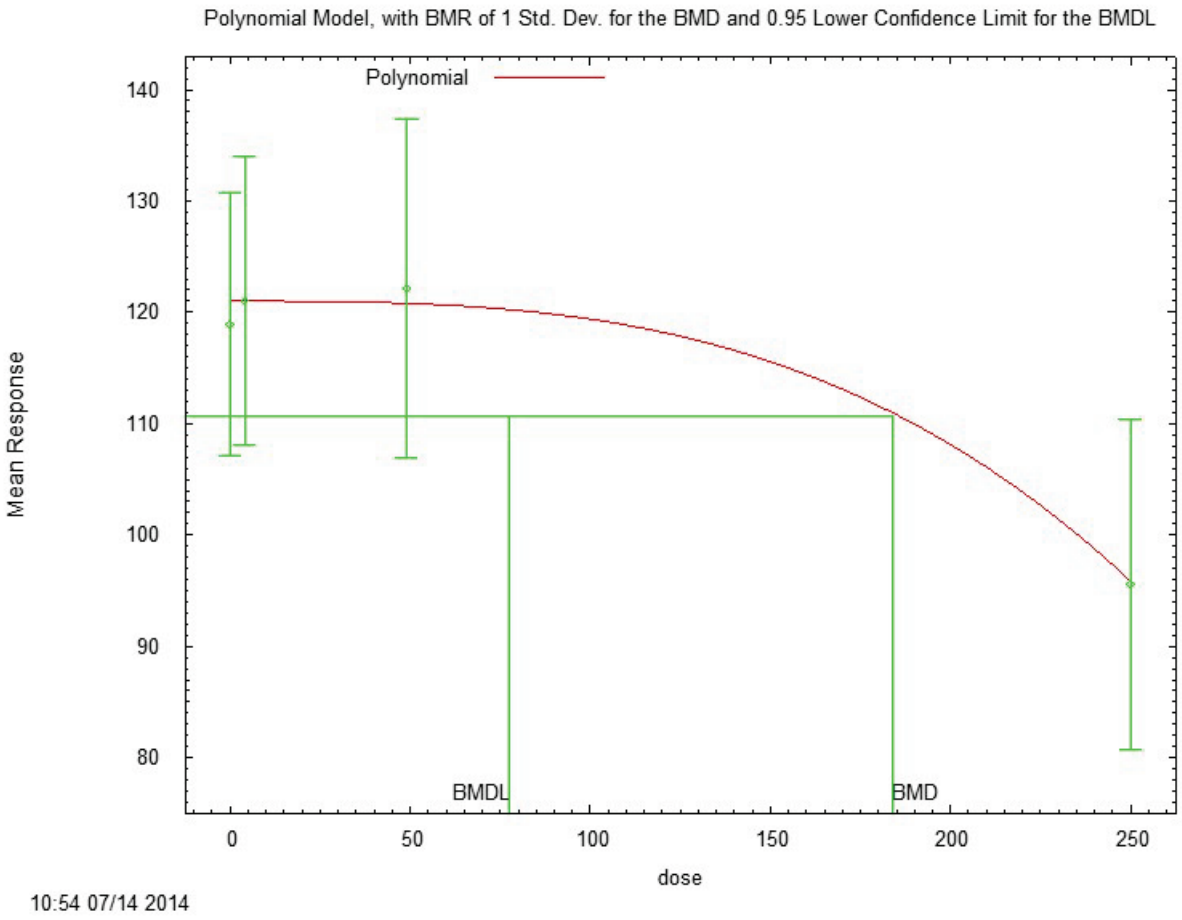

Figure D-6. Constant Variance Polynomial (3-Degree) (Total Bone Marrow Cell Counts, Male Mice)

```
================================================================
        Polynomial Model. (Version: 2.18;  Date: 05/19/2014)
        Input Data File:
C:/USEPA/PTV/NaTungstate/bonemarrowcellct/ply_bonemarrowcellct_Ply3-ConstantVariance-B
MR1Std-RestrictUp.(d)
        Gnuplot Plotting File:
C:/USEPA/PTV/NaTungstate/bonemarrowcellct/ply_bonemarrowcellct_Ply3-ConstantVariance-B
MR1Std-RestrictUp.plt
                                          Mon Jul 14 10:54:58 2014
 ================================================================

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

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   The polynomial coefficients are restricted to be negative
   A constant variance model is fit
```

```
   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                          alpha =      122.889
                            rho =            0   Specified
                         beta_0 =       118.89
                         beta_1 =            0
                         beta_2 =   -0.0124316
                         beta_3 =            0

           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho    -beta_1    -beta_2
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                   alpha       beta_0       beta_3

     alpha             1     2.5e-007    -1.8e-007

    beta_0     2.6e-007            1         -0.5

    beta_3    -1.8e-007         -0.5            1

                                Parameter Estimates

                                                                  95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
          alpha          99.8021          31.5602             37.9452
161.659
         beta_0          120.736          2.58593             115.668
125.804
         beta_1               -0              NA
         beta_2    -2.74523e-024              NA
         beta_3    -1.61114e-006
3.3099e-007       -2.25987e-006       -9.62413e-007

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

     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     5        119          121         9.51         9.99         -0.413
    4     5        121          121         10.4         9.99         0.0636
   49     5        122          121         12.2         9.99          0.352
  250     5       95.5         95.6           12         9.99       -0.00265

 Model Descriptions for likelihoods calculated

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

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

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

 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

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

                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1          -55.881351            5     121.762702
             A2          -55.677704            8     127.355408
             A3          -55.881351            5     121.762702
         fitted          -56.031890            3     118.063780
              R          -63.846659            2     131.693318

