ADDENDUM TO THE TOXICOLOGICAL PROFILE FOR TUNGSTEN

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Supplement to the 2005 Toxicological Profile for Tungsten

Background Statement

This addendum to the [Toxicological Profile for Tungsten](#) supplements the profile that was released in 2005.

Toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). CERCLA mandates that the Administrator of ATSDR prepare toxicological profiles on substances on the CERCLA Priority List of Hazardous Substances. CERCLA further states that the Administrator will "establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" [Title 42, Chapter 103, Subchapter I, § 9604 (i)(1)(B)].

The purpose of this addendum is to provide to the public and other federal, state, and local agencies a non-peer reviewed supplement of the scientific data that were published in the open peer-reviewed literature since the release of the profile in 2005.

Chapter numbers in this addendum coincide with the [Toxicological Profile for Tungsten](#) (2005). This document should be used in conjunction with the profile. It does not replace it.

3. HEALTH EFFECTS

3.1 INTRODUCTION

Health Effects information in this Addendum for Tungsten does not conflict with conclusions presented in the 2005 Toxicological Profile for Tungsten, including the determination that available data are insufficient for MRL derivation.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

The information in Section 3.2.1 (Inhalation Exposure) may apply to individuals exposed to tungsten in ambient air or airborne tungsten released from tools (e.g., tungsten carbide cutting and grinding wheels) during their use. Some studies (Mendy et al. 2012; Navas-Acien et al. 2005; Tyrrell et al. 2013) evaluated relationships between urinary tungsten and the prevalence of selected medical conditions among subjects in National Health and Nutrition Examination Survey (NHANES) reports (ongoing cross-sectional surveys of the civilian, environmentally exposed, noninstitutionalized U.S. population). The results of these studies are summarized in Section 3.2.2 (Oral Exposure) because most background environmental exposures to tungsten are the likely result of tungsten in the drinking water (CDC 2013). In a recent review of tungsten (Lemus and Venezia 2015), information from unpublished industry studies included evaluations of selected tungsten compounds for skin irritation, eye irritation, and skin sensitization. These studies were not peer-reviewed or available to ATSDR; therefore, results are not included in this Addendum to the 2005 Toxicological Profile for Tungsten.

3.2.1 Inhalation Exposure

3.2.1.2 Systemic Effects

Respiratory Effects.   Roedel et al. (2012) instilled military-relevant metal powder mixtures (tungsten alloys) consisting of 92% tungsten/5% nickel/3% cobalt (W-Ni-Co) or 92% tungsten/5% nickel/3% iron (W-Ni-Fe) into the trachea of male Sprague-Dawley rats at 10, 20, or 40 mg/kg and evaluated pulmonary toxicity by measuring differential cell counts, lactate dehydrogenase (LDH) activity, albumin content, and inflammatory cytokine levels in bronchoalveolar (BAL) fluid collected 24 hours postinstillation. Other groups of rats received intratracheal instillation of pure tungsten at doses of 9.2, 18.4, or 36.8 mg/kg (equivalent to the proportion of tungsten in the 10, 20, and 40 mg/kg doses of tungsten alloys, respectively), or one of the other component metals. Controls consisted of rats receiving intratracheal

instillation of saline. Compared to controls, both tungsten alloys (W-Ni-Co and W-Ni-Fe) caused dose-related significant increases in multiple markers of pulmonary inflammation (percent of neutrophils; inflammatory cytokines CINC-1, CINC-3, TNF-α, and/or IL-1; albumin, and/or LDH activity). No significant changes in markers of pulmonary inflammation were observed in rats treated with pure tungsten at doses of 9.2 or 18.4 mg/kg, although signs of mild inflammation (BAL consisting of 30% neutrophils; mildly elevated albumin content) were noted at the highest pure tungsten dose (36.8 mg/kg). Intratracheal instillation results in relatively high lung burdens within a short time period; the relevance of these findings to natural inhalation exposure to tungsten is uncertain.

Contributions of individual metals (W, Ni, Co, and Fe) were evaluated by intratracheal instillation of 20 mg W-Ni-Co or W-Ni-Fe alloys in male Sprague-Dawley rats or intratracheal instillation of W, Ni, Co, or Fe at levels equivalent to those contained in the W-Ni-Co or W-Ni-Fe alloys (18.4 mg/kg W, 1 mg/kg Ni, 0.6 mg/kg Co, or 0.6 mg/kg Fe); control rats received intratracheal instillation of saline (Roedel et al. 2012). Instillation of 20 mg W-Ni-Co/kg resulted in significant increases in total cells, albumin, and percent of neutrophils in BAL; instillation of 20 mg W-Ni-Fe/kg resulted in significant increases in inflammatory cytokines (CINC-1, CINC-3, and IL-1β), albumin, and percent of neutrophils in BAL. There were no significant changes in any of the markers of pulmonary inflammation in rats receiving intratracheal instillation of individual metals (W, Ni, Co, or Fe).

Rajendran et al. (2012) exposed groups of male and female Sprague-Dawley rats (10/sex/group) to filtered air (controls) or tungsten blue oxide (a mixture of tungsten oxide species used as an intermediate in the manufacture of tungsten powder and consisting of 69% WO_3, 8.0% $W_{25}O_{73}$, and 23.0% $W_{20}O_{58}$; $WO_{2.97}$ overall) aerosol (particle size 2.63–2.87 µm mass median aerodynamic diameter [MMAD]; 1.89–1.94 µm geometric standard deviation [GSD]) at gravimetrically-measured concentrations of 0, 0.081, 0.331, or 0.652 mg/L air, 6 hours/day for 28 consecutive days. Ten rats per group (5 males and 5 females) were sacrificed at the end of the exposure period; the remaining rats were maintained for 14 days following cessation of exposures to evaluate recovery. All groups of tungsten blue oxide-exposed male rats exhibited significantly increased mean absolute and relative lung weight (20–45% greater than control means), which persisted in the highest exposure group at the end of the 14-day recovery period (14–19% greater than control means). The two highest exposure groups of female rats also exhibited significantly increased mean absolute and relative lung weight (25–33% greater than control means), which persisted in the highest exposure group at the end of the 14-day recovery period (32–33% greater than control means). The increased lung weights were attributed to deposition of tungsten blue oxide, which elicited a macrophage influx as evidenced by exposure concentration-related

increases in alveolar pigmented macrophages, alveolar foreign material, and individual alveolar foamy macrophages.

Hematological Effects.   Rajendran et al. (2012) exposed groups of male and female Sprague-Dawley rats to filtered air (controls) or tungsten blue oxide at gravimetrically-measured concentrations of 0, 0.081, 0.331, or 0.652 mg/L air, 6 hours/day for 28 consecutive days. Among rats sacrificed at the end of the exposure series, significantly increased white blood cells and eosinophils were noted in male rats of the 0.331 mg/L exposure group only. Males of the highest exposure group exhibited significantly increased blood levels of eosinophils, neutrophils, and monocytes (1.9–2.2 times greater than controls). There were no significant exposure-related effects on hematological parameters of the female rats. Among rats maintained for 14 days following cessation of tungsten blue oxide exposure, males of the highest exposure level exhibited significantly increased hemoglobin, hematocrit, mean corpuscular volume, and numbers of white blood cells; however, the study authors noted that these values were within the range of historical controls. Recovery female rats of the highest exposure level exhibited significantly increased numbers of reticulocytes (30% greater than that of controls).

3.2.2   Oral Exposure

3.2.2.1   Death

No deaths were observed among male Wistar rats administered sodium tungstate by gavage for 60 days at 50 mg/kg/day (31.2 mg tungsten/kg/day; tungsten accounts for 62.4% of the molecular weight of sodium tungstate) (Pandey et al. 2011). No deaths occurred among healthy (non-diabetic) male Wistar rats administered sodium tungstate in the drinking water for 3 months at 2,000 mg/L (1,248 mg tungsten/L; estimated dose of 172 mg tungsten/kg/day)[1] (Ballester et al. 2005). No deaths were observed among healthy (non-diabetic) female Wistar rats administered sodium tungstate in drinking water for 12 weeks at 2,000 mg/L (1,248 mg tungsten/L; estimated dose of 185 mg tungsten/kg/day)[2] (Ballester et al. 2007). No deaths occurred among male C57BL/6J mice receiving drinking water from the tap to which tungsten was added at concentrations of 0, 15, 200, or 1,000 mg tungsten/L (3.7, 49.5, and 247.5 mg tungsten/kg/day,

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[1]Average body weight of tungsten-exposed male rats = mean starting weight of 0.200 kg + ½ mean body weight gain [0.195 kg/2] = 0.298 kg. Water consumption calculated using the U.S. Environmental Protection Agency (EPA 1988) allometric equation for laboratory mammals (i.e., water consumption = 0.1[0.298 $kg^{0.7377}$] = 0.041 L/day). Daily tungsten dose = (1,248 mg tungsten/L x 0.041 L/day) / 0.298 kg = 172 mg tungsten/kg/day.

[2]Average body weight of tungsten-exposed female rats = mean starting weight of 0.200 kg + ½ mean body weight gain [0.0457 kg/2] = 0.223 kg. Water consumption calculated using the EPA (1988) allometric equation for laboratory mammals (i.e., water consumption = 0.1[0.223 $kg^{0.7377}$] = 0.033 L/day). Daily tungsten dose = (1,248 mg tungsten/L x 0.033 L/day) / 0.223 kg = 185 mg tungsten/kg/day.

respectively)[3] for up to 16 weeks (Kelly et al. 2013). No deaths were reported among male and female Sprague-Dawley rats administered sodium tungstate by gavage at 5, 75, or 125 mg/kg/day (3.1, 29, or 78 mg tungsten/kg/day, respectively) for a total of 70 days including 14 days prior to mating, 14 days of mating, 22 days of gestation, and 20 days of lactation (McInturf et al. 2011). In a similarly-designed study that employed sodium tungsten gavage doses of 5 or 125 mg/kg/day (3.1 and 78 mg tungsten/kg/day, respectively) for 70 days, no deaths were observed (McInturf et al. 2008; U.S. Navy 2007). In a study in which groups of male and female Sprague-Dawley rats were administered gavage doses of sodium tungstate at 0, 10, 75, 125, or 200 mg/kg/day (0, 6.24, 46.8, 78, or 124.8 mg tungsten/kg/day, respectively) for up to 90 days, 2 of 10 high-dose male Sprague-Dawley rats died on study days 55 and 56, respectively (U.S. Army 2007).

3.2.2.2 Systemic Effects

Respiratory Effects.   Mendy et al. (2012) evaluated relationships between urinary tungsten and the prevalence of a variety of self-reported medical conditions (including respiratory effects) among a nationally represented group of 922 male and 935 female subjects in the 2007–2008 NHANES report (an ongoing cross-sectional survey of the civilian, non-occupational, noninstitutionalized U.S. population). A statistically significant association was noted between elevated creatinine-adjusted urinary tungsten and asthma (odds ratio [OR] 1.72; 95% confidence interval [CI] 1.15, 2.59). The study authors noted that elevated urinary tungsten levels can result from either recent oral exposure or previous occupational inhalation exposure; potential associations between tungsten and asthma have mainly involved occupational exposure to cemented tungsten carbides (tungsten carbide with cobalt as a binder) (Stefaniak et al. 2009).

Cardiovascular Effects.   Several groups of investigators have evaluated possible relationships between urinary levels of tungsten and risk of selected cardiovascular effects among nationally-represented groups of human subjects.

Agarwal et al. (2011) evaluated relationships between urinary levels of various heavy metals (including tungsten) and the prevalence of cardio- and/or cerebrovascular disease among a nationally represented group of male and female subjects in pooled 1999–2006 NHANES reports. Individuals with cardio- and/or cerebrovascular disease were identified by pooling responses on a Centers for Disease Control and Prevention (CDC) questionnaire to questions related to cardiac and stroke history. Of the

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[3]Based on EPA (1988) subchronic reference values for body weight (0.0316 kg) and water consumption (0.00782 L/day) for the male mouse.

