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National Toxicology Program
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NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Tungstate Dihydrate (CASRN 10213-10-2) in Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice (Drinking Water Studies)

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Foreword

The National Toxicology Program (NTP), established in 1978, is an interagency program within the Public Health Service of the U.S. Department of Health and Human Services. Its activities are executed through a partnership of the National Institute for Occupational Safety and Health (part of the Centers for Disease Control and Prevention), the Food and Drug Administration (primarily at the National Center for Toxicological Research), and the National Institute of Environmental Health Sciences (part of the National Institutes of Health), where the program is administratively located. NTP offers a unique venue for the testing, research, and analysis of agents of concern to identify toxic and biological effects, provide information that strengthens the science base, and inform decisions by health regulatory and research agencies to safeguard public health. NTP also works to develop and apply new and improved methods and approaches that advance toxicology and better assess health effects from environmental exposures.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to NTP. The studies described in the NTP Technical Report series are designed and conducted to characterize and evaluate the toxicological potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances (e.g., chemicals, physical agents, and mixtures) selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of commercial production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are derived solely from the results of these NTP studies, and extrapolation of the results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection for study per se is not an indicator of a substance's carcinogenic potential.

NTP conducts its studies in compliance with its laboratory health and safety guidelines and the Food and Drug Administration Good Laboratory Practice Regulations and meets or exceeds all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. Studies are subjected to retrospective quality assurance audits before they are presented for public review. Draft reports undergo external peer review before they are finalized and published.

The NTP Technical Reports are available free of charge on the NTP website and cataloged in PubMed, a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health). Data for these studies are included in NTP's Chemical Effects in Biological Systems database.

For questions about the reports and studies, please email NTP or call 984-287-3211.

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Explanation of Levels of Evidence of Carcinogenic Activity

The National Toxicology Program (NTP) describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, in as much as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence)**; one category for uncertain findings **(equivocal evidence)**; one category for no observable effects **(no evidence)**; and one category for experiments that cannot be evaluated because of major flaws **(inadequate study)**. These categories of interpretative conclusions were first adopted in June 1983 and then revised on March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been

adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as "were also related" to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as "may have been" related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

Peer Review

The National Toxicology Program (NTP) convened a virtual external ad hoc panel to peer review the draft *NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Tungstate Dihydrate (CASRN 10213-10-2) in Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice (Drinking Water Studies)* on April 2, 2021. NTP announced the peer-review meeting in the Federal Register (86 FR. 9947. February 17, 2021). The public could view the proceedings online, and opportunities were provided for submission of written and oral public comments. The selection of panel members and conduct of the peer review were in accordance with federal policies and regulations. The panel was charged to:

(1) Review and evaluate the scientific and technical elements of each study and its presentation.

(2) Determine whether each study's experimental design, conduct, and findings support the NTP's conclusions regarding the conditions of each study.

NTP carefully considered the panel's recommendations in finalizing the report. The peer-review report is provided in Appendix F. Other meeting materials are available on the NTP website (https://ntp.niehs.nih.gov/go/meeting).

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Abstract

Sodium tungstate dihydrate (ST) is present naturally in the environment and can enter waterways through the weathering of rocks and soils. ST also is a high-production volume compound that is used in a variety of commercial applications including fire- and waterproofing fabrics, in the preparation of complex compounds (e.g., phosphotungstate and silicotungstate), as a reagent for biological products, and as a precipitant for alkaloids. Tungsten was nominated to the National Toxicology Program (NTP) by the Centers for Disease Control and Prevention to evaluate its potential to cause chronic toxicity and carcinogenicity because of concern about potential human exposure via contaminated drinking water (e.g., in the form of salts like tungstate) and inadequate data to assess human health implications of elevated exposures. ST was selected for study because it is the most prevalent water-soluble form of tungsten. In these studies, Sprague Dawley (Hsd:Sprague Dawley® SD®) rat dams were exposed to ST in drinking water from gestation day (GD) 6 through lactation day (LD) 20. Their pups were exposed to the same exposure concentrations in drinking water from postnatal day (PND) 12 through 3 months or 2 years. Adult male and female B6C3F1/N mice were exposed to ST in drinking water for 3 months or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, rat and mouse peripheral blood erythrocytes, and cells from liver, kidney, and ileum; peripheral blood leukocytes from rats and mice also were assessed for DNA damage.