                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              16.3379          6         0.01205
   Test 2             0.407294          3          0.9387
   Test 3             0.407294          3          0.9387
   Test 4             0.301078          2          0.8602

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

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

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

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

             Benchmark Dose Computation

Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        183.715


            BMDL =        77.7506
```

The 28-day percentage CD71+ helper T cell data sets were amenable to BMD modeling (see Tables D-7). With constant variance model applied, none of the models provided an adequate fit to the variance. After applying the model for nonconstant variance, all models provided an adequate fit to the means, though the BMDL computation failed for several models. BMDLs for models providing adequate fit were sufficiently close (differed by less than two- to three-fold), so the model with the lowest AIC was selected (Exponential [Models 2 and 3] and Power all yielded the same results).

Table D-7. Modeling Results for Percentage CD71+ Helper T Cells in the Spleen of Adult C57BL/6J Mice Exposed to Sodium Tungstate in Drinking Water for 28 Days[a]

Model	Test for Significant Difference *p*-Value[b]	Variance *p*-Value[c]	Means *p*-Value[c]	Scaled Residuals[d]	AIC	BMD_{1SD} (mg W/kg-d)	$BMDL_{1SD}$ (mg W/kg-d)
Constant variance							
Exponential (Model 2)[e]	0.001	0.0005	0.84	0.03	144.35	183.65	74.18
Exponential (Model 3)[e]	0.001	0.0005	0.84	0.03	144.35	183.65	74.18
Exponential (Model 4)[e]	0.001	0.0005	0.61	0.00	146.27	NA	NA
Exponential (Model 5)[e]	0.001	0.0005	0.61	0.00	146.27	NA	NA
Hill[e]	0.001	0.0005	0.63	−0.18	146.24	342.58	0.00001
Linear[f]	0.001	0.0005	0.80	0.07	144.44	168.11	89.53
Polynomial (2-degree)[f]	0.001	0.0005	0.80	0.07	144.44	168.11	89.53
Polynomial (3-degree)[f]	0.001	0.0005	0.80	0.07	144.44	168.11	89.53
Power[e]	0.001	0.0005	0.80	0.07	144.44	168.11	89.53
Nonconstant variance							
Exponential (Model 2)[e]	***0.001***	***0.33***	***0.19***	***−0.05***	***133.68***	***333.62***	***118.36***
Exponential (Model 3)[e]	0.001	0.33	0.19	−0.05	133.68	333.62	118.36
Exponential (Model 4)[e]	0.001	0.33	0.73	0	132.43	NA	NA
Exponential (Model 5)[e]	0.001	0.33	NA	0	134.32	NA	NA
Hill[e]	0.001	0.33	NA	0	134.32	NA	NA
Linear[g]	0.001	0.33	0.11	−0.17	134.69	266.99	130.94
Polynomial (2-degree)[f]	0.001	0.33	0.11	−0.17	134.69	266.99	130.94
Polynomial (3-degree)[f]	0.001	0.33	0.11	−0.17	134.69	266.99	130.94
Polynomial (4-degree)[f]	0.001	0.33	0.11	−0.17	134.69	266.99	130.94
Power[e]	0.001	0.33	0.19	−0.05	133.68	333.62	118.36

[a]Osterburg et al. (2014)
[b]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[d]Scaled residuals for dose group near the BMD.
[e]Power restricted to ≥1.
[f]Coefficients restricted to be negative.

NA = not applicable (BMDL computation failed or the BMD was higher than the highest dose tested).

The BMDS output for the selected model follows.