15,167 individuals canvassed, 1,601 subjects (53.5% males, 46.5% females) were considered to have cardio- and/or cerebrovascular disease. Within a subpopulation of the study group (573 subjects with cardio- and/or cerebrovascular disease and 4,464 subjects without cardio- and/or cerebrovascular disease), a statistically significant association was noted between elevated urinary tungsten and risk of cardio- and/or cerebrovascular disease (OR 1.78; 95% CI 1.28, 2.48) after adjustment for demographic factors (age, sex, race, education), clinical factors (hypertension, diabetes, hypercholesterolemia, chronic kidney disease, body mass index, C-reactive protein), smoking status, and serum cotinine. Significant associations between risk of cardio- and/or cerebrovascular disease and elevations of other individual urinary metals (antimony, cadmium, cobalt) resulted in ORs that were higher than the OR for tungsten.

Tyrrell et al. (2013) evaluated relationships between urinary tungsten levels and the prevalence of cardiovascular disease or stroke among 8,614 adults (18–74 years of age) in pooled 1999–2010 NHANES reports for whom urinary tungsten data were available. The primary study group included 193 reported cases of stroke and 428 reported cases of cardiovascular disease. Elevated urinary tungsten was significantly associated with prevalence of stroke in crude and adjusted models (crude OR 1.53; 95% CI 1.14, 2.05; adjusted OR 1.66; 95% CI 1.17, 2.34; adjusted for age, sex, ethnicity, socio-economic status, smoking status, alcohol consumption, occupation, body mass index, hypertension, hypercholesterolemia, and concentrations of molybdenum and cobalt). Inclusion of other heavy metals (e.g., barium, cadmium, lead, uranium, thallium) resulted in an OR of 2.10 (95% CI 1.24, 3.57). Stratification by age resulted in an OR of 2.17 (95% CI 1.33, 3.53) for those subjects under 50 years of age. Stratification by sex resulted in an OR of 2.07 (95% CI 1.20, 3.60) for females and an OR of 1.51 (95% CI 0.92, 2.46) for males. A significant association between urinary tungsten and the prevalence of cardiovascular disease was noted in crude models (OR 1.17; 95% CI 1.02, 1.34), but not in adjusted models (OR 0.94; 95% CI 0.67, 1.31).

Mendy et al. (2012) evaluated relationships between urinary tungsten and the prevalence of a variety of self-reported medical conditions (including cardiovascular effects, thyroid effects, liver effects, and cancer) among a nationally represented group of 922 male and 935 female subjects in the 2007–2008 NHANES report. No significant associations were found between elevated urinary tungsten and conditions that included congestive heart failure, coronary heart disease, angina pectoris, heart attack, or stroke. The source of urinary tungsten was not addressed.

Navas-Acien et al. (2005) evaluated relationships between urinary tungsten and the prevalence of peripheral arterial disease (PAD) among a nationally represented group of 51 PAD cases and 700 noncases. Tungsten was evaluated because accounting for traditional PAD confounders did not fully

account for PAD distribution in the study group. Subjects were part of the 1999–2000 NHANES report. Although 49% of the PAD cases exhibited higher urinary tungsten levels than noncases, no statistically significant association was found between urinary tungsten and prevalence of PAD (OR 2.25; 95% CI 0.97, 5.24) when comparing the 75th and 25th percentiles of tungsten distribution in the study populations.

Gastrointestinal Effects.   Male and female Sprague-Dawley rats (10/sex/dose) were administered sodium tungstate by gavage for 90 days at 0, 10, 75, 125, or 200 mg/kg/day (0, 6.24, 46.8, 78, or 124.8 mg tungsten/kg/day, respectively) (U.S. Army 2007). Histopathological evaluations revealed subacute inflammation and goblet cell metaplasia of the mucosa in the glandular stomach. In the 0, 10, 75, 125, and 200 mg sodium tungstate/kg/day groups, incidences of rats with subacute inflammation were 0/10, 2/10, 1/10, 5/9, and 4/10, respectively, for males and 0/10, 0/10, 1/10, 8/10, and 9/10, respectively, for females; incidences of rats exhibiting goblet cell metaplasia of the mucosa were 0/10, 1/10, 4/10, 8/9, and 8/10, respectively, for males and 0/10, 0/10, 4/10, 8/10, and 10/10, respectively, for females. According to Fisher's exact test, incidences of male and female rats exhibiting subacute inflammation were significantly increased in the two highest dose groups compared to controls; incidences of male and female rats exhibiting goblet cell metaplasia were significantly increased in the three highest dose groups compared to controls.

Hematological Effects.   No treatment-related hematological effects were observed following administration of sodium tungstate to male and female Sprague-Dawley rats by gavage for 90 days at doses up to 200 mg/kg/day (124.8 mg tungsten/kg/day) (U.S. Army 2007). Kelly et al. (2013) added sodium tungstate dihydrate to the tap drinking water of male C57BL/6J mice at levels resulting in reported tungsten concentrations of 0, 15, 200, or 1,000 mg/L (estimated doses of 0, 3.7, 49.5, and 247.5 mg tungsten/kg/day, respectively) for up to 16 weeks. The authors did not specify whether the tap water used in the study had been evaluated for the presence of tungsten; estimated tungsten doses are based solely on tungsten added to the tap drinking water. There were no effects on erythrocyte or platelet counts or hemoglobin or hematocrit levels. Peripheral white blood cell counts in low-, mid-, and high-dose groups were significantly lower (ca. 10, 25, and 50% lower than that of controls) after 1 week of treatment; white cell differential counts revealed significantly decreased lymphocytes in the mid-dose group and significantly decreased lymphocytes, granulocytes, and monocytes in the high-dose group. Significantly decreased white blood cell count was noted in the high-dose group at treatment week 12 as well (approximately 60% lower than controls). There were no statistically significant differences between controls and treatment groups at 4, 8, or 16 weeks. The transient nature of the changes in selected hematological values renders the hematological results of questionable toxicological significance.

Hepatic Effects.   Mendy et al. (2012) evaluated relationships between urinary tungsten and the prevalence of a variety of self-reported medical conditions (including liver condition) among a nationally represented group of 922 male and 935 female subjects in the 2007–2008 NHANES report. No significant association was found between elevated urinary tungsten and liver condition (OR 0.64; 95% CI 0.27, 1.54).

In a study of male Wistar rats receiving sodium tungstate orally at 119 or 238 mg/kg/day (74.2 or 148.5 mg tungsten/kg/day, respectively) for 14 days, the high-dose group exhibited significantly increased plasma alanine transaminase (ALT) and aspartate transaminase (AST) activity (Sachdeva et al. 2013). The increased plasma ALT and AST activity, along with significantly increased concentration of reactive oxygen species as indicated by decreased tissue ratio of reduced glutathione (GSH) to oxidized GSH in the liver of high-dose rats, are suggestive of potential for tungsten-related liver damage.

No treatment-related effects on liver weight were observed among male Wistar rats administered sodium tungstate (in distilled water) by gavage for 60 days at 50 mg/kg/day (31.2 mg tungsten/kg/day) (Pandey et al. 2011). No treatment-related effects on serum ALT or AST activities were observed among male C57BL/6J mice receiving tungsten from the drinking water at estimated doses of 3.7, 49.5, or 247.5 mg/kg/day for up to 16 weeks (Kelly et al. 2013).

U.S. Army (2007) reported significantly decreased absolute (but not relative) liver weight in male (but not female) Sprague-Dawley rats administered sodium tungstate by gavage for 90 days at 200 mg/kg/day (124.8 mg tungsten/kg/day). Histopathologic examination of livers revealed no evidence of treatment-related lesions.

Renal Effects.   No treatment-related effects on kidney weight were observed among male Wistar rats administered sodium tungstate by gavage for 60 days at 50 mg/kg/day (31.2 mg tungsten/kg/day) (Pandey et al. 2011). In a study of male Wistar rats receiving sodium tungstate orally at 119 or 238 mg/kg/day (74.3 or 148.5 mg tungsten/kg/day, respectively) for 14 days, the high-dose group exhibited significantly increased plasma urea (Sachdeva et al. 2013). However, because the increase was of small magnitude and was not accompanied by a significant effect on the tissue ratio of reduced GSH to oxidized GSH in the kidney, the tungsten treatment was not considered to be indicative of an adverse kidney effect.

U.S. Army (2007) administered sodium tungstate to male and female Sprague-Dawley rats by gavage at 0, 10, 75, 125, or 200 mg/kg/day (0, 6.24, 46.8, 78, or 124.8 mg tungsten/kg/day, respectively) for 90 days. Significantly increased absolute (but not relative) kidney weight was noted in high-dose female (but not male) rats; the study authors stated that the increased kidney weight may have been associated with renal tubular regeneration. Histopathologic examination of kidneys revealed mild to severe regeneration of renal cortical tubules in 1/9 male and 1/10 female rats at 78 mg tungsten/kg/day and 10/10 high-dose males and 8/10 high-dose females compared to no incidences in other groups of male or female rats. The study authors stated that minimal regeneration of renal cortical tubules (consistent with chronic progressive nephropathy) was observed in all groups, including controls, but did not include incidence data. Affected tubules were characterized as rare or few and minimally affected. Urinalysis revealed no treatment-related changes.

No treatment-related effects on adrenal weight were observed among male Wistar rats administered sodium tungstate by gavage for 60 days at 50 mg/kg/day (31.2 mg tungsten/kg/day) (Pandey et al. 2011).

Endocrine Effects.   Yorita Christensen (2013) evaluated relationships between urinary levels of various heavy metals (including tungsten) and serum levels of thyroid hormones among a nationally represented group of 1,587 subjects in the 2007–2008 NHANES report who reported no history of thyroid disease or use of medications that might affect serum thyroid hormone levels and for whom data on urinary metals and serum thyroid hormone levels were available. A significant association was reported between increasing urinary tungsten levels and increasing thyroid-stimulating hormone (TSH) levels (but not other serum thyroid hormones); conversely, increasing urinary levels for most other metals (lead, mercury, barium, cobalt, cesium, molybdenum, antimony, thallium, and uranium) was significantly associated with decreasing TSH levels.

Mendy et al. (2012) evaluated relationships between urinary tungsten and the prevalence of a variety of self-reported medical conditions (including thyroid effects) among a nationally represented group of 922 male and 935 female subjects in the 2007–2008 NHANES report. No significant association was found between elevated urinary tungsten and thyroid problems (OR 1.58; 95% CI 0.93, 2.70).

Ocular Effects.   Ophthalmologic evaluations of male and female rats administered sodium tungstate by gavage for 90 days at up to 200 mg/kg/day (124.8 mg tungsten/kg/day) revealed no signs of treatment-related effects (U.S. Army 2007).

Body Weight Effects.   No treatment-related effects on body weight were observed among male Wistar rats administered sodium tungstate by gavage for 60 days at 50 mg/kg/day (31.2 mg tungsten/kg/day) (Pandey et al. 2011). McInturf et al. (2011) evaluated body weight effects on male and female Sprague-Dawley rats administered sodium tungstate by gavage for a total of 70 days including 14 days prior to mating, 14 days of mating, 22 days of gestation, and 20 days of lactation at doses of 0, 5, 62.5, or 125 mg/kg/day (0, 3.1, 39, or 78 mg tungsten/kg/day, respectively). Mean body weight gains in the mid-dose group of parental male and female rats were approximately 15% less than that of controls; the difference in mean weight gain was statistically significant in the male rats (p<0.05), but not statistically significant in the female rats. Mean body weight gains in the low- and high-dose groups of males and females were slightly higher (not significantly different from that of respective controls), indicating the lack of a tungsten-related dose response. The apparent tungsten-related effect on mean body weight gain in the mid-dose rats is of questionable toxicological significance due to the lack of a clear dose-response. In a similarly-designed study that employed sodium tungsten gavage doses of 0, 5, or 125 mg/kg/day (McInturf et al. 2008; U.S. Navy 2007), there was no evidence of tungsten-related effects on mean maternal body weight gain (body weight data were not presented for the parental male rats). No significant tungsten-related effects on body weight were seen in a study of male and female C57BL6 mice administered sodium tungstate in the drinking water for 28 days at estimated doses of 62.5–200 mg/kg/day (39–124.8 mg tungsten/kg/day), or among mice similarly treated at estimated doses of 2–200 mg/kg/day (1.2–124.8 mg tungsten/kg/day) for 90 days prior to mating and 7 weeks of mating, gestation, and lactation or their F1 offspring treated for an additional 90 days after weaning (Osterburg et al. 2014).