Perinatal and Three-month Study in Rats

Beginning on GD 6, groups of eight F_0 time-mated female rats were exposed to ST in drinking water throughout gestation and lactation at one of five exposure concentrations (125, 250, 500, 1,000, or 2,000 mg/L) or were provided the vehicle control (deionized water). Groups of 10 F_1 rats per sex continued on in the study after weaning and were given drinking water containing the same respective ST concentrations for 3 months. There were no significant effects of ST exposure on pregnancy status, maternal survival, or littering parameters. By the end of lactation, dams in the 1,000 and 2,000 mg/L groups showed significant decreases in group mean body weight of approximately 10% and 18%, respectively, and water consumption was significantly decreased for the 500, 1,000, and 2,000 mg/L groups relative to the vehicle control group over the LD 17 to LD 21 interval. When adjusted for litter size, the mean body weight of male and female pups in the 2,000 mg/L group on PND 21 was significantly decreased by approximately 16% and 11%, respectively, compared to the corresponding vehicle control groups.

There were no early deaths during the 3-month study. When compared to the vehicle control group, final mean body weights were lower for the 1,000 and 2,000 mg/L males and 2,000 mg/L females. Water consumption was lower for the 1,000 and 2,000 mg/L males and females. The urine xanthine/creatinine ratios were significantly increased in all male and female exposed groups. Serum insulin concentrations were significantly decreased in the 2,000 mg/L males relative to the vehicle control males. Significantly decreased absolute weights were observed in several organs but were considered secondary to body weights reductions. Exposure-related histological lesions were limited to the kidneys and included increased incidences of renal tubule regeneration in the 1,000 and 2,000 mg/L males and females; the increases in the 2,000 mg/L groups were significant relative to the vehicle control group.

Perinatal and Two-year Study in Rats

Beginning on GD 6, F_0 time-mated females were exposed to ST in drinking water throughout gestation and lactation at one of three exposure concentrations (250, 500, or 1,000 mg/L) or were

provided the vehicle control (deionized water). Groups of 50 F_1 rats/sex/group continued on in the study after weaning and were provided drinking water containing the same respective ST concentration as their dam for 2 years. An additional 40 F_1 rats/sex/exposure group were used for interim evaluations and were provided dosed drinking water or the vehicle control for 3, 6, 12, or 18 months. There were no significant effects on reproductive performance, including the percentage of mated females producing pups. During gestation and lactation, the mean body weight of dams in the 1,000 mg/L group was lower than that of the vehicle control group. There were no exposure-related differences between the vehicle control group and the ST-exposed groups in the number of litters, litter size, mean litter weights, sex ratio, or the pup mean weights of males and females.

Interim evaluations were performed on male and female rats at 3, 6, 12, or 18 months for organ weights and tungsten concentrations in plasma, kidney, and urine. Although there was no consistent pattern of changes in kidney weights across sex or over time, kidney tungsten concentrations increased with exposure concentration, and the kidney/plasma ratios were higher than 1 at all exposure concentrations and time points demonstrating retention of tungsten in the kidney. This finding was consistent with the nephrotoxicity observed in the 2-year study.

Survival to study termination was significantly increased in all groups of exposed male rats compared to the vehicle control males, with survival of the vehicle control males being lower than that typically seen in groups of control male Sprague Dawley rats in previous 2-year NTP studies. There were no significant differences in the survival of female groups. At study termination, mean body weights of all groups of exposed males were within 10% of the vehicle control group. In females, mean body weights of the 500 mg/L and 1,000 mg/L groups at study termination were approximately 11% and 21% less than those of the vehicle control group, respectively. Over the course of the 2-year study, mean water consumption for the 250, 500, and 1,000 mg/L groups averaged 93%, 99%, and 84% of the vehicle control males and averaged 95%, 100%, and 91% of the vehicle control females.