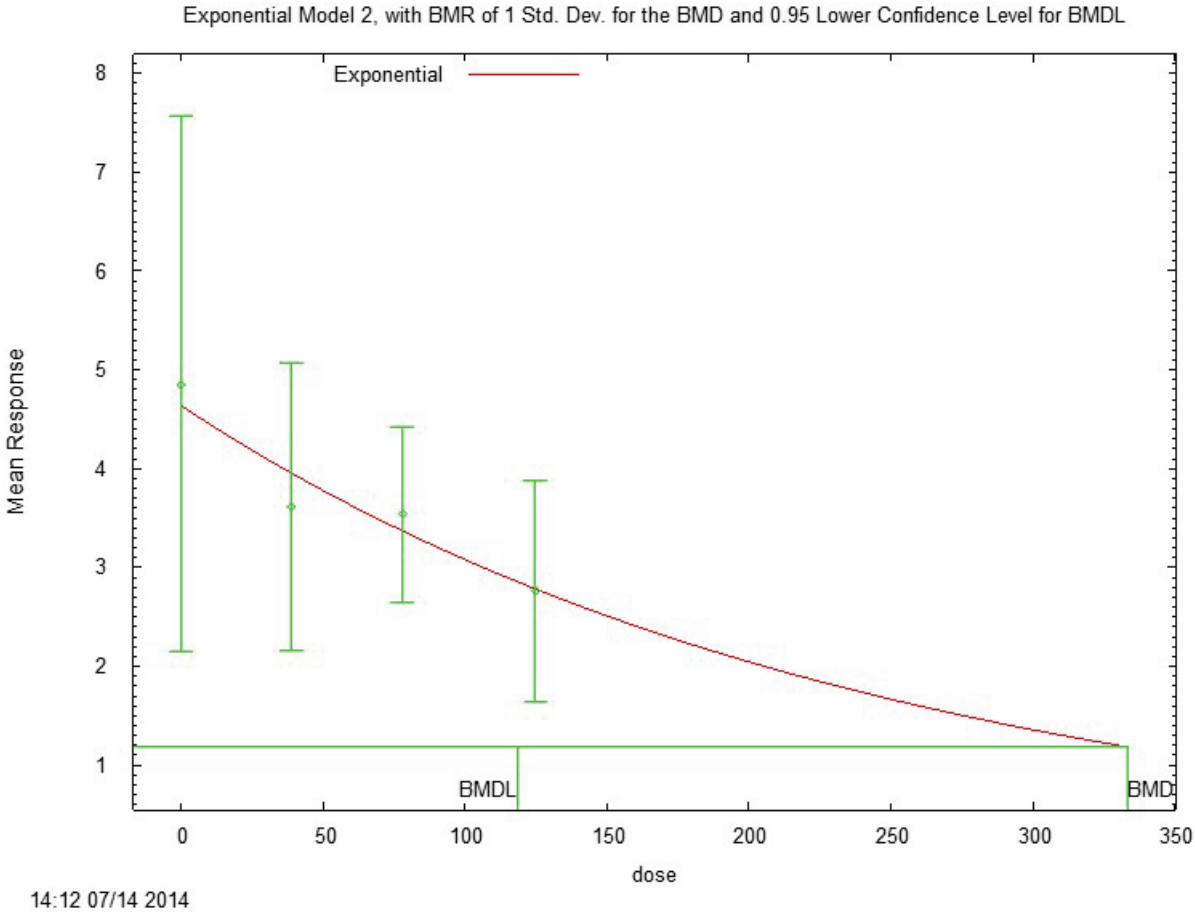

Figure D-7. Nonconstant Variance Exponential Model 2 (Percentage CD71+ Helper T Cells, Mice, 28 Days, Drinking Water)

```
================================================================
        Exponential Model. (Version: 1.9;  Date: 01/29/2013)
        Input Data File:
C:/USEPA/PTV/NaTungstate/Tcell/adult28day/exp_Tcell_adult_28day_Exp-ConstantVariance-B
MR1Std-Up.(d)
        Gnuplot Plotting File:
                                              Mon Jul 14 14:12:37 2014
 ================================================================

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

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

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

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

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

              Initial Parameter Values

              Variable          Model 2
              --------          --------
                lnalpha            -2.63791
                    rho             3.28816
                      a             2.81592
                      b           0.00413591
                      c                    0
                      d                    1

                 Parameter Estimates

               Variable          Model 2
               --------          -------
                lnalpha          -2.85622
                    rho           3.47741
                      a           4.63872
                      b        0.00409388
                      c                 0
                      d                 1

  NC = No Convergence

        Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
      0     12         4.85         4.26
     39     12         3.61         2.29
     78     12         3.54         1.39
    125     12         2.76         1.77

              Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
      0         4.639        3.455           0.2118
     39         3.954        2.618          -0.4555
     78         3.371        1.983           0.2958
    125         2.781        1.419         -0.05053

Other models for which likelihoods are calculated:

  Model A1:        Yij = Mu(i) + e(ij)
```