In a study of male Wistar rats receiving sodium tungstate at 119 or 238 mg/kg/day (74.3 or 148.5 mg tungsten/kg/day, respectively) for 14 days, both treated groups exhibited mean weight loss (1.6–2.7% of initial body weight); mean weight gain was 15% for a group of concurrently-treated vehicle controls (Sachdeva et al. 2013). In a study of male Wistar rats that were made diabetic via Streptozotocin injection and subsequently administered sodium tungstate in the drinking water for 3 months at a concentration resulting in an estimated dose of 172 mg tungsten/kg/day, mean body weight gain was significantly depressed (17% less than that of controls) (Ballester et al. 2005). Among female Wistar rats administered sodium tungstate in drinking water for 12 weeks at a concentration resulting in an estimated dose of 185 mg tungsten/kg/day, significantly depressed mean body weight (41% less than that of controls) was noted among both healthy (non-diabetic) rats and those with diabetes (Ballester et al. 2007). Among male C57BL/6J mice receiving tungsten from tap drinking water at estimated doses of 3.7, 49.5, or 247.5 mg/kg/day for up to 16 weeks, the high-dose group exhibited significantly lower (p<0.001) mean

body weight as early as the first week of treatment (approximately 13% less than that of controls) and 20–30% less than that of controls at other time points; body weights of low- and mid-dose mice were generally slightly to significantly higher than that of controls throughout most of the treatment period (Kelly et al. 2013).

Groups of male and female Sprague-Dawley rats were administered sodium tungstate by gavage for 90 days at up to 200 mg/kg/day (124.8 mg tungsten/kg/day) (U.S. Army 2007). There were no significant treatment-related effects on female body weight. High-dose male rats exhibited significantly depressed mean body weight from days 70 to 90 (12–15% less than that of controls); however, the high-dose male rats also exhibited significantly decreased food consumption (quantitative food consumption data were not presented in the study report).

3.2.2.3 Immunological and Lymphoreticular Effects

Male C57BL/6J mice received tap drinking water to which sodium tungstate was added at concentrations resulting in estimated tungsten doses of 3.7, 49.5, or 247.5 mg/kg/day for up to 16 weeks (not including a possible tungsten contribution from the tap water) (Kelly et al. 2013). The study authors reported dose-dependent increases in tungsten concentration in bone and statistically significant increases in percentages of mature IgD+ B-cell populations in bone marrow (2-fold increase in the high-dose group at week 1, 1.5-fold increase in the mid-dose group at week 4, and 1.6-fold increase in mid- and high-dose groups at week 8) and significantly increased percentages of B-cells in late pro-/large pre-B developmental stages in low-, mid-, and high-dose groups only at week 16 (1.8–2.2-fold greater than controls). However, when expressed as total number of B-cells normalized to bone marrow cellularity, the only statistically significant effect was that of increased number of late pro-/large pre-B cells in low- and mid-dose groups only at week 16. There were no significant differences between controls and tungsten-treated groups regarding CD43- or IgD- B-cell populations or early pre-pro B-cell or early pro B-cell populations. At 16 weeks, the high-dose group exhibited a significantly increased number of pre-B cell colony forming units and significantly decreased total bone marrow cellularity; there was no apparent effect on the population of common lymphoid precursors. The study authors suggested that these results, together with findings of treatment-related increased DNA damage in bone marrow cells, indicate the potential for tungsten-induced adverse immunological effects and provide a possible pathway for tungsten-induced leukemogenesis.

Groups of male and female C57BL6 mice were administered sodium tungstate in the drinking water for 28 days at concentrations resulting in estimated doses of 0, 62.5, 125, or 200 mg sodium tungstate/kg/day (0, 39, 78, or 124.8 mg tungsten/kg/day, respectively) (Osterburg et al. 2014). At 24 hours prior to scheduled necropsy, selected mice from each treatment group received intraperitoneal injection of Staphylococcal enterotoxin B (SEB) to induce an immune response; other mice received intraperitoneal injection of saline. At necropsy, splenocytes and blood were harvested for analysis. The quantities of activated cytotoxic T-cells (CD3+, CD8+, CD71+) and helper T-cells (CD3+, CD8+, CD71+) were measured in splenocytes from the SEB-treated and saline-treated mice. The highest tungsten dose induced a statistically significant reduction in CD71+ T-helper and T-cytocoxic cells of the SEB-treated mice. The results are suggestive of possible tungsten-related effects on the immune system.

In a study designed to test tungsten-induced delayed-type hypersensitivity, groups of male and female C57BL6 mice were administered sodium tungstate in the drinking water for 28 days at concentrations resulting in estimated doses of 0, 0.2, 2, 20, or 200 mg sodium tungstate/kg/day (0, 0.12, 1.2, 12.5, or 124.8 mg tungsten/kg/day, respectively) (Osterburg et al. 2014). Mice were administered solubilized 4-hydroxy-3-nitrophenylacetic acid active ester (in 2% dimethyl sulfoxide [DMSO]) via subcutaneous injection (50 µL) followed by 0.1 mL of borate-buffered saline solution to enhance haptenization and primary immune responses. Mice were continued on sodium tungstate treatment for another 10 days and challenged with an injection of 20 µL of 4-hydroxy-3-nitrophenylacetic acid active ester in phosphate-buffered saline into the right hind foot pad; an equal volume of phosphate-buffered saline was injected into the left hind foot pad. The extent of footpad swelling as a result of Type IV cell-mediated delayed type hypersensitivity response was measured at 24 hours postchallenge. Significantly reduced footpad swelling in the 4-hydroxy-3-nitrophenylacetic acid active ester-challenged foot pad was noted within the two highest sodium tungstate treatment groups (20 and 200 mg sodium tungstate/kg/day). The results are suggestive of tungsten-related immune suppression.

The National Toxicology Program (NTP 2012) summarized results from a range-finding study to assess the immunotoxicity of sodium tungsten dihydrate in female B6C3F1/N mice. NTP noted that the study results had not been peer reviewed. Quantitative data were not included in the available summary (NTP 2012), and database searches did not identify other sources for results of this range-finding study. Mice were administered sodium tungsten dihydrate in the drinking water for 28 days at concentrations of 125, 250, 500, 1,000, or 2,000 mg/L. There were no treatment-related effects on thymus or spleen weights, total splenocyte number, spleen cell phenotypes, T-dependent antibody responses (evaluated using antibody-forming cell response, the sheep red blood cell enzyme-linked immunosorbent assay, and

keyhole limpet hemocyanin assay; the results of which suggested a lack of effect on humoral immunity), or natural killer cell activity or functional activity of the mononuclear phagocytic system (indicating a lack of effect on innate immunity). There was some evidence of treatment-related effects on bone marrow cells at the highest exposure level (2,000 mg/L). Bone marrow cell numbers were increased at the highest sodium tungsten exposure level in one study, but not in a second study. Absolute bone marrow cell differentials were increased for all markers evaluated except the CD3+ population. When expressed as percent values, the only apparent effect was that of increased numbers of TER-119+ erythroid lineage cells. In the summary, it was stated that some significant differences were observed in two *ex vivo* cell-mediated assays (mixed leukocyte response, cytotoxic T-lymphocyte response), but that the differences were not dose-responsive. It was further stated that no effects were observed in two *in vivo* cell-mediated assays (delayed-type hypersensitivity response to *Candida albicans*; anti-CD3 mediated proliferation).

U.S. Army (2007) reported significantly increased absolute (but not relative) spleen weight in female (but not male) rats administered sodium tungstate by gavage for 90 days at 200 mg/kg/day (124.8 mg tungsten/kg/day). Histopathologic examination livers revealed no evidence of treatment-related lesions.

3.2.2.4 Neurological Effects

Adams et al. (2013) evaluated possible associations between urinary tungsten levels and severity of autism in a study of children (5–16 years of age; 89% male) with diagnosed autism (n=54) and 44 "neurotypical" children. The autism group exhibited higher levels of urinary tungsten (44% higher than that of controls; p=0.00005), lead (74% higher than that of controls; p=0.02), thallium (77% higher than that of controls; p=0.0001), and tin (115% higher than that of controls; p=0.01). Study limitations include the following: (1) the designation of "neurotypical" was based on parental reporting; (2) sample sizes were small; (3) nearly half of the autistic children were taking medications that may have influenced detoxification pathways; and (4) some measurements of the other elevated urinary metals were at or below limits of detection, limiting evaluation of their possible associations with autism.

McInturf and coworkers (McInturf et al. 2008, 2011; U.S. Navy 2007) administered sodium tungstate to male and female Sprague-Dawley rats by gavage for a total of 70 days including 14 days prior to mating, 14 days of mating, 22 days of gestation, and 20 days of lactation. The study reports of McInturf et al. (2008) and U.S. Navy (2007) included three groups of male and female rats: vehicle controls (water only), low dose (5 mg sodium tungstate/kg/day; ca. 3 mg tungsten/kg/day), and high dose (125 mg

sodium tungstate/kg/day; ca. 78 mg tungsten/kg/day). The study of McInturf et al. (2011) was similarly designed, but included controls and an intermediate dose group (62.5 mg sodium tungstate/kg/day=39 mg tungsten/kg/day) as well. The study reports of McInturf et al. (2008, 2011) and U.S. Navy (2007) present identical data for spontaneous locomotor activity among control and high-dose maternal rats and apparently identical results from other neurobehavioral tests performed on controls, low-dose, and high-dose maternal rats. There were no apparent treatment-related effects on maternal retrieval latency, water maze navigation (latency or distance traveled), or acoustic startle/prepulse inhibition. Low-dose dams exhibited significantly more ambulatory time and distance traveled and decreased time in stereotypies than controls or high-dose dams. High-dose dams exhibited significantly less time resting and more time in stereotypic movements than controls or low-dose dams. Refer to Section 3.2.2.6 (Developmental Effects) for information regarding neurobehavioral results for the F1 progeny.

3.2.2.5 Reproductive Effects

Male Wistar rats were administered sodium tungstate by gavage for 60 days at 0 or 50 mg/kg/day (0 or 31.2 mg tungsten/kg/day) (Pandey et al. 2011). After 55 days of treatment, males were paired with untreated parous females for evaluation of mating success and fertility; inseminated females were allowed to deliver their pups. At sacrifice following the final dosing period, reproductive organs and tissues were removed for evaluation. There were no treatment-related effects on fertility, serum hormone levels, weights of testis, epididymis, or seminal vesicles, or testicular histology.

No exposure-related adverse effects were seen regarding mating success, serum hormone levels, or testicular histology among healthy (non-diabetic) male Wistar rats administered sodium tungstate in the drinking water for 3 months at 2,000 mg/L (estimated dose of 172 mg tungsten/kg/day) and paired with untreated females for up to 9 days after 10 weeks of treatment (Ballester et al. 2005). In contrast, among male rats exhibiting signs of reduced reproductive function after having been made diabetic via Streptozotocin injection, sodium tungstate treatment resulted in a recovery from diabetes-associated adverse reproductive function, a likely result of increased density and function of Leydig cells and increased levels of serum testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH).

There were no exposure-related effects regarding mating success, fertility, litter size, serum hormone levels, ovarian histology, or expression of ovarian or uterine estrogen, progesterone, FSH, LH, or prolactin levels in a study in which healthy (non-diabetic) female Wistar rats were administered sodium

tungstate in the drinking water for 12 weeks at 2,000 mg/L (estimated dose of 185 mg tungsten/kg/day) (Ballester et al. 2007). Among female rats exhibiting signs of reduced reproductive function after having been made diabetic via Streptozotocin injection, sodium tungstate treatment resulted in significantly increased serum FSH and significantly decreased serum progesterone. Females had been paired with untreated males after 10 weeks of treatment, and allowed to deliver their pups.

McInturf and coworkers (McInturf et al. 2008, 2011; U.S. Navy 2007) administered sodium tungstate to male and female Sprague-Dawley rats by gavage for a total of 70 days including 14 days prior to mating, 14 days of mating, 22 days of gestation, and 20 days of lactation. The study reports of McInturf et al. (2008) and U.S. Navy (2007) included three groups of male and female rats: vehicle controls (water only), low dose (5 mg sodium tungstate/kg/day; ca. 3 mg tungsten/kg/day), and high dose (125 mg sodium tungstate/kg/day; ca. 78 mg tungsten/kg/day). The study of McInturf et al. (2011) was similarly designed, but included an intermediate dose group (62.5 mg sodium tungstate/kg/day=39 mg tungsten/kg/day) as well. McInturf et al. (2008) reported significantly increased average gestation length in the high-dose group (22.08 ± 0.09 days compared to 21.55 ± 0.1 days for controls) and no treatment-related effects on average number of pups born per litter (data not included in the study report). McInturf et al. (2011) reported significantly increased average gestation length in high-dose (but not low- or mid-dose) groups.