The incidences of thyroid gland C-cell adenomas were higher in all exposed groups of female rats, and the increase was significant in the 500 mg/L group relative to the vehicle control group. Although not significant, the incidence of C-cell carcinomas was higher in the 1,000 mg/L females. The incidences of C-cell adenoma or carcinoma (combined) exceeded the historical control range in the 250 and 500 mg/L females.

In the kidney, the incidences of suppurative inflammation of the renal tubules were significantly increased in the 1,000 mg/L males and females, and the incidence of renal tubule regeneration was significantly increased in the 1,000 mg/L females, relative to the respective vehicle control groups.

In the uterus, there was a significant increase in the incidence of atypical hyperplasia, relative to the vehicle control group, in the 500 mg/L females.

Three-month Study in Mice

Groups of 10 male and 10 female mice were exposed to ST in drinking water for 3 months at one of five exposure concentrations (125, 250, 500, 1,000, or 2,000 mg/L) or were provided the vehicle control (deionized water). All mice survived to the end of the study. Over the course of the study, mean body weights were below 90% of the vehicle control group for the 250, 1,000, and 2,000 mg/L females and the 2,000 mg/L males. At study termination, the mean body weights of all exposed groups of males and females were within 10% of the vehicle control groups.

Weekly mean water consumption values were <90% of the vehicle control groups in the 1,000 and 2,000 mg/L males and the 2,000 mg/L females. Lower absolute organ weights were attributed to body weight reductions.

The only histological lesion associated with exposure was in the kidney. The incidences of renal tubule regeneration were higher in the 1,000 and 2,000 mg/L male and female groups compared to the respective vehicle control groups. The increases in the male groups were significant.

Two-year Study in Mice

Groups of 50 male and 50 female mice were exposed to ST in drinking water for 2 years at one of three exposure concentrations (500, 1,000, or 2,000 mg/L) or were provided the vehicle control (deionized water). An additional 40 mice/sex/exposure group were included for interim evaluations at 3, 6, 12, and 18 months.

More males in the ST-exposed groups survived to study termination than did the vehicle control males; however, the differences were not significant. Survival in females was similar across all groups. At study termination, the mean body weight of the 2,000 mg/L males was 88% of the vehicle control group, and water consumption was approximately 78% of the vehicle control group; all other groups of exposed males and all groups of exposed females had mean body weights within 10% of their respective vehicle control groups. Clinical observations included more occurrences of thinness and ruffled fur in exposed males compared to vehicle control males.

Kidney tungsten concentrations increased with exposure concentration and the kidney/plasma ratios were higher than 1 at all exposure concentrations and time points, demonstrating retention of tungsten in the kidney. Renal tubule neoplasms were only recorded in exposed males; one renal tubule adenoma was observed in the 1,000 mg/L males, and two renal tubule carcinomas were observed in the 2,000 mg/L males. Compared to the respective vehicle control groups, there were significantly increased incidences of renal tubule regeneration in all exposed groups of males and in the 1,000 and 2,000 mg/L groups of females.

In the large intestine, the incidences of pigment in the cecum were significantly increased in the 1,000 and 2,000 mg/L males and females.

In the testes, there was a significantly increased incidence of germinal epithelium degeneration in the 500 mg/L group relative to the vehicle control group; the incidences were increased, but not significant, in the 1,000 and 2,000 mg/L groups.

Genetic Toxicology

ST was not mutagenic in any of several bacterial tester strains, with or without exogenous metabolic activation (S9 mix). No increases in micronucleated erythrocytes were seen in male and female rats and mice administered ST in drinking water for 3 months. An exposure concentration-related significant increase in the percent of circulating immature erythrocytes was seen in male and female rats and in male mice, whereas there were no changes in this population of cells in female mice. Significantly increased DNA damage, as measured by the comet assay following administration of ST in drinking water for 3 months, was seen in liver cells of male and female rats and male mice; it also was seen in cells from the ileum of male mice. No increases in the levels of DNA damage were observed in blood leukocytes from either species or in kidney cells from mice.

Conclusions

Under the conditions of these 2-year drinking water studies, there was *no evidence of carcinogenic activity* of sodium tungstate dihydrate (ST) in male Hsd:Sprague Dawley® SD® rats at exposure concentrations of 250, 500, or 1,000 mg/L. There was *equivocal evidence of carcinogenic activity* of ST in female Hsd:Sprague Dawley® SD® rats based on increased incidences of C-cell adenoma or carcinoma (combined) of the thyroid gland.