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

  Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

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

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

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-69.00187	5	148.0037
A2	-60.04898	8	136.098
A3	-61.15947	6	134.3189
R	-70.97856	2	145.9571
2	-62.84106	4	133.6821

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

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	21.86	6	0.001284
Test 2	17.91	3	0.00046
Test 3	2.221	2	0.3294
Test 4	3.363	2	0.1861

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

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

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

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

Benchmark Dose Computations:

Specified Effect = 1.000000

Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD = 333.62

```
BMDL =      118.363
```

The 19-week (F0 mice) percentage of CD71+ helper T cells data sets were not amenable to modeling, because the available constant-variance and nonconstant-variance models did not provide adequate fits to the variances (see Table D-8).

Table D-8. Modeling Results for Percentage of CD71+ Helper T Cells in the Spleen of F0 C57BL/6J Exposed to Sodium Tungstate in Drinking Water for ~19 Weeks[a]

Model	Test for Significant Difference *p*-Value[b]	Variance *p*-Value[c]	Means *p*-Value[c]	Scaled Residuals[d]	AIC	BMD_{1SD} (mg W/kg-d)	$BMDL_{1SD}$ (mg W/kg-d)
Constant variance							
Exponential (Model 2)[e]	0.0001	0.0002	0.80	0.78	89.64	74.46	38.58
Exponential (Model 3)[e]	0.0001	0.0002	0.65	0.66	91.50	82.99	39.17
Exponential (Model 4)[e]	0.0001	0.0002	0.80	0.78	89.64	74.46	24.04
Exponential (Model 5)[e]	0.0001	0.0002	0.35	0.66	93.50	82.99	39.17
Hill[e]	0.0001	0.0002	0.40	0.58	93.34	84.80	52.13
Linear	0.0001	0.0002	0.87	0.65	89.36	81.07	52.18
Polynomial (2-degree)[f]	0.0001	0.0002	0.72	0.51	91.29	87.38	52.42
Polynomial (3-degree)[f]	0.0001	0.0002	0.74	0.42	91.23	90.30	52.64
Polynomial (4-degree)[f]	0.0001	0.0002	0.76	0.37	91.18	92.47	52.82
Power[e]	0.0001	0.0002	0.70	0.58	91.34	84.83	52.25
Nonconstant variance							
Exponential (Model 2)[e]	0.0001	0.001	0.07	0.73	91.57	69.74	30.79
Exponential (Model 3)[e]	0.0001	0.001	0.07	0.73	91.57	69.74	30.79
Exponential (Model 4)[e]	0.0001	0.001	NA	0.68	85.25	4.75	0.77
Exponential (Model 5)[e]	0.0001	0.001	0.26	0.04	89.74	119.30	93.36
Hill[e]	0.0001	0.001	0.54	−0.51	87.71	95.10	82.91
Linear[f]	0.0001	0.001	0.09	0.79	90.88	87.08	53.80
Polynomial (2-degree)[f]	0.0001	0.001	0.44	0.13	87.19	111.67	90.86
Polynomial (3-degree)[f]	0.0001	0.001	0.67	0.08	86.04	117.50	95.67

Polynomial (4-degree)[f]	0.0001	0.001	0.73	0.05	85.78	119.86	109.07
Power[e]	0.0001	0.001	0.53	0.04	87.75	120.77	95.89

[a]Osterburg et al. (2014)
[b]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[d]Scaled residuals for dose group near the BMD.
[e]Power restricted to ≥1.
[f]Coefficients restricted to be negative.

NA = not applicable (BMDL computation failed or the BMD was higher than the highest dose tested).

The 32-week (F1 mice) percentage of CD71+ helper T cells data sets were not amenable to modeling, because the available constant-variance and nonconstant-variance models did not provide adequate fits to the variances (see Table D-9).