Groups of male and female C57BL6 mice were administered sodium tungstate dihydrate in the drinking water for 90 days prior to mating and 7 weeks of mating, gestation, and lactation at concentrations resulting in estimated doses of 0, 2, 62.5, 125, or 200 mg sodium tungstate/kg/day (0, 1.2, 39, 78, or 124.8 mg tungsten/kg/day, respectively) (Osterburg et al. 2014). At weaning of the pups (F1), parental mice were sacrificed; F1 pups continued sodium tungstate treatment for 90 days postweaning, followed by terminal sacrifice. There was no indication of treatment-related effects on number of live births, litter size, or sex ratio.

3.2.2.6 Developmental Effects

In the studies of McInturf and coworkers described in Section 3.2.2.5 (Reproductive Effects), no statistically significant dose-related effects were observed regarding pup righting reflex, although righting reflex latency among males was significantly shorter than that of females.

3.2.2.7 Cancer

The CDC led a multiagency cross-sectional, case-comparison study to determine whether ongoing environmental exposures might be responsible for a cluster of childhood leukemia cases (16 cases between the years 1997 and 2002; <2 were expected based on county population and statewide cancer rates) in Churchill County, Nevada (CDC 2003b). The results were briefly summarized in the Toxicological Profile for Tungsten (Agency for Toxic Substances and Disease Registry 2005) and subsequently published in the open literature (Rubin et al. 2007). The large-scale multiagency study used advanced analytical methods and enrolled 16 childhood leukemia cases and all other people currently living in the home of each case (as well as biologic parents not living full time in the case child's home) (Rubin et al. 2007). A strict case definition was constructed based on age being 0–6 years old at time of diagnosis, diagnosis of acute lymphocytic leukemia (ALL, pre-B cell), and residence of at least 6 months at time of diagnosis; a total of nine children met this case definition. The comparison group included 55 age- and sex-matched children (without leukemia) and their parents. Participants provided blood, urine, and cheek swab samples for analysis of a wide range of substances, including tungsten, and completed a 500-item questionnaire. Environmental and household sampling included collection of samples from indoor air, play yard soil, household dust, and tap water. Concentrations of tungsten in tap water samples ranged from not detectable to 290 µg/L (mean 4.66 µg/L; 95% CI 2.98, 7.30). Tungsten was detectable in urine from 98.5% of the study participants at a geometric mean level of 1.19 µg/L (95% CI 0.89, 1.59). By comparison, tungsten was detected within the general U.S. population (participants of NHANES 1999–2000) at geometric mean levels of 0.081 µg/L (95% CI 0.074, 0.088) among 2,465 subjects ≥6 years of age, 0.145 µg/L (95% CI 0.117, 0.181) among 340 children 6–11 years of age, 0.099 µg/L (95% CI 0.085, 0.115) among 719 subjects 12–19 years of age, and 0.072 µg/L (95% CI 0.066, 0.079) among 1,406 subjects ≥20 years of age (CDC 2003a). Median urinary tungsten was 1.93 µg/L for the leukemia cases and 2.35 µg/L for the comparison children; median urinary tungsten was 0.61 µg/L for the case families and 0.62 µg/L for the comparison families (Rubin et al. 2007). Using conditional logistic regression on data from the Churchill County, Nevada assessment, no significant association was found between leukemia and tungsten exposure among leukemia case children (n=13) and comparison children (n=55) (OR 0.78; 95% CI 0.33, 1.86), among case parents (n=24) and comparison parents (n=92) (OR 1.06; 95% CI 0.66, 1.70), or after limiting analysis of tungsten exposure to case families (excluding siblings) (n=14) and their matched comparison families (n=51) (OR 0.96; 95% CI 0.77, 1.24) (CDC 2003b). This study was limited for purposes of risk assessment because it involved only 13 leukemia cases.

The CDC, in collaboration with the Arizona Department of Health and Cochise County Health Department, conducted a cross-sectional biological exposure assessment in Sierra Vista, Arizona, due to the incidence rate of childhood leukemia in that area (9.9 cases per 100,000 versus 4.5 cases per 100,000 for the state of Arizona). The study included a total of 44 participants consisting of 5 families (children, parents, and other care-taking adults in the home) who had been residents prior to the diagnosis of ALL or acute myelocytic leukemia (AML) in a child between the years 1997 and 2003 and 9 comparison children (without leukemia) and their parents as a reference population (CDC 2006). One case of childhood leukemia died prior to the commencement of the study; therefore, only four cases were available for biological sampling. Blood, urine, and cheek-swab samples were collected for analysis of a wide range of substances including tungsten. Tungsten was detectable in 86% of the study participant's urine. Mean urinary tungsten was 0.41 µg/L (95% CI 0.06, 2.6) for the 4 leukemia cases, 0.29 µg/L (95% CI 0.17, 0.50) for the 9 comparison children, and 0.13 µg/L (95% CI 0.08, 0.20) for the 44 subjects combined; the differences were not statistically significant. These levels were less than the 95th percentile values for the general U.S. population. Mean urinary tungsten in the U.S. population was 0.093 µg/L (95% CI 0.087, 0.100) for subjects ≥6 years of age and 0.158 µg/L (95% CI 0.123, 0.204) for children 6–11 years of age for survey years 1999–2000. Mean urinary levels in the general U.S. population for survey years 2001–2002 were 0.082 µg/L (95% CI 0.073, 0.092) for children ≥6 years of age and 0.137 µg/L (95% CI 0.110, 0.170) for children 6–11 years of age for the survey years 2001–2002 (CDC 2005). Because no community-wide environmental exposure of concern was identified among the 128 substances evaluated, it was suggested that elevated mean urinary tungsten in the Sierra Vista, Arizona, study subjects was most likely due to individual variation. Furthermore, the study was limited for purposes of risk assessment because it involved only four leukemia cases.

A study was conducted in Saskatchewan, Canada, on the relationship between 64 elements (including tungsten) in drinking water and the incidence of non-Hodgkin's Lymphoma (NHL). Tungsten was detected in 22 water samples (<15% of the water samples tested, detection limit not specified in study report). Tungsten concentrations ranged from 0.10 to 3.0 ppm, with a mean of 0.61±0.8 ppm and a median of 0.21 ppm. The log-transformed concentrations were stratified for 71 NHL case and 83 control subjects, and no correlation (log W_{cases} = -1.330±0.28; log $W_{controls}$ = -0.719±0.44; p=0.27) was found between the incidence of NHL and the concentrations of tungsten in drinking water (Witmans et al. 2008).

3.2.4   Other Routes of Exposure

In a study designed to evaluate the carcinogenicity of embedded tungsten alloys in male B6C3F1 mice (20/group), two groups of mice received intramuscular implantation of pellets of tungsten-nickel-cobalt alloy (W-Ni-Co; 91.1% W, 6.0% Ni, 2.9% Co) or tungsten-nickel-iron alloy (W-Ni-Fe; 91% W, 7.0% Ni, 2.0% Fe) (Emond et al. 2015; U.S. Army 2011). The tungsten pellets measured 1 mm in diameter and 2 mm in length (cylindrical shape). Each low-dose rat received two pellets; each high-dose group received four pellets. Other groups were similarly treated by implantation of pellets consisting of tantalum (inert metal used for implantation control), Ni (positive controls; intramuscular injection of Ni is known to cause rhabdomyosarcomas at the injection site), and groups implanted with pellets containing mixtures of tantalum and one or more of the individual components in the tungsten alloys (individual components were present at the levels observed in the tungsten alloys). A group of sham-operated mice was included in the study. The mice were observed for up to 24 months postimplantation. The major findings of this study were: (1) implantation site tumors (rhabdomyosarcomas) developed in W-Ni-Co alloy-implanted groups (95% incidence) and low- and high-dose Ni alone groups (100% incidence); (2) no pellet-associated tumors developed in other groups; (3) the mice implanted with W-Ni-Co alloy exhibited slower and less aggressive tumor development than similarly-treated male F344 rats (Kalinich et al. 2005); and (4) tumors in the mice did not metastasize to other organs as noted in the similarly-treated rats. These results indicate that tungsten itself was not the source of tumorigenicity in the rats and mice with embedded W-Ni-Co.

Schuster et al. (2012) evaluated the tumorigenicity of intramuscularly-implanted pure tungsten or tungsten alloys (W-Ni-Co or W-Ni-Fe) in male F344 rats. The study included a group implanted with the inert metal, tantalum, and a sham-operated control group. Rats received either 4 pellets (low dose) or 20 pellets (high dose), with the exception of the W-Ni-Co alloy-treated rats that included only a low-dose group. All rats with embedded W-Ni-Co alloy developed aggressive implantation site rhabdomyosarcomas, typically encapsulating the pellets, within 6–12 months following implantation; no implantation site malignant tumors developed in any of the rats with embedded W-Ni-Fe alloy, pure tungsten, or tantalum. Benign pellet-encapsulating sebaceous adenomas were observed at 2 months in 3–14 rats with embedded pure tungsten at the high dose; however, the adenomas remained benign throughout the remainder of the 24-month evaluation period. The W-Ni-Co alloy-treated group exhibited progressive corrosion of the matrix phase of W-Ni-Co pellets (as revealed via electron microscopy) and high urinary concentrations of W, Ni, and Co (resulting from galvanic corrosion and anodic dissolution). Microarray analysis of tumors revealed changes in expression of genes involved in cell cyclicity and

muscle development and differentiation. Non-carcinogenic W-Ni-Fe pellets were minimally corroded and urinary metals were low. The results indicate that the tumorigenic response was the likely result of mobilization of Ni and/or Co resulting from the corrosion of W-Ni-Co alloy.

Roedel et al. (2012) instilled W-Ni-Co alloy (92% W, 5% Ni, and 3% Co) or W-Ni-Fe alloy (92% W, 5% Ni, and 3% Fe) into the trachea of groups of male Sprague-Dawley rats at doses of 10, 20, or 40 mg/kg. Other groups of rats received intratracheal instillation of one of the individual components of the alloys (W, Ni, Co, or Fe) at doses according to their percentages in the alloy mixtures. Following sacrifice at 24 hours postinstillation, BAL fluid was collected and evaluated for signs of tungsten-induced pulmonary inflammation. There were no signs of treatment-related effects in low- or mid-dose tungsten-only treated rats. The high-dose tungsten-only treated rats exhibited signs of mild pulmonary inflammation (elevated neutrophils and albumin content in BAL), but no elevation in inflammatory cytokines or lactate dehydrogenase activity. All doses of tungsten alloys induced altered gene expression in BAL cells and produced signs of inflammatory response (increased IL-1β protein levels in BAL fluid); however, IL-6 and CYP2A3 were the only genes that differentially expressed in lung tissue.

Single case reports were published in which granuloma formation was associated with metal fragments that had apparently been embedded in subcutaneous tissues from a chain saw blade in one case (fragment size 3.5x4.0 mm, nodule size 25x20 mm) (Osawa et al. 2006) and a lawn mower blade in another case (fragment size 2x3 mm, tumor size 20x30 mm) (Saruwatari et al. 2009). The metal fragments consisted primarily of tungsten, with lesser amounts of Co, Ag, Zn, Cd, and Cu in the chain saw blade and S, P, Co, and Cs in the lawn mower blade.