There was *equivocal evidence of carcinogenic activity* of ST in male B6C3F1/N mice based on the occurrences of renal tubule adenoma or carcinoma (combined) in exposed animals. There was *no evidence of carcinogenic activity* of ST in female B6C3F1/N mice at exposure concentrations of 500, 1,000, or 2,000 mg/L.

Exposure to ST in drinking water caused increased incidences of nonneoplastic lesions in the kidney of male and female rats and mice, in the uterus of female rats, in the large intestine of male and female mice, and in the testes of male mice.

Synonyms: Tungstic acid sodium salt dihydrate

Summary of the Perinatal and Two-year Carcinogenesis and Genetic Toxicology Studies of Sodium Tungstate Dihydrate

	Male Sprague Dawley Rats	Female Sprague Dawley Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Concentrations in Drinking Water	0, 250, 500, or 1,000 mg/L	0, 250, 500, or 1,000 mg/L	0, 500, 1,000, or 2,000 mg/L	0, 500, 1,000, or 2,000 mg/L
Survival Rates	12/50, 26/50, 24/50, 29/50	30/50, 33/50, 31/50, 31/50	26/50, 31/50, 35/50, 35/50	38/50, 41/50, 38/50, 40/50
Body Weights	Exposed groups similar to the vehicle control group	500 mg/L group 11% less than the vehicle control group; 1,000 mg/L group 22% less than the vehicle control group	2,000 mg/L group 12% less than the vehicle control group	Exposed groups similar to the vehicle control group
Nonneoplastic Effects	Kidney: renal tubule, inflammation, suppurative (25/50, 33/50, 35/50, 41/50)	Kidney: renal tubule, inflammation, suppurative (8/50, 9/50, 6/50, 19/50); renal tubule, regeneration (0/50, 0/50, 0/50, 18/50) Uterus: atypical hyperplasia (4/50, 7/50, 19/50, 8/50)	Kidney: renal tubule, regeneration (2/50, 21/50, 32/50, 38/50) Large intestine: cecum, pigment (3/50, 7/50, 17/50, 32/50) Testis: germinal epithelium, degeneration (11/50, 20/50, 20/50, 20/50)	Kidney: renal tubule, regeneration (0/50, 1/50, 7/50, 7/50) Large intestine: cecum, pigment (0/50, 3/50, 7/50, 14/50)
Neoplastic Effects	None	None	None	None
Equivocal Findings	None	Thyroid gland: C-cell adenoma (5/50, 13/50, 13/49, 8/50); C-cell carcinoma (2/50, 2/50, 2/49, 4/50); C-cell adenoma or carcinoma (combined) (7/50, 15/50, 14/49, 11/50)	Kidney: renal tubule adenoma (0/50, 0/50, 1/50, 0/50); renal tubule carcinoma (0/50, 0/50, 0/50, 2/50); renal tubule adenoma or carcinoma (combined) (0/50, 0/50, 1/50, 2/50)	None
Level of Evidence of Carcinogenic Activity	No evidence	Equivocal evidence	Equivocal evidence	No evidence

Genetic Toxicology

Bacterial gene mutations: Negative in *Salmonella typhimurium* strains TA98 and TA100 and *Escherichia coli* strain WP2 *uvrA* (pKM101) with and without S9

Micronucleated erythrocytes (in vivo)

Rat peripheral blood: Negative in males and females

Mouse peripheral blood: Negative in males and females

DNA damage

Rat: Positive in liver (males and females); negative in leukocytes (males and females) and ileum (females); not reported in ileum (males) and kidney (males and females)

Mouse: Positive in liver and ileum (males); negative in liver and ileum (females); negative in kidney and leukocytes (males and females)

Introduction

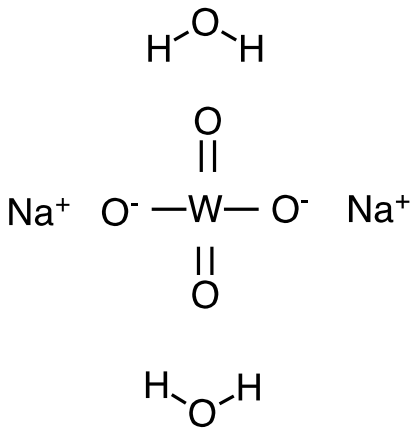


Figure 1. Sodium Tungstate Dihydrate (CASRN 10213-10-2; Chemical Formula: $Na_2WO_4 \cdot 2H_2O$; Molecular Weight: 329.86)

Synonyms: Tungstic acid sodium salt dihydrate.