Table D-9. Modeling Results for Percentage of CD71+ Helper T Cells in the Spleen of F1 C57BL/6J Exposed to Sodium Tungstate in Drinking Water for ~32 Weeks[a]

Model	Test for Significant Difference *p*-Value[b]	Variance *p*-Value[c]	Means *p*-Value[c]	Scaled Residuals[d]	AIC	BMD_{1SD} (mg W/kg-d)	$BMDL_{1SD}$ (mg W/kg-d)
Constant variance							
Exponential (Model 2)[e]	<0.0001	0.46	<0.0001	1.621	69.41	84.55	44.20
Exponential (Model 3)[e]	<0.0001	0.46	<0.0001	1.621	69.41	84.55	44.20
Exponential (Model 4)[e]	<0.0001	0.46	0.009	-3.70×10^{-07}	58.03	0.32	0.00
Exponential (Model 5)[e]	<0.0001	0.46	0.002	1.13×10^{-07}	60.03	0.56	0.00
Hill[e]	<0.0001	0.46	0.002	-1.72×10^{-07}	60.03	0.79	0.00
Linear[f]	<0.0001	0.46	<0.0001	1.47	69.64	93.61	57.54
Polynomial (2-degree)[f]	<0.0001	0.46	<0.0001	1.47	69.64	93.61	57.54
Polynomial (3-degree)[f]	<0.0001	0.46	<0.0001	1.47	69.64	93.61	57.54
Polynomial (4-degree)[f]	<0.0001	0.46	<0.0001	1.4	71.62	96.67	57.59
Power[e]	<0.0001	0.46	<0.0001	1.47	69.64	93.61	57.54
Nonconstant variance							
Exponential (Model 2)[e]	<0.0001	0.66	<0.0001	−0.52	69.28	108.34	53.81
Exponential (Model 3)[e]	<0.0001	0.66	<0.0001	−0.52	69.28	108.34	53.81
Exponential (Model 4)[e]	<0.0001	0.66	0.0043	−0.01	59.54	0.36	0.003
Exponential (Model 5)[e]	<0.0001	0.66	0.0010	−0.01	61.54	0.49	0.003
Hill[e]	<0.0001	0.66	0.0010	0.13	61.54	0.82	1.25×10^{-13}
Linear[f]	<0.0001	0.66	<0.0001	−0.40	69.27	111.00	66.71
Polynomial (2-degree)[f]	<0.0001	0.66	<0.0001	−0.40	69.27	111.00	66.71
Polynomial (3-degree)[f]	<0.0001	0.66	<0.0001	−0.28	71.19	113.29	67.15

Polynomial (4-degree)[f]	<0.0001	0.66	<0.0001	−0.21	71.07	114.45	67.83
Power[e]	<0.0001	0.66	<0.0001	−0.40	69.27	111.00	66.71

[a]Osterburg et al. (2014)
[b]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[d]Scaled residuals for dose group near the BMD.
[e]Power restricted to ≥1.
[f]Coefficients restricted to be negative.

For the percentage CD71+ cytotoxic T cell data sets from the 28-day study were amenable to BMD modeling (see Table D-10). With constant variance model applied, none of the models provided an adequate fit to the variance. After applying the model for nonconstant variance, all models provided an adequate fit to the means except for the Exponential Models 3 and 5. BMDLs for models providing adequate fit were sufficiently close (differed by less than two- to three-fold), so the model with the lowest AIC was selected (Hill, with nonconstant variance model applied).

Table D-10. Modeling Results for Percentage CD71+ Cytotoxic T Cells in the Spleen of Adult C57BL/6J Mice Exposed to Sodium Tungstate in Drinking Water for 28 Days[a]

Model	Test for Significant Difference *p*-Value[b]	Variance *p*-Value[c]	Means *p*-Value[c]	Scaled Residuals[d]	AIC	BMD_{1SD} (mg W/kg-d)	$BMDL_{1SD}$ (mg W/kg-d)
Constant variance							
Exponential (Model 2)[e]	0.0004	0.02	0.91	−0.23	216.74	59.78	33.10
Exponential (Model 3)[e]	0.0004	0.02	0.91	−0.23	216.74	59.78	33.10
Exponential (Model 4)[e]	0.0004	0.02	0.86	0.10	218.59	51.83	18.81
Exponential (Model 5)[e]	0.0004	0.02	NA	0.00	220.56	51.73	18.95
Hill[e]	0.0004	0.02	NA	0.00	220.56	51.01	12.18
Linear[f]	0.0004	0.02	0.54	−0.62	217.79	82.06	56.46
Polynomial (2-degree)[f]	0.0004	0.02	0.54	−0.62	217.79	82.06	56.46
Polynomial (3-degree)[f]	0.0004	0.02	0.54	−0.62	217.79	82.06	56.46
Power[e]	0.0004	0.02	0.54	−0.62	217.79	82.06	56.46
Nonconstant variance							
Exponential (Model 2)[e]	0.0004	0.11	0.28	−0.32	215.32	83.12	41.32
Exponential (Model 3)[e]	0.0004	0.11	<0.0001	−1.90	235.08	15,535.20	31.77
Exponential (Model 4)[e]	0.0004	0.11	0.19	−0.05	216.55	71.93	27.36
Exponential (Model 5)[e]	0.0004	0.11	NA	−0.31	216.92	56.44	36.86
Hill[e]	**0.0004**	**0.11**	**0.74**	**−0.31**	**214.92**	**49.07**	**39.87**
Linear[f]	0.0004	0.11	0.12	0.36	217.06	106.04	66.23
Polynomial (2-degree)[f]	0.0004	0.11	0.12	0.36	217.06	106.04	66.23