3.3   GENOTOXICITY

Guilbert et al. (2011) observed significant dose-related 2–2.2-fold increases in DNA damage (increases in COMET tail moment) within bone marrow of C57BL/6J mice (unspecified sex) receiving tap drinking water to which tungsten (form not specified) was added at 15 or 200 mg/L for 8 weeks (estimated doses of 3.75 and 50 mg/kg/day, respectively), when compared to tap water controls. It is unclear whether these dose levels were cytotoxic. In an *in vitro* portion of the study in which murine BU-11 early pro/preB-cells (grown on BMS2 bone marrow stromal cells) were exposed to solutions containing 0, 50, 100, 250, or 500 mg tungsten/L, tungsten exposure-related effects included dose- and time-dependent decreases in numbers of BU-11 cells, changes in cell cycle (increased percentage of cells in the G0/G1 phase and decreased percentage of cells in the G2/M phase at high dose), increased numbers of apoptotic

cells (as indicated by increased percentage of cells with $subG_0$ DNA content and confirmed by increased caspase 3/7 activity and annexin B staining), and increased DNA strand breaks in primary murine bone marrow-derived developing B lymphocytes. Kelly et al. (2013) reported treatment-related increased DNA damage (increases in COMET tail moment) within bone marrow cells from male C57BL/6J mice receiving tap drinking water to which sodium tungstate dihydrate was added at concentrations resulting in estimated doses of 3.7, 49.5, or 247.5 mg tungsten/kg/day for 4 weeks; significantly increased DNA damage was noted in low- and mid-dose (but not high-dose) groups after 1 week of treatment, mid-dose (but not low- or high-dose) groups after 8 weeks of treatment, low- and high-dose (but not mid-dose) groups after 12 weeks of treatment, and low-dose (but not mid- or high-dose) groups after 16 weeks of treatment. The study authors noted that DNA damage at the highest tungsten dose level was often less than that observed at lower dose levels and suggested that the highest dose level may have increased DNA repair efficiency or caused significant apoptosis of damaged cells that was undetected in the assays performed. Sodium tungstate dihydrate did not induce gene mutations in *Salmonella typhimurium* strains TA98 or TA100 or *Escherichia coli* strain pKM101 in the presence or absence of exogenous metabolic activation (NTP 2010). Sodium tungsten dihydrate did not induce micronuclei in polychromatic erythrocytes from male or female Harlan Sprague-Dawley rats or B6C3F1 mice administered the chemical in the drinking water for 13 weeks at concentrations in the range of 125–2,000 mg/L (NTP 2014a, 2014b).

3.4   TOXICOKINETICS

3.4.1   Absorption

3.4.1.1   Inhalation Exposure

Increased urinary and blood tungsten levels among workers exposed to airborne tungsten in a hard metal plant is evidence that inhaled tungsten is absorbed. In a facility producing hard metal cutting tools, measured airborne tungsten levels collected for 6–7 hours ranged from 0.001 $\mu g/m^3$ in mounting and shipping areas to as high as 6.54 $\mu g/m^3$ in the wet grinding area (De Palma et al. 2010). Among 55 workers, before-shift and end-of-shift urinary tungsten averaged 4.20 and 7.06 $\mu g/L$, respectively, compared to 0.08 $\mu g/L$ within a group of 34 unexposed control subjects. Before-shift and end-of-shift urinary tungsten were highest in the wet-grinding area, averaging 11.32 and 20.49 $\mu g/L$, respectively. End-of-shift plasma tungsten levels in blood collected from workers were 2–3 times higher among those working in the wet-grinding area than among workers in other areas.

Radiolabeled sodium tungstate ($Na_2{}^{188}WO_4$) powder with a 1.50 µm MMAD and aerosolized to a concentration of 256 mg W/m^3 was readily absorbed from the lungs of male CD rats as evidenced by its presence in a range of organs and tissues immediately following a 90-minute nose-only exposure (Radcliffe et al. 2010).

In a study designed to evaluate olfactory transport of inhaled tungsten to the brain, male CD rats were exposed (nose-only) to aerosols of $Na_2{}^{188}WO_4$ for 90 minutes with one nostril plugged to prevent nasal deposition of ^{188}W to that side (Radcliffe et al. 2009). It was determined that olfactory transport plays only a minimal role in delivery of tungsten to the rat brain because concentrations of ^{188}W in the ipsilateral olfactory bulb was only 1–3% of the concentration observed in the olfactory epithelium of the unoccluded nostril. The detection of ^{188}W in blood, trigeminal nerve, cerebellum, striatum, and pituitary gland provides evidence of absorption from the respiratory tract.

3.4.1.2   Oral Exposure

Detection of elevated levels of tungsten in bone marrow of male C57BL/6J mice receiving tap drinking water to which tungsten (as sodium tungstate dihydrate) was added at concentrations in the range of 15–1,000 mg tungsten/L for as little as 1 week and up to 16 weeks demonstrates that ingested tungsten is absorbed (Kelly et al. 2013).

3.4.2   Distribution

3.4.2.1   Inhalation Exposure

Young adult male CD rats were exposed (nose-only) to radiolabeled sodium tungstate ($Na_2{}^{188}WO_4$; MMAD 1.5 µm, GSD 1.33) for 90 minutes at an aerosol concentration of 256 mg W/m^3 (Radcliffe et al. 2010). Levels of ^{188}W were measured in a range of tissues immediately following cessation of exposure and 1, 3, 7, and 21 days later. Immediately following exposure, the highest mean ^{188}W activity was measured in thyroid gland (5,939 nCi/g) and lung (809 nCi/g); lesser amounts of ^{188}W activity were noted in other tissues (155 nCi/g in kidney, 146 nCi/g in blood, 100 nCi/g in femur, 93.7 nCi/g in pituitary, 86.1 nCi/g in liver, 69.5 nCi/g in spleen, 67.2 nCi/g in pancreas, 58.1 nCi/g in adrenal, 55.4 nCi/g in testis, 54.5 nCi/g in lymph node, 47.9 nCi/g in thymus, and 46.4 nCi/g in heart). Activity of ^{188}W in all tissues decreased rapidly in all tissues examined; however, mean ^{188}W activity in femur, lung, and spleen at postexposure day 21 was approximately 12.9, 11, and 9%, respectively, of the activity measured in each of these tissues immediately following exposure, indicating some degree of retention. Sodium

tungstate redistributed among the tissues in a multi-phase manner, with bone and lung representing the largest long-term repositories.

In the toxicokinetics portion of a 28-day inhalation study, Rajendran et al. (2012) exposed groups of male and female Sprague-Dawley rats to filtered air (controls) or aerosols of tungsten blue oxide for 6 hours/day for up to 28 consecutive days at target concentrations of 0.08 or 0.65 mg/L of air (low- or high-exposure groups, respectively; author-estimated inhaled tungsten blue oxide target doses were 15 or 124 mg/kg/day, respectively). Tungsten concentrations were reported for lung, liver, kidney, spleen, testes, brain, and femur. Blood tungsten concentrations peaked at approximately 0.8 and 10.9 µg/g blood in the low- and high-exposure groups, respectively, at the end of a single 6-hour exposure and decreased by approximately 100-fold by 6 days postexposure. Following 14 days of repeated exposures at low- and high-exposure levels, tungsten concentrations in tissues were highest in lung (at least 10-fold higher than in femur and kidney) and were attributed to pulmonary deposition of tungsten blue oxide; slightly increased concentrations were observed in liver, spleen, brain, and testes. However, the relative tungsten concentrations for high to low dose groups in all of the tissues was much less than the factor of 8 ratio of high to low exposures. Between days 14 and 28 of exposure, tungsten concentrations continued increasing in the lung, remained unchanged in the femur, and decreased in the other tissues. The study authors stated that clearance of deposited tungsten blue oxide from the lung occurred slowly.

In a study designed to evaluate olfactory transport of inhaled tungsten to the brain, male CD rats were exposed (nose-only) to aerosols of $Na_2{}^{188}WO_4$ for 90 minutes with one nostril plugged to prevent nasal deposition of ^{188}W to that side (Radcliffe et al. 2009). Detection of ^{188}W in blood, selected brain tissues, and pituitary gland provides evidence that tungsten was absorbed from the respiratory tract and distributed by the blood. Mean end-of-exposure pituitary ^{188}W concentration was higher than concentrations in trigeminal nerve, cerebellum, or striatum.

3.4.2.2 Oral Exposure

Disposition of tungsten was evaluated in female Sprague-Dawley rats and C57BL/6N mice at 1, 2, 4, or 24 hours following single-dose gavage administration of tungsten (as sodium tungstate dihydrate in water) at 1, 10, or 100 mg/kg (0.6, 5.6, or 55.7 mg tungsten/kg, respectively; tungsten comprises 55.7% of sodium tungstate dihydrate) (McDonald et al. 2007). Dose-related increased tungsten concentrations were observed in plasma, intestine, liver, kidney, femur, and uterus of the gavage-treated rats and mice (not always proportional to dose). In rats and mice tungsten concentrations at 1 hour postdosing were

highest in plasma and intestine; lesser amounts kidney, femur, uterus, and liver (decreasing order of concentration). Tungsten concentrations in rat tissues typically peaked at 4 hours postdosing; tungsten concentrations in most mouse tissues peaked at 1–2 hours postdosing. Femoral tungsten concentrations in high-dose rats and mice increased during the first 4 hours postdosing and reached baseline levels by 24 hours postdosing. During the first 4 hours, plasma and intestine levels peaked more quickly and reached higher values in mice, while levels in rats remained stable or slowly increased. During this period, levels in kidney, liver, and uterus peaked higher and were achieved faster in rats. Levels in the femur of both species tended to build up slowly or remain constant. By 24 hours postexposure, the femur showed some tungsten retention, while levels in the other tissues returned to baseline. These results demonstrate basic differences in rat and mouse absorption, distribution, and elimination kinetics.

In a repeated-dose oral study, tissue distribution of tungsten was evaluated in groups of male and female C57BL/6 mice at 24 hours following exposure for 28 days to drinking water containing sodium tungstate dihydrate (estimated doses of 0, 62.5, 125, or 200 mg sodium tungstate/kg/day; 0, 39, 78, or 124.8 mg tungsten/kg/day, respectively) (Guandalini et al. 2011). Dose-related significantly increased tungsten levels were observed in kidney, liver, bone, spleen, and brain; dose-related increases in the colon did not reach the level of statistical significance. At the highest dose level, tungsten accumulation was highest in bone (ca. 61–63 mg tungsten/kg tissue); lesser amounts were noted in spleen (ca. 6 and 16 mg/kg for males and females, respectively, which correlates with iron levels), colon (ca. 14 and 5 mg/kg for males and females, respectively), kidney (ca. 5 and 7 mg/kg for males and females, respectively), liver (ca. 2 and 3 mg/kg for males and females, respectively), and brain (ca. 0.11 mg/kg for males and females).

Weber et al. (2008) examined the disposition of tungsten in non-gestating Sprague-Dawley rats and C57BL/6N mice at 24 hours following gavage administration of sodium tungsten dihydrate (in water) at 10 mg/kg/day or in the drinking water (560 mg/L) for 14 days; the study included evaluation of tungsten disposition within groups of gestating rats and mice exposed via the drinking water during gestation days (GDs) 6–14 (mice) and GDs 6–15 (rats). Tungsten concentrations were evaluated in plasma, intestine, liver, kidney, femur, uterus, and fetus at 24 hours following cessation of treatment. Tungsten was detected in all tissues examined in both species and accumulated in intestine, kidneys, and femur. There were no remarkable species differences regarding baseline tungsten levels in various tissues prior to the initiation of gavage treatment, with the exception of the femur for which the mean levels for the rat and mouse were 0.01±0.02 and 0.15±0.13 μg/g, respectively. Following 14 days of treatment, rats exhibited slightly increased tungsten levels in plasma, liver, and uterus and more pronounced increases in intestine,

kidney, and femur; mice exhibited slightly increased tungsten levels in plasma, intestine, liver, and femur and a large increase in the uterus. Rat-to-mouse tungsten concentration ratios for femur, kidney, and uterus were 25, 20, and 0.15, respectively. Among non-gestating and gestating rats treated with tungsten via the drinking water, the highest tungsten levels were noted in the femur, intestine, and kidney; similarly treated non-gestating and gestating mice exhibited the highest tungsten levels in femur and intestine. Uterine tungsten levels were lower in gestating than non-gestating rats and mice. Maternal tungsten transfer to the developing fetus was evidenced by detection of tungsten in fetal tissue; tungsten levels were approximately 8 times higher in mouse fetuses than rat fetuses. Based on observed differences in distribution and elimination of orally-administered tungsten in rats and mice in the study of Weber et al. (2009), the lack of quantitative data regarding kinetics of ingested tungsten in humans precludes meaningful interspecies extrapolation.