Chemical and Physical Properties

Tungsten, also called wolfram, is a steel-gray to tin-white metal with a high melting point and good electrical conductivity. Along with chromium and molybdenum (Mo), it is in group VI of the periodic table. It can replace Mo in Mo-containing enzymes,[1; 2] such as aldehyde oxidase, sulfite oxidase,[3] and xanthine oxidase,[4] and renders the enzymes inactive. Sodium tungstate dihydrate (ST) is a chemical intermediate for tungsten and tungsten compounds.[5] ST has high solubility in water and is not volatile. It effloresces in dry air and loses its water at 100°C. As an aqueous solution, it is slightly alkaline (pH 8–9). When heated to decomposition, it emits toxic fumes of sodium oxide.

Production, Use, and Human Exposure

Tungsten and its salts are present naturally in the environment and can enter waterways through the weathering of rocks and soils.[6] Atmospheric tungsten-containing particulates eventually settle to the earth's surface by dry deposition or can be removed from the atmosphere by wet deposition (i.e., precipitation). Upon reaching water and soil, tungsten will be in either soluble (e.g., tungstate ion, WO_4^{2-}) or insoluble forms (e.g., tungsten trioxide) in sediment and soil. Environmental exposure to ST by the general public occurs mainly through contaminated drinking water. For example, tungsten has been detected in the municipal water of Fallon, Nevada. However, the amount of tungsten in drinking water is generally not known.[6]

ST is a high-production volume compound and is produced for industrial purposes by the reaction of a mixture of soft and hard tungsten carbide when combined with a mixture of sodium nitrate and sodium hydroxide in a fusion process. ST is used in a variety of commercial applications including fire- and waterproofing fabrics, in the preparation of complex compounds (e.g., phosphotungstate and silicotungstate), as a reagent for biological products, and as a

precipitant for alkaloids. Occupational exposure can occur through inhalation of dusts and dermal contact during the production or use of tungsten-containing compounds.[6; 7]

Clinically, ST is used as an antidiabetic agent to improve pancreatic function through a combination of hyperglycemia-independent pathways and by its own direct and indirect effects[8; 9]; it is also used to treat infertility in people with diabetes.[10]

Regulatory Status

The American Conference of Governmental Industrial Hygienists limits for tungsten and its soluble compounds include a time-weighted average (TWA) air concentration of 1 mg/m^3 and a short-term exposure limit (STEL) of 3 mg/m^3. The National Institute for Occupational Safety and Health (NIOSH) recommends a 10-hour TWA air concentration of 1 mg/m^3 for tungsten and its soluble compounds. When developing a final rule, the Occupational Safety and Health Administration (OSHA) proposed an 8-hour TWA permissible exposure limit of 1 mg/m^3 and a 15-minute STEL of 3 mg/m^3 for tungsten. NIOSH concurred with OSHA's addition of the STEL, and therefore the final rule established limits for tungsten and its soluble compounds of 1 mg/m^3 as an 8-hour TWA and 3 mg/m^3 as a 15-minute STEL, measured as tungsten.[11] No federal drinking water standard or ambient water quality criterion have been established for tungsten.[12]