Polynomial (3-degree)[f]	0.0004	0.11	0.12	0.36	217.06	106.04	66.23
Power[e]	0.0004	0.11	0.12	0.36	217.06	106.04	66.23

[a]Osterburg et al. (2014)
[b]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[d]Scaled residuals for dose group near the BMD.
[e]Power restricted to ≥1.
[f]Coefficients restricted to be negative.

NA = not applicable (BMDL computation failed or the BMD was higher than the highest dose tested).

The BMDS output for the selected model follows:

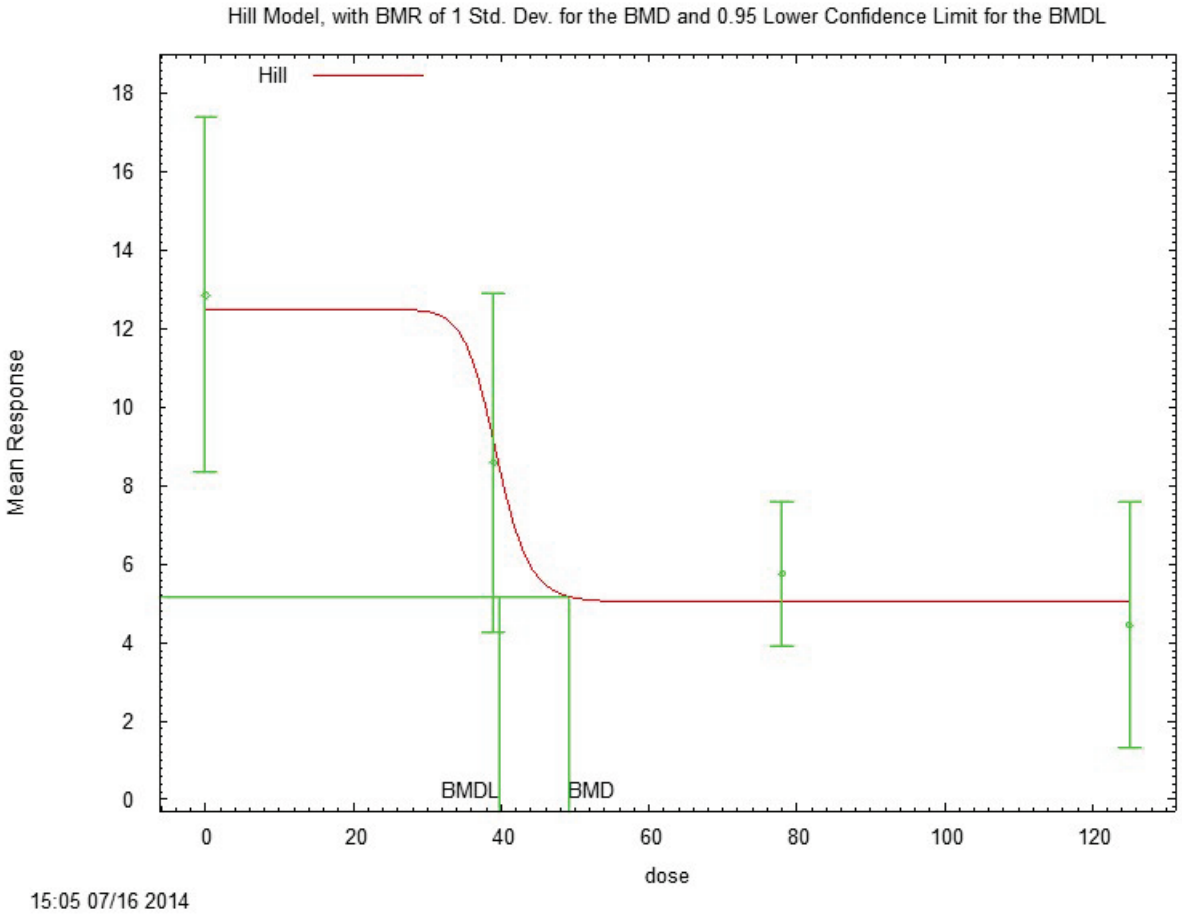

Figure D-8. Nonconstant Variance Hill (Percentage CD71+ Cytotoxic T Cells, Mice, 28 Days, Drinking Water)

```
 ================================================================
        Hill Model. (Version: 2.17;  Date: 01/28/2013)
        Input Data File:
C:/USEPA/PTV/NaTungstate/cytotoxic_Tcell/adult28day/hil_cytotox_Tcell_adult_28day_Hil-
ModelVariance-BMR1Std-Restrict.(d)
```

```
        Gnuplot Plotting File:
C:/USEPA/PTV/NaTungstate/cytotoxic_Tcell/adult28day/hil_cytotox_Tcell_adult_28day_Hil-
ModelVariance-BMR1Std-Restrict.plt
                                                  Wed Jul 16 15:05:32 2014
 ========================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)

   Dependent variable = Mean
   Independent variable = Dose
   Power parameter restricted to be greater than 1
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                 Default Initial Parameter Values
                        lalpha =      3.47363
                           rho =            0
                     intercept =        12.87
                             v =        -8.43
                             n =      11.8827
                             k =      38.4977

           Asymptotic Correlation Matrix of Parameter Estimates

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

                 lalpha          rho    intercept            v            k

    lalpha            1        -0.98         0.35        -0.49        -0.15

       rho        -0.98            1        -0.41         0.52         0.15

 intercept         0.35        -0.41            1        -0.94        -0.54

         v        -0.49         0.52        -0.94            1         0.48

         k        -0.15         0.15        -0.54         0.48            1

                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
        lalpha         0.612805          1.33743            -2.00851
3.23412
           rho          1.33432          0.65039           0.0595841
2.60907
     intercept          12.4839          2.09917             8.36961
16.5982
             v         -7.46343
2.29559             -11.9627            -2.96414
             n               18                NA
```

```
             k          39.4424          2.17384             35.1817
43.703

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

     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    12       12.9         12.5          7.1         7.32          0.183
   39    12        8.6         9.13         6.79         5.94         -0.309
   78    12       5.75         5.02         2.88         3.99          0.634
  125    12       4.44         5.02         4.92         3.99         -0.504

 Model Descriptions for likelihoods calculated

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

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

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

                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1         -105.278932            5     220.557864
             A2         -100.231462            8     216.462923
             A3         -102.403795            6     216.807590
         fitted         -102.458954            5     214.917909
              R         -112.540270            2     229.080539

                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              24.6176          6       0.0004018
   Test 2              10.0949          3         0.01778
   Test 3              4.34467          2          0.1139
   Test 4             0.110319          1          0.7398

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