Kelly et al. (2013) observed significant accumulation of tungsten in tibiae from male C57BL/6N mice (4 weeks of age at initiation of treatment) receiving tap drinking water to which tungsten (as sodium tungstate dihydrate) was added at 15, 200, or 1,000 mg tungsten/L (estimated doses of 3.7, 47.5, or 247.5 mg tungsten/kg/day, respectively) for 1–16 weeks. For control animals, tibial tungsten measured approximately 1 ppm at week 1, 0.8 ppm at week 4, and dropped to approximately 0.3 ppm from weeks 8 through 16. Tungsten in the control animals likely reflects trace amounts of tungsten in the pre-exposure diet and/or tap water. A 3-fold decrease in tungsten concentration in tibiae of control mice during the 16-week treatment period is likely the result of, at least in part, increasing bone mass during rapid growth from the young weanling stage.

In male and female Sprague-Dawley rats fed sodium tungstate by gavage for a total of 70 days including 14 days prior to mating, 14 days of mating, gestation, lactation, and postweaning at 62.5 or 125 mg/kg/day (39 or 78 mg tungsten/kg/day), tungsten was primarily found in bone and spleen, followed by kidney and gastrointestinal tract; lesser amounts were observed in lungs, liver, brain, blood, thymus, testes, and ovaries (McInturf et al. 2011). Among their 20-day-old pups, tungsten was found mainly in bone and gastrointestinal tract, with much lesser amounts in other organs and tissues. The high-dose group generally exhibited higher tungsten concentrations than the low-dose group.

3.4.2.4 Other Routes of Exposure

Disposition of tungsten was evaluated in female Sprague-Dawley rats and C57BL/6N mice at 1, 2, 4, or 24 hours following intravenous administration of tungsten (as sodium tungstate dihydrate in water) at

1 mg/kg (McDonald et al. 2007). In rats, peak tungsten concentrations were observed at 1–2 hours postdosing and were highest in kidney and intestine, followed by lesser amounts in femur, uterus, liver, and plasma, respectively. Tungsten levels measured in rats at 24 hours postdosing had dropped to approximately 2% (intestine and kidney), 5% (liver), and 21% (femur) of the concentrations measured at 1 hour postdosing; tungsten was not detected in plasma or uteri of rats at 24 hours postdosing. In mice, peak tungsten concentrations were observed at 1–2 hours postdosing and were highest in kidney, intestine, and femur, followed by lesser amounts in uterus, plasma, and liver. Tungsten levels measured in mice at 24 hours postdosing had dropped to approximately 5% (intestine), 10% (femur), 34% (kidney), and 50% (liver) of the concentrations measured at 1 hour postdosing; tungsten was not detected in plasma or uteri of mice at 24 hours postdosing. These findings indicate significant differences in rat and mouse absorption, distribution, and elimination kinetics.

In a study designed to evaluate urinary and serum levels of tungsten, nickel, and cobalt as indicators of embedded tungsten alloy fragments, Kalinich et al. (2008) implanted weapons-grade W-Ni-Co alloy (91.1% W, 6.0% Ni, and 2.9% Co) as 4 pellets (low dose) or 20 pellets (high dose) into muscle tissues of male F344 rats. Although pellets removed at various times up to 22 months showed little change in physical appearance, the mass of pellets decreased slightly over 6 months (a statistically significant decrease of approximately 0.7 mg/pellet), and both urinary and serum levels of tungsten, nickel, and cobalt were significantly higher in W-Ni-Co alloy-implanted rats than tantalum pellet-implanted control rats. A lower fraction of tungsten was mobilized compared to the other metals. Urinary tungsten in the low-dose group was approximately 360 ng/mg creatinine at 1 month postimplantation, increased to approximately 410 ng/mg creatinine at 3 months postimplantation, and decreased to approximately 275 ng/mg creatinine at 6 months postimplantation. Urinary tungsten in the high-dose group was highest at 1 month postimplantation (approximately 2,400 ng/mg creatinine) and decreased to approximately 1,270 ng/mg creatinine by 6 months postimplantation. Serum tungsten in the low-dose group was approximately 410 ng/mL at 1 month postimplantation, decreased to approximately 32 ng/mL at 3 months postimplantation, and appeared to be nondetectable at 6 months postimplantation. Tungsten levels were higher in urine than in serum throughout the study.

3.4.4   Elimination and Excretion

3.4.4.1   Inhalation Exposure

In a facility producing hard metal cutting tools, measured airborne tungsten levels ranged from 0.001 µg/m^3 in mounting and shipping areas to as high as 6.54 µg/m^3 in the wet grinding area (De Palma

et al. 2010). Among 55 workers, before-shift and end-of-shift urinary tungsten averaged 4.20 and 7.06 µg/L, respectively, compared to 0.08 µg/L within a group of 34 unexposed control subjects.

Young adult male CD rats were exposed (nose-only) to aerosols of radiolabeled sodium tungstate ($Na_2{}^{188}WO_4$; MMAD 1.5 µm, GSD 1.33) for 90 minutes (Radcliffe et al. 2010). Within 1 day following exposure, at least 90% of the [188]W observed immediately following cessation of exposure had been cleared from most tissues (thyroid, blood, pituitary, liver, pancreas, adrenal, testis, lymph node, thymus, and heart); however, 34, 20, 16, and 11% of the initial [188]W concentration was noted in lung, femur, spleen, and kidney, respectively. Elimination from most tissues followed two-phase kinetics consisting of an initial-phase half-time of approximately 0.2 days, followed by second-phase half-times ranging from 6.1 days (pituitary) to 66.7 days (femur). By day 21 postexposure only trace amounts of [188]W were found in tissues other than lung, femur, thyroid, spleen, and kidney. Urinary excretion was considered to be the primary route of [188]W elimination; a mean of 4,382 nCi/g was observed in urine samples from rats sacrificed immediately following cessation of exposure. However, the study report did not include data regarding fecal [188]W levels. Urinary excretion exhibited an initial-phase half-time of 0.6 days; from day 7 postexposure onward, mean urinary concentration remained relatively constant at approximately 40 nCi/g.

Rajendran et al. (2012) reported information regarding urinary and fecal levels of tungsten in samples collected from male Sprague-Dawley rats exposed to airborne tungsten blue oxide for 6 hours at target concentrations of 0.08 or 0.65 mg/L air. Tungsten concentrations in fecal samples measured 1.9 and 15 µg/g for the low- and high-exposure groups, respectively. Urinary tungsten levels were reported to be 3 orders of magnitude lower than fecal levels. The time course for fecal and urine sampling was not specified in the study report. Systemic elimination half-lives were estimated as 23±4.3 and 154±92.8 hours for the low- and high-dose groups, respectively. The relatively high level of fecal excretion is a likely result of pulmonary deposition followed by mucociliary clearance from the respiratory tract and subsequent ingestion. This is supported by the finding that absolute lung weight was statistically significantly elevated at the end of exposure, but decreased rapidly during a 14-day recovery period for all but the high-dose group.

In a study designed to evaluate olfactory transport of inhaled tungsten to the brain, male CD rats were exposed (nose-only) to aerosols of $Na_2{}^{188}WO_4$ for 90 minutes with one nostril plugged to prevent nasal deposition of [188]W to that side (Radcliffe et al. 2009). Elimination kinetics of [188]W from blood, pituitary gland, trigeminal nerve, cerebellum, and striatum were best described by a two-compartment

pharmacokinetic model with initial half-times of approximately 0.2 days. Initial elimination half-times of [188]W from olfactory epithelium, respiratory epithelium, and the olfactory bulb from the unoccluded side were longer (0.38, 0.29, and 0.3 days, respectively) than those from the occluded side (0, 0.19, 0.27, and 0.22 days, respectively. Terminal phase half-lives were generally shorter for the occluded side (11.1, 16.3, and 11.1 days, respectively) than the unoccluded side (10.6, 19.9, and 31.5 days, respectively). The olfactory nerve did not effectively transport sodium tungstate to the brain; systemic circulation was considered to be the main source of brain tungsten. An extreme terminal phase half-life of 777 days was reported for the trigeminal nerve, but its significance was not addressed.

3.4.4.2 Oral Exposure

Maternal milk secretions on postnatal days (PNDs) 10–14 from female Sprague-Dawley rats administered sodium tungstate by gavage for a total of 70 days including 14 days prior to mating, 14 days of mating, gestation, lactation, and postweaning at 5 or 125 mg/kg/day (3.1 or 78 mg tungsten/kg/day) were significantly elevated in high-dose animals (0.45 ± 0.01 ppm) relative to either the low-dose group (0.021 ± 0.001 ppm) or controls (0.005 ± 0.0 ppm) (McInturf et al. 2008; U.S. Navy 2007).

3.4.4.4 Other Routes of Exposure

In a study designed to evaluate urinary and serum levels of tungsten, nickel, and cobalt as indicators of absorption and elimination of metals from embedded tungsten alloy fragments, Kalinich et al. (2008) implanted weapons-grade W-Ni-Co alloy (91.1% W, 6.0% Ni, and 2.9% Co) as 4 pellets (low dose) or 20 pellets (high dose) into muscle tissues of male F344 rats. Although pellets removed at various times up to 22 months showed little change in physical appearance, the mass of pellets decreased slightly over 6 months (a statistically significant decrease of approximately 0.7 mg/pellet), and both urinary and serum levels of tungsten, nickel, and cobalt were significantly higher in W-Ni-Co alloy-implanted rats than tantalum pellet-implanted control rats. A lower fraction of tungsten was mobilized compared to the other metals. Urinary tungsten in the low-dose group was approximately 360 ng/mg creatinine at 1 month postimplantation, increased to approximately 410 ng/mg creatinine at 3 months postimplantation, and decreased to approximately 275 ng/mg creatinine at 6 months postimplantation. Urinary tungsten in the high-dose group was highest at 1 month postimplantation (approximately 2,400 ng/mg creatinine) and decreased to approximately 1,270 ng/mg creatinine by 6 months postimplantation. Serum tungsten in the low-dose group was approximately 410 ng/mg/mL at 1 month postimplantation, decreased to approximately 32 ng/mL at 3 months postimplantation, and appeared to be nondetectable at 6 months postimplantation. Tungsten levels were higher in urine than in serum throughout the study.

3.5   MECHANISMS OF ACTION

3.5.2   Mechanisms of Toxicity

Osterburg et al. (2014) evaluated effects of sodium tungstate on an adaptive immunological response in male and female C57BL6 mice. The mice were administered sodium tungstate in the drinking water for 28 days at estimated doses of 62.5–200 mg sodium tungstate/kg/day (39–124.8 mg tungsten/kg/day). Tungsten bioaccumulated in important immunological organs (bone and spleen) and reduced the numbers of activated CD71+ helper and cytotoxic T-cells in the spleen, resulting in reduced lymphocyte production in mice challenged with *Staphylococcal* enterotoxin B. The study authors proposed that inhibition of intracellular reactive-oxygen species production (via inhibition of xanthine and/or NADPH oxidases might explain the observed reduction in T-cell proliferation in response to challenge.

In a study of male Wistar rats receiving sodium tungstate at 119 or 238 mg/kg/day (74.2 or 148.5 mg tungsten/kg/day, respectively) for 14 days, the high-dose group exhibited significantly increased reactive oxygen species (measured as a decrease in reduced GSH:oxidized GSH ratio) in blood, liver, kidney, and spleen; increased reactive oxygen species were also noted in spleens from the low-dose group (Sachdeva et al. 2013). However, there was no change in erythrocyte thiobarbituric acid reactive substances (TBARS) level, which is a marker of lipid peroxidation and another sensitive biomarker of oxidative stress. The study authors suggested that oxidative stress may be a major mechanism for sodium tungstate toxicity.

Bolt et al. (2015) utilized a mouse mammary tumor cell line (66C14) to study *in vivo* and *in vitro* effects of tungsten exposure on mammary tumor growth and metastasis. Groups of female BALB/c mice received tap water only or tap water to which 15 ppm sodium tungstate dihydrate was added for up to 8 weeks. After 4 weeks of treatment, selected mice from each group received 66C14 cell injections into mammary fat pads; tumor size was determined every 3–4 days. When the tumor volume end point from the groups receiving 66C14 cell injections was reached (25–36 days), mice were euthanized and tumors were excised for evaluation. The tungsten-exposed tumor-bearing mice exhibited a significant delay in primary mammary tumor growth (tumor size approximately 600 mm^3 among tungsten-exposed mice versus 900 mm^3 among tumor-bearing tap water-treated controls); there were no significant differences between controls and tungsten-exposed mice regarding numbers of Ki67-positive nuclei in primary mammary tumors. Numbers of lung metastases per lung lobe were not statistically significantly increased by tungsten exposure; however, the average size of lung tumors in the tungsten-exposed mice was 3-fold

greater that of tumor-bearing tap water-treated control mice (p<0.001). Tungsten increased numbers of activated α-SMA positive fibroblasts at the metastatic site, increased circulating levels of matrix metalloproteinases, and increased numbers of myeloid-derived suppressor cells in peripheral blood. *In vitro* testing of 66C14 cells revealed that tungsten exposure did not enhance 66C14 proliferation, but did increase metastases. These results suggest that tungsten caused alterations in the tumor microenvironment rather than acting directly on the 66C14 primary tumor cells.