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

The National Toxicology Program (NTP) previously evaluated the disposition of tungsten (administered as ST) in female Sprague Dawley rats or C57BL/6N mice.[13; 14] Following a single gavage administration of 1, 10, or 100 mg ST/kg body weight (mg/kg), animals were euthanized 1, 2, 4, and 24 hours postadministration.[13] Plasma, liver, kidney, femur, and uterus concentrations of tungsten increased with increasing dose in both species, with higher tissue concentrations relative to plasma at each respective time point. Tissue concentrations were generally higher in rats relative to mice, with some variation by tissue and time point. In general, tungsten concentrations peaked approximately 4 hours postadministration in rats and 1–4 hours postadministration in mice; concentrations then decreased over time with values approaching background/endogenous concentrations detected in the study (0.04–0.15 µg/g, depending on the matrix) by 24 hours postadministration. After a single intravenous administration of ST at 1 mg/kg in rats and mice, concentrations of tungsten peaked at approximately 1 hour postadministration and steadily decreased through 24 hours postadministration.[13]

After 14 days of gavage administration of ST to rats and mice (10 mg/kg; animals necropsied 24 hours after the last administration), tungsten concentrations in plasma, liver, kidney, femur, and uterus were either similar to or slightly higher than those in animals receiving a single administration of a similar dose and necropsied 24 hours postadministration; this observation suggests minimal accumulation of tungsten following repeat gavage dosing in rats and mice.[14] After exposure via drinking water for 14 days (560 mg/L; dosed water offered until study termination) in rats and mice, tungsten was detected in plasma and tissues; however, a direct comparison between repeat gavage dosing and drinking water exposure cannot be established due to the differences in the time of necropsy after the last exposure. In pregnant rats and mice,

following a similar drinking water exposure paradigm, tungsten was also detected in the fetus, which demonstrated gestational transfer.[14]

Humans

Studies of human exposure to tungsten compounds are limited. The daily dietary intake of tungsten is about 0.01 mg (0.05 µmol), whereas the median for daily urinary excretion is 0.007 mg (0.04 µmol).[15] Tungsten (VI) is well absorbed, and approximately 75% of the amount ingested is excreted in the urine.[16] In a limited study with no specific exposure, four healthy young adults eliminated trace quantities of tungsten in urine (2.0–13.0 µg [0.01–0.07 µmol]) and feces (1.6–5.7 µg [8.7–31 nmol]) over 24-hour periods.[17]

Toxicity

Experimental Animals

Acute toxicity values (i.e., median lethal dose [LD_{50}]) for ST range from 240 to 1,904.1 mg/kg in mice and from 1,904 to 1,928 mg/kg in rats.[18; 19] Acute oral or intravenous administration of ST in mice and rats decreased motor activity and muscle tone, and the animals exhibited ataxia, palpebral ptosis, hunched back, pallor, prostration, and dyspnea.[19]

In a relatively recent study, the subchronic toxicity of an aqueous ST solution in male and female Sprague Dawley rats was evaluated following daily administration via oral gavage to 0, 10, 75, 125, or 200 mg ST/kg body weight/day (mg/kg/day) for 90 days.[20] The kidney was noted as the main target organ of toxicity in both male and female rats dosed at 125 or 200 mg/kg/day, with mild to severe cortical tubule basophilia observed in those dose groups. In males, intraluminal hypospermia with cell debris was observed in the epididymis after administration of 200 mg/kg/day. In both sexes, histopathological changes were observed in the glandular stomach and included inflammation and metaplasia in the high-dose rats (125 and 200 mg/kg/day). The histopathological effects seen in the kidneys indicate that the lowest-observed-adverse-effect level from this study was 125 mg/kg/day and the no-observed-adverse-effect level was 75 mg/kg/day in both sexes of rats for oral subchronic toxicity. There was a significant decrease in feed consumption and body weight gain in males at 200 mg/kg/day from days 77 to 90; however, there was no effect on feed consumption and body weight in females. There were no changes in the hematological or clinical parameters in this study. Histopathological changes were seen in the kidney of male and female rats and in the epididymis of male rats.