```
The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

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

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

        Benchmark Dose Computation

Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       49.0739

            BMDL =       39.8748
```

The percentage CD71+ cytotoxic T cell data sets from the 19-week (F0 mice) study were not amenable to modeling, because the available constant-variance and nonconstant-variance models did not provide adequate fits to the variances (see Table D-11).

Table D-11. Modeling Results for Percentage CD71+ Cytotoxic T Cells in the Spleen of F0 C57BL/6J Mice Exposed to Sodium Tungstate in Drinking Water for 19 Weeks[a]

Model	Test for Significant Difference *p*-Value[b]	Variance *p*-Value[c]	Means *p*-Value[c]	Scaled Residuals[d]	AIC	BMD_{1SD} (mg W/kg-d)	$BMDL_{1SD}$ (mg W/kg-d)
Constant variance							
Exponential (Model 2)[e]	<0.0001	<0.0001	0.72	0.79	102.85	42.25	22.76
Exponential (Model 3)[e]	<0.0001	<0.0001	0.62	0.33	104.45	52.56	23.77
Exponential (Model 4)[e]	<0.0001	<0.0001	0.72	0.79	102.85	42.25	19.26
Exponential (Model 5)[e]	<0.0001	<0.0001	0.50	0.0005	105.96	52.43	22.83
Hill[e]	<0.0001	<0.0001	0.50	9.06×10^{-06}	105.96	50.08	20.56
Linear[f]	<0.0001	<0.0001	0.68	−0.87	103.00	60.21	41.93
Polynomial (2-degree)[f]	<0.0001	<0.0001	0.68	−0.87	103.00	60.21	41.93
Polynomial (3-degree)[f]	<0.0001	<0.0001	0.68	−0.87	103.00	60.21	41.93
Polynomial (4-degree)[f]	<0.0001	<0.0001	0.68	−0.87	103.00	60.21	41.93
Power[e]	<0.0001	<0.0001	0.68	−0.87	103.00	60.21	41.93
Nonconstant variance							
Exponential (Model 2)[e]	<0.0001	0.0004	0.02	0.78	104.72	38.97	19.32
Exponential (Model 3)[e]	<0.0001	0.0004	0.74	0.00	97.75	124.35	111.94
Exponential (Model 4)[e]	<0.0001	0.0004	0.03	0.66	104.10	0.96	0.13
Exponential (Model 5)[e]	<0.0001	0.0004	0.44	0.00	99.75	124.35	86.45

Hill[e]	<0.0001	0.0004	0.02	0.31	104.96	39.75	NA
Linear[f]	<0.0001	0.0004	0.04	−0.86	103.57	72.04	NA
Polynomial (2-degree)[f]	<0.0001	0.0004	0.16	0.55	100.37	119.54	94.66
Polynomial (3-degree)[f]	<0.0001	0.0004	0.41	0.26	98.02	124.05	107.52
Polynomial (4-degree)[f]	<0.0001	0.0004	0.62	0.14	96.91	124.28	111.85
Power[e]	<0.0001	0.0004	0.90	0.00	95.75	124.67	115.50

[a]Osterburg et al. (2014)
[b]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[d]Scaled residuals for dose group near the BMD.
[e]Power restricted to ≥1.
[f]Coefficients restricted to be negative.

NA = not applicable (BMDL computation failed or the BMD was higher than the highest dose tested).

The percentage CD71+ cytotoxic T cell data sets from the 32-week (F1 mice) study were not amenable to modeling because none of the constant or nonconstant variance models provided adequate fits to the means (see Table D-12).

Table D-12. Modeling Results for Percentage CD71+ Cytotoxic T Cells in the Spleen of F1 C57BL/6J Mice Exposed to Sodium Tungstate in Drinking Water for 32 Weeks[a]

Model	Test for Significant Difference *p*-Value[b]	Variance *p*-Value[c]	Means *p*-Value[c]	Scaled Residuals[c]	AIC	BMD_{1SD} (mg W/kg-d)	$BMDL_{1SD}$ (mg W/kg-d)
Constant variance							
Exponential (Model 2)[e]	<0.0001	0.21	<0.0001	3.13	56.09	62.07	37.18
Exponential (Model 3)[e]	<0.0001	0.21	<0.0001	3.13	56.09	62.07	37.18
Exponential (Model 4)[e]	<0.0001	0.21	<0.0001	3.13	56.09	62.07	0.46
Exponential (Model 5)[e]	<0.0001	0.21	<0.0001	3.13	58.09	62.07	0.42
Hill[e]	<0.0001	0.21	<0.0001	0.02	56.36	2.40	0.35
Linear[f]	<0.0001	0.21	<0.0001	3.02	55.82	68.19	46.07
Polynomial (2-degree)[f]	<0.0001	0.21	<0.0001	2.77	57.60	78.97	46.70
Polynomial (3-degree)[f]	<0.0001	0.21	<0.0001	2.41	56.73	89.78	49.71
Polynomial (4-degree)[f]	<0.0001	0.21	<0.0001	2.15	55.86	95.91	54.37
Power[e]	<0.0001	0.21	<0.0001	3.02	55.82	68.19	46.07
Nonconstant variance							
Exponential (Model 2)[e]	<0.0001	0.66	<0.0001	−2.65	57.62	53.84	26.91
Exponential (Model 3)[e]	<0.0001	0.66	<0.0001	−0.00016	50.81	119.63	99.51
Exponential (Model 4)[e]	<0.0001	0.66	<0.0001	−0.30	55.28	1.61	0.51
Exponential (Model 5)[e]	<0.0001	0.66	<0.0001	−0.00016	52.81	119.63	98.90
Hill[e]	<0.0001	0.66	<0.0001	−0.35	55.12	1.76	0.39

Linear[f]	<0.0001	0.66	<0.0001	2.95	57.78	66.90	41.52
Polynomial (2-degree)[f]	<0.0001	0.66	<0.0001	2.58	54.60	94.70	80.35
Polynomial (3-degree)[f]	<0.0001	0.66	<0.0001	−0.15	51.60	103.16	89.83
Polynomial (4-degree)[f]	<0.0001	0.66	<0.0001	−0.08	50.26	107.85	100.00
Power[e]	<0.0001	0.66	<0.0001	-8.65×10^{-05}	48.81	120.83	100.81

[a]Osterburg et al. (2014)
[b]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[d]Scaled residuals for dose group near the BMD.
[e]Power restricted to ≥1.
[f]Coefficients restricted to be negative.

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