Guilbert et al. (2011) observed significant apoptosis and increased DNA strand breaks in primary murine bone marrow-derived developing B lymphocytes following *in vitro* exposure to tungsten at ≥100 μg/mL. Tungsten-induced DNA damage (measured as the COMET tail moment) was also elicited in bone marrow of C57BL/6J mice exposed to tungsten at ≥15 μg/mL in the drinking water for 8 weeks. The results implicate B lymphocytes as targets of tungsten toxicity.

Osterburg et al. (2010) evaluated tungsten-induced effects on human peripheral blood lymphocytes *in vitro*. Exposure of cells to 0.1–10 mM sodium tungstate resulted in dose-and time-dependent increases in numbers of cells in early apoptosis; reductions in the number of cells entering the cell cycle; reduced numbers of cells in S and G_2/M phases at 1 mM; changes in the expression of cellular proteins; and reductions in the quantity of interleukin-10, tumor necrosis factor-α, and interleukin-6 at 1 mM. Effects on cell cycle were also noted in THP-1 (immortalized monocytic leukemia line) cells exposed to sodium tungstate. These results indicate that sodium tungstate increased apoptosis in peripheral blood lymphocytes, altered cell cycle progression, and reduced cytokine production.

Paget et al. (2015) exposed human lung (A549), liver (Hep3B), and kidney (Caki-1) cell lines to commercially-available tungsten carbide-cobalt nanoparticles (60 nm in diameter) at concentrations of 5–300 mg/L for 15 minutes or 24, 48, or 72 hours. Flow cytometric measurements at each time point confirmed that the nanoparticles had entered cells and that their concentrations increased with time. However, a decrease at 72 hours only for lung cells indicated that those cells began expelling nanoparticles, perhaps as a protective mechanism. The tungsten carbide-cobalt nanoparticles induced cell mortality in kidney and liver cells at 75 mg/L, but not in lung cells at 150 mg/L. The nanoparticles induced DNA double-strand breaks (based on nuclear γ–H2Ax foci counts) in liver cells and especially in kidney cells starting at 25 mg/L (lung cell line was not evaluated). The nanoparticles also induced cell cycle arrest in liver cells at 25 mg/L and kidney cells at 75 mg/L, but not in the lung cell line. The combined results indicate that WC-Co-nanoparticles induced reactive oxygen species (ROS) production in liver and kidney cells, but that lung cells were more resistant and also expelled the nanoparticles. The

specific contribution of tungsten to the observed effects could not be established because exposure included both tungsten and cobalt.

3.12.3 Ongoing Studies

Short-term toxicity studies (13 weeks) and long-term carcinogenicity studies (2 years) of Harlan Sprague-Dawley rats and B6C3F1/N mice (10/species/sex/dose) administered sodium tungstate dihydrate in the drinking water have been performed for NTP (2014c). As of September 14, 2014, histopathologic evaluations were reported to be in progress, but study reports were not publicly available. A continuous breeding study of male and female Harlan Sprague-Dawley rats with conventional teratology assessment was identified as having been selected; however, additional information regarding study status was not available at the time of the September 14, 2014 update of the website.

4. CHEMICAL AND PHYSICAL INFORMATION

Tungsten metal can exist in two allotropic forms, both with cubic lattice crystal structures; α-tungsten is the more stable allotrope (A2 cell; a body-centered cubic with a=3.16 Å dimension) and β-tungsten is the metastable allotrope (A15 cell; dimension a=5.04 Å) (Charlton and Davis 1955; Kiss 1998). Solute-free exclusion-zones were detected on the surface of tungsten foil, with an average fractional coverage of 58% and average size of 72 µm (Chai et al. 2012).

5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.1 PRODUCTION

No information is available in the Toxics Release Inventory (TRI) database on facilities that manufacture or process tungsten because this chemical is not required to be reported under Section 313 of the Emergency Planning and Community Right-to-Know Act (Title III of the Superfund Amendments and Reauthorization Act of 1986) (EPA 2005).

According to the United States Geological Survey (USGS) Mineral Commodities Report, a single mine located in the state of California produced tungsten concentrates in the United States in 2014, while seven companies in the United States processed tungsten concentrates, ammonium paratungstate, tungsten oxide, and/or scrap to make tungsten powder, tungsten carbide powder, and/or tungsten chemicals (USGS 2015). U.S. production volumes for the years 2006, 2007, 2008, and 2009 (USGS 2011) were reported as

4,490, 4,330, 4,790, and 3,550 metric tons, respectively. U.S. production volumes for the years 2010,
2011, 2012, and 2013 (USGS 2015) were reported as 5,680, 11,000, 9,180, and 8,610 metric tons,
respectively; the estimated U.S. production for 2014 was 8,300 metric tons (USGS 2015).

5.2   IMPORT/EXPORT

Imports and exports of tungsten from 2006 to 2013 are shown in Table 5-1. The major import sources for
2006–2009 were China, Canada, Germany, and Bolivia.

Table 5-1. Import and Export Volumes (Metric Tons) of Tungsten

	2006	2007	2008	2009	2010	2011	2012	2013	2014[a]
Imports:									
Concentrate	2,290	3,880	3,990	3,590	2,740	3,640	3,650	3,690	4,100
Other forms	9,700	9,050	9,060	6,410	9,690	9,600	8,060	8,480	8,900
Exports:									
Concentrate	130	109	496	38	276	169	203	1,060	1,500
Other forms	6,310	5,950	5,480	2,730	4,350	6,960	6,530	6,670	5,800

[a]Estimated.

Sources: USGS 2011 (for 2006–2009 data); USGS 2014 (for 2010–2014 data)

5.3   USE

According to the USGS (2015), more than half of the tungsten consumed in the United States was used in
cemented carbide parts for cutting and wear resistant materials, primarily in the construction,
metalworking, mining, and oil- and gas-drilling industries. The remainder of the tungsten was used to
make tungsten heavy alloys for applications requiring high-density electrodes, filaments, wires, and other
components for electrical, electronic, heating, lighting, and welding applications; steels, superalloys, and
wear-resistant alloys; and chemicals for various applications. Tungsten carbides are employed as co-
catalysts in small-scale hydrogen production from water reactions (Esposito et al. 2012; Garcia-
Esparza et al. 2013). Tungsten carbide is also being used in the fabrication of jewelry (Penrice 2010)
due to its extreme hardness, high polish, and resistance to scratching (e.g., Forever Metals 2009); the
jewelry is sometimes plated with other metals.

6. POTENTIAL FOR HUMAN EXPOSURE

6.2   RELEASES TO THE ENVIRONMENT

No information is available in the TRI database on facilities that release tungsten because this chemical is not required to be reported under Section 313 of the Emergency Planning and Community Right-to-Know Act (Title III of the Superfund Amendments and Reauthorization Act of 1986).

6.4   LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

6.4.1   Air

Tungsten levels in particulate matter ranged from approximately 1.5 to 3.0 ng/m^3 in samples obtained near a metal milling and processing facility in Sweet Home, Oregon, while levels located within 20 km in the nearby towns of Brownsville-Crawfordsville and Lebanon that did not have any industrial tungsten sources were <0.5 ng/m^3. Air concentrations decreased sharply with distance from the mill site, dropping more than 3 orders of magnitude in a distance of 100 m from the mill facility (Sheppard et al. 2007).

6.4.2   Water

Tap water from private wells for 253 households that consumed water in Churchill County, Nevada had tungsten levels ranging from 2 to 510 µg/L, with average and median levels of 11 and 2 µg/L, respectively. When nonconsuming families were included, the range, average, and median levels were below the limits of detection to 610, 26, and 2 µg/L, respectively (Walker and Fosbury 2009). A study was conducted in Saskatchewan, Canada on the relationship between 64 elements (including tungsten) in drinking water and the incidence of NHL. Tungsten concentrations ranged from 0.10 to 3.0 ppm, with a mean of 0.61 ppm and median of 0.21 ppm, for the fewer than 15% of samples that had concentrations above the limit of detection (Witmans et al. 2008).

Migration of tungsten from soil to groundwater was measured at small arms ranges at Camp Edwards on the Massachusetts Military Reservation. Tungsten was detected at concentrations of 0.001–400 mg/L in small arms range wells and at concentrations of 0.001–0.56 mg/L in groundwater monitoring well samples collected approximately 30 m below the ground surface. These levels are not representative of background conditions based upon downgradient samples with concentrations below the limit of detection of <0.0002 mg/L (Clausen and Korte 2009). Tungsten concentrations ranged from 298 to

430 mg/L in Keya Stream, a short river that moves wastewater from industrial complexes in Hsinchu City, Taiwan to Taiwan Strait and then the Pacific Ocean (Hsu et al. 2011).

6.4.3   Sediment and Soil

Tungsten levels in soils near large tungsten mining operations in China ranged from 3.99 to 43.7 mg/kg, with >90% of it in non-soluble residual forms (Lin et al. 2014). Surface dust in Sweet Home, Oregon was collected of waste metal in a hard metal processing plant and from pavement 75 m to the east side of the mill and analyzed for tungsten. The median particle size for both was 1.5 µm, but the maximum size within the facility (51 µm) was much larger than on the pavement (29 µm). This decrease in maximum particle size with distance from the plant and blending with the ambient tungsten was observed on high volume air sample filters (15 µm at 400 m and 21 µm in the town area) (Sheppard et al. 2012). High concentrations of tungsten, up to 2,080 mg/kg, were detected in soil at Camp Edwards on the Massachusetts Military Reservation small arms ranges. Elevated levels were the result of firing tungsten/nylon rounds between 2000 and 2005 in response to an EPA ban on training with lead bullets. Tungsten concentration decreased rapidly with depth, at least by a factor of 10 at 25 cm, but still at detectable levels to a depth of 150 cm. This indicates that tungsten powder is mobile in soils that are aerobic and slightly acidic. These levels were elevated compared to other off- and on-site background soil tungsten levels of 1.3–1.5 mg/kg (Clausen and Korte 2009). Tungsten concentrations ranged from 5.5 to 394 mg/L in sediments from the Keya Stream in Taiwan. The sampling area contains treated industrial effluent discharges from several nearby semiconductor manufacturers (Hsu et al. 2011).

6.4.4   Other Environmental Media

An elevated tungsten concentration of 1,317 ppm was measured in lichen and moss samples near a metal milling operation located in Sweet Home, Oregon, compared to 0.93 ppm in samples collected approximately 20 km away in the nearby town of Crawfordsville, Oregon that does not have such a facility. The presence of tungsten inside the lichen was presumed but not confirmed, no evaluation was reported that assessed surface deposition versus uptake (Sheppard et al. 2007). Levels of tungsten in agricultural soil near the Xihuashan mine, the world's largest tungsten mine operating in Ganzhou, China were 3.99–43.65 mg/kg (Lin et al. 2014). Average tungsten levels in rice root, stem, leaf, and grain grown near the mine were 7.06, 2.34, 4.76, and 0.17 mg/kg. Average enrichment factor values were 0.39, 0.13, 0.28, and 0.01 mg/kg for root, stem, leaf, and grain, respectively, indicating that tungsten from ore is bioavailable but does not bioconcentrate in rice grains. Residual soil (spoil) samples at an abandoned

tungsten mine located in Cumbia, United Kingdom contained 944–1,637 mg/kg (Wilson and Pyatt 2006). Mean levels of tungsten in root, woody tissue, and annual tissue (leaf) from *Calluna vulgaris* and from sphagnum grown in the tungsten spoil were 655, 48.9, 124, and 93.7 mg/kg, respectively. Accumulation factors for woody and leaf samples from *C. vulgaris* were 4.2 and 10.6%, respectively, demonstrating the bioavailability of tungsten; however, tungsten does not bioconcentrate in this species. Control values were not provided in the studies of Lin et al. (2014) and Wilson and Pyatt (2006).