In an older study, ST (equivalent to 2% tungsten) administered via the diet to young rats caused the death of all animals within 10 days.[17; 21] When dietary concentrations were reduced to an equivalent of 0.5% tungsten, death occurred in 75% of rats by the end of the 70-day exposure period. When given by gavage or in drinking water to young rats, ST (15–1,000 mg/kg/day [0.051–3.403 mmol/kg/day]) for 4 or 13 weeks produced emesis, anorexia, cachexia, pallor, and dyspnea.[22; 23] At the highest dose, concentrations of urea, creatinine, and total cholesterol were increased, whereas erythrocyte count, glucose, aspartate aminotransferase/alanine aminotransferase, protein, hematocrit, and hemoglobin levels were decreased. All parameter values returned to normal after a recovery period of 6 weeks. Another study in male rats noted effects on spermatogenesis after inhalation exposure to ST (504 µg/m^3 [41.9 ppb]) for 24 hours per day for 17 weeks.[24]

In a 28-day study of B6C3F1/N mice exposed to ST via drinking water at concentrations of 125–2,000 mg/L, NTP found limited effects on humoral and innate immunity, on developing hematopoietic cells in the bone marrow, and on unstimulated splenocyte phenotypes. These data indicated that, under conditions of co-exposure to an immune-stimulating agent, such as tumor cells or genetically dissimilar leukocytes, ST may modulate the normal cell-mediated immune response.[25]

Chronic oral exposure to 5 ppm ST in drinking water has been shown to significantly reduce longevity in male Long-Evans rats.[26] In male Wistar rats, daily gavage administration of <150 mg/kg for up to 300 days produced no significant effects on body weights, organ weights, or survival.[27]

Humans

The data implicating ST as toxic, hazardous, or carcinogenic in humans are limited, although tungsten poisoning has been reported following continuous occupational exposure to dusts and vapors during the refining of tungsten metal.[28]

Reproductive and Developmental Toxicity

Experimental Animals

Although some reproductive and teratological effects have been previously reported in rats and mice exposed to tungsten, those studies are not well characterized. In mice, a single dose of ST (concentration not specified) provided to dams at early fetal organogenesis was shown to produce a high frequency of resorptions but did not induce any fetal malformations.[29] In pregnant rats, doses that did not produce maternal toxicity increased embryo lethality and inhibited bone ossification in fetuses.[30] Another study evaluated the reproductive and neurobehavioral effects of ST in Sprague Dawley rats after 70 days of gavage administration with 0, 5, 62.5, or 125 mg/kg/day starting prior to mating and continuing through gestation and weaning (postnatal day 20). The perinatally exposed offspring showed subtle neurobehavioral effects related to motor activity and emotionality.[31]

Humans

The literature contains no studies about reproductive or developmental toxicity in humans following exposure to tungsten or tungsten compounds.

Carcinogenicity

The literature contains no carcinogenicity studies of ST in experimental animals or epidemiology studies in humans.

Genetic Toxicity

Few reports have been published on the genotoxicity of ST. Of these reports, although ST did not induce gene mutations or chromosomal damage, it was capable of causing DNA damage in vivo. ST did not induce morphological transformation of Syrian hamster embryo (SHE) cells exposed in culture or in a host-mediated in vivo/in vitro assay in which cells from embryos excised from

Syrian golden hamster dams administered 2.5 or 5 mg ST per 100 g maternal weight (intraperitoneal injection) were grown in a transformation assay.[32] Chromosomal aberrations and sister chromatid exchanges were not induced in human lymphocytes or in SHE cells exposed to up to 10 µg/mL ST.[33]

In a series of industry-sponsored studies that followed OECD test guidelines, ST was evaluated in bacterial mutagenicity assays, two mammalian cell mutagenicity assays, and an in vivo micronucleus test.[34] ST was negative in *Salmonella typhimurium* tester strains TA1535, TA1537, TA98, and TA100, and in *Escherichia coli* WP2 *uvrA* when tested up to 5,000 µg/plate in the absence or presence of induced rat liver S9 mix. Chromosomal aberrations were not induced in Chinese hamster ovary cells that were exposed to concentrations of up to 3,500 µg/ml ST for 3 hours (followed by 20 hours of culture) in the absence or presence of S9 mix, or for 20 hours in the absence of S9 mix, which were also up to a top concentration of 3,500 µg/ml ST. ST was negative in the L5178Y $Tk^{+/-}$ mouse lymphoma forward mutation assay when cells were exposed to concentrations of up to 3,500 µg/ml ST for 4 hours in the absence or presence of S9 mix. Lastly, in a bone marrow micronucleus test, micronucleated polychromatic erythrocytes were not induced in male Crl:CD-1 mice exposed