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

The geometric mean concentrations of tungsten in the breathing zone of workers employed at a cemented tungsten carbide manufacturing plant were measured in various work areas of two of the plant's three facilities. The metal separation facility reduced tungsten oxide powder to tungsten metal powder and then carburized it with carbon black to form tungsten carbide, the powder-handling facility then added alloying agents, and the forming and machining facility compacted and sintered the mixtures and machined the material to final dimensions. Levels in the metal separation facility were not reported due to a laboratory error; however, the reclamation work areas are likely to yield the highest tungsten exposures based on cobalt measurements in the facility. Tungsten levels in the powder handling facility ranged from 15.0 to 431.6 $\mu g/m^3$, with the highest levels in the screening area followed by spray drying, milling, powder maintenance, and powder inventory. Levels in the forming and machining facility were generally lower and ranged from 11 to 198 $\mu g/m^3$, with the highest levels in the pressing work area followed by shaping, production control, and grinding. Using an American Conference of Governmental Industrial Hygienists/ International Organization for Standardization/Comité Européen de Normalization (ACGIH/ISO/CEN) lung deposition model, it was estimated that the greatest exposures to respirable tungsten would occur in the metal reclamation areas, followed by screening and powder mixing areas. Levels of cobalt and tungsten did not correlate well, so the authors recommended that epidemiological studies should use material-relevant metrics (rather than surrogates) and respirable particle sizes (over total particulates) to avoid biasing exposure-response relationships (Stefaniak et al. 2009).

The updated tables (February 2015) of the Fourth National Report on Human Exposure to Environmental Chemicals includes measurements of urinary tungsten in 2-year increments during the years from 1999 to 2012 (CDC 2015). Geometric mean urinary tungsten and creatinine-adjusted urinary tungsten concentrations for the U.S. population are presented in Table 6-1. For all ages combined, mean urinary tungsten ranged from 0.071 to 0.099 µg/L; creatinine-adjusted mean urinary tungsten ranged from 0.070 to 0.103 µg/g creatinine. Urinary tungsten concentrations decreased by age group in each study

year group and were approximately 2-fold higher in children ages 6–11 years compared to subjects ≥20 years of age (0.130–0.160 versus 0.062–0.088 µg/L; 0.151–0.209 versus 0.063–0.093 µg/g creatinine).

Table 6-1. Geometric Mean Urinary Tungsten Concentrations and Creatinine-Adjusted Mean Urinary Tungsten Concentrations for the U.S. Population from the National Health and Nutrition Examination Survey

Group	Years	Geometric mean urinary tungsten (95% CI)	
		Unadjusted (µg/L)	Creatinine-adjusted (µg/g creatinine)
Age group			
All ages	1999–2000	0.093 (0.087–0.100)	0.087 (0.080–0.095)
	2001–2002	0.082 (0.073–0.092)	0.078 (0.069–0.087)
	2003–2004	0.071 (0.064–0.078)	0.070 (0.063–0.078)
	2005–2006	0.085 (0.079–0.092)	0.084 (0.080–0.089)
	2007–2008	0.099 (0.093–0.105)	0.103 (0.096–0.111)
	2009–2010	0.081 (0.075–0.087)	0.086 (0.080–0.093)
	2011–2012	0.074 (0.067–0.082)	0.084 (0.076–0.093)
6–11 years of age	1999–2000	0.158 (0.123–0.204)	0.174 (0.150–0.201)
	2001–2002	0.137 (0.110–0.170)	0.168 (0.144–0.197)
	2003–2004	0.130 (0.111–0.151)	0.151 (0.131–0.174)
	2005–2006	0.142 (0.121–0.166)	0.175 (0.152–0.202)
	2007–2008	0.160 (0.138–0.186)	0.209 (0.185–0.235)
	2009–2010	0.151 (0.126–0.180)	0.205 (0.177–0.239)
	2011–2012	0.136 (0.120–0.155)	0.194 (0.174–0.217)
12–19 years of age	1999–2000	0.113 (0.097–0.132)	0.084 (0.078–0.091)
	2001–2002	0.113 (0.095–0.135)	0.081 (0.071–0.092)
	2003–2004	0.105 (0.090–0.122)	0.075 (0.065–0.086)
	2005–2006	0.131 (0.108–0.158)	0.101 (0.086–0.117)
	2007–2008	0.150 (0.136–0.167)	0.118 (0.104–0.132)
	2009–2010	0.101 (0.089–0.115)	0.094 (0.085–0.106)
	2011–2012	0.107 (0.088–0.130)	0.103 (0.091–0.116)
≥20 years	1999–2000	0.084 (0.078–0.091)	0.080 (0.072–0.089)
	2001–2002	0.073 (0.065–0.082)	0.070 (0.063–0.079)
	2003–2004	0.062 (0.056–0.068)	0.063 (0.057–0.071)
	2005–2006	0.075 (0.070–0.081)	0.075 (0.071–0.079)
	2007–2008	0.088 (0.081–0.095)	0.093 (0.087–0.101)
	2009–2010	0.073 (0.068–0.079)	0.078 (0.072–0.084)
	2011–2012	0.066 (0.058–0.074)	0.074 (0.067–0.083)
Gender			
Males	1999–2000	0.107 (0.096–0.120)	0.083 (0.074–0.094)
	2001–2002	0.088 (0.074–0.105)	0.071 (0.060–0.083)
	2003–2004	0.081 (0.071–0.093)	0.068 (0.059–0.079)
	2005–2006	0.095 (0.090–0.101)	0.078 (0.073–0.083)
	2007–2008	0.109 (0.102–0.116)	0.097 (0.090–0.106)
	2009–2010	0.090 (0.082–0.098)	0.082 (0.074–0.089)
	2011–2012	0.084 (0.074–0.095)	0.079 (0.068–0.090)

Table 6-1. Geometric Mean Urinary Tungsten Concentrations and Creatinine-Adjusted Mean Urinary Tungsten Concentrations for the U.S. Population from the National Health and Nutrition Examination Survey

Group	Years	Geometric mean urinary tungsten (95% CI)	
		Unadjusted (µg/L)	Creatinine-adjusted (µg/g creatinine)
Females	1999–2000	0.082 (0.077–0.087)	0.091 (0.085–0.098)
	2001–2002	0.076 (0.069–0.084)	0.085 (0.077–0.094)
	2003–2004	0.062 (0.056–0.069)	0.072 (0.065–0.079)
	2005–2006	0.077 (0.069–0.085)	0.090 (0.085–0.096)
	2007–2008	0.090 (0.083–0.098)	0.109 (0.100–0.118)
	2009–2010	0.074 (0.067–0.081)	0.091 (0.083–0.100)
	2011–2012	0.066 (0.060–0.073)	0.090 (0.083–0.097)
Race/ethnicity			
Mexican Americans	1999–2000	0.113 (0.095–0.133)	0.106 (0.093–0.120)
	2001–2002	0.101 (0.093–0.109)	0.098 (0.090–0.108)
	2003–2004	0.086 (0.073–0.100)	0.079 (0.065–0.096)
	2005–2006	0.102 (0.088–0.118)	0.096 (0.083–0.111)
	2007–2008	0.106 (0.084–0.135)	0.106 (0.083–0.137)
	2009–2010	0.099 (0.089–0.109)	0.103 (0.096–0.111)
	2011–2012	0.097 (0.082–0.114)	0.110 (0.090–0.134)
Non-Hispanic blacks	1999–2000	0.113 (0.101–0.126)	0.073 (0.064–0.083)
	2001–2002	0.096 (0.080–0.116)	0.066 (0.056–0.077)
	2003–2004	0.092 (0.082–0.104)	0.066 (0.059–0.074)
	2005–2006	0.101 (0.088–0.116)	0.073 (0.065–0.082)
	2007–2008	0.120 (0.099–0.146)	0.088 (0.076–0.102)
	2009–2010	0.093 (0.086–0.100)	0.074 (0.067–0.080)
	2011–2012	0.102 (0.087–0.119)	0.079 (0.068–0.092)
Non-Hispanic whites	1999–2000	0.092 (0.084–0.100)	0.091 (0.083–0.100)
	2001–2002	0.076 (0.066–0.088)	0.078 (0.068–0.088)
	2003–2004	0.065 (0.058–0.073)	0.069 (0.060–0.078)
	2005–2006	0.082 (0.075–0.089)	0.085 (0.080–0.090)
	2007–2008	0.093 (0.087–0.101)	0.104 (0.096–0.114)
	2009–2010	0.077 (0.070–0.083)	0.086 (0.078–0.094)
	2011–2012	0.068 (0.060–0.076)	0.082 (0.072–0.094)
All Hispanics	1999–2000	No data	No data
	2001–2002	No data	No data
	2003–2004	No data	No data
	2005–2006	No data	No data
	2007–2008	No data	No data
	2009–2010	No data	No data
	2011–2012	0.082 (0.072–0.092)	0.092 (0.078–0.108)

Table 6-1. Geometric Mean Urinary Tungsten Concentrations and Creatinine-Adjusted Mean Urinary Tungsten Concentrations for the U.S. Population from the National Health and Nutrition Examination Survey

		Geometric mean urinary tungsten (95% CI)	
Group	Years	Unadjusted (µg/L)	Creatinine-adjusted (µg/g creatinine)
Asians	1999–2000	No data	No data
	2001–2002	No data	No data
	2003–2004	No data	No data
	2005–2006	No data	No data
	2007–2008	No data	No data
	2009–2010	No data	No data
	2011–2012	0.067 (0.058–0.077)	0.089 (0.075–0.107)

Source: CDC 2015

7. ANALYTICAL METHODS

7.2 ENVIRONMENTAL SAMPLES

The detection of tungsten at the sub-ppb levels and the speciation of its forms in groundwater and extracts of soil was determined using high-performance liquid chromatography-inductively coupled plasma-mass spectrometry. The detection level for individual species was 0.4–5 µg/L (Bednar et al. 2007).

8. REGULATIONS AND ADVISORIES

Table 8-1. Regulations and Guidelines Applicable to Tungsten

Agency	Description	Information	Reference
NATIONAL			
Regulations and guidelines:			
a. Air			
DOE	PACs[a]		DOE 2012a
	Tungsten		
	PAC-1	10 mg/m^3	
	PAC-2	10 mg/m^3	
	PAC-3	2,000 mg/m^3	
	Tungsten trioxide		
	PAC-1	13 mg/m^3	
	PAC-2	13 mg/m^3	
	PAC-3	210 mg/m^3	
	Tungsten oxide		
	PAC-1	12 mg/m^3	
	PAC-2	130 mg/m^3	
	PAC-3	790 mg/m^3	

Table 8-1. Regulations and Guidelines Applicable to Tungsten

Agency	Description	Information	Reference
	Tungsten hexafluoride		
	PAC-1	4.9 mg/m^3	
	PAC-2	4.9 mg/m^3	
	PAC-3	64 mg/m^3	
	Tungsten carbide		
	PAC-1	11 mg/m^3	
	PAC-2	120 mg/m^3	
	PAC-3	730 mg/m^3	
	Sodium tungstate dihydrate		
	PAC-1	5.4 mg/m^3	
	PAC-2	13 mg/m^3	
	PAC-3	81 mg/m^3	
	Ammonium tungstate		
	PAC-1	4.1 mg/m^3	
	PAC-2	45 mg/m^3	
	PAC-3	270 mg/m^3	
	Tungsten chloride		
	PAC-1	5.3 mg/m^3	
	PAC-2	58 mg/m^3	
	PAC-3	350 mg/m^3	
b. Other	Superfund, emergency planning, and community right-to-know		
EPA	Designated CERCLA hazardous substance and reportable quantity	Final RQ	EPA 2013 40 CFR 302.4
	^{176}W	1,000 Ci	
	^{177}W	100 Ci	
	^{178}W	100 Ci	
	^{179}W	1,000 Ci	
	^{181}W	100 Ci	
	^{185}W	10 Ci	
	^{187}W	100 Ci	
	^{188}W	10 Ci	

[a]Definitions of PAC terminology are available from U.S. Department of Energy (DOE 2012b).

CERCLA = Comprehensive Environmental Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; Ci = Curie; DOE = Department of Energy; EPA = Environmental Protection Agency; PAC = protective action criteria; RQ = reportable quantity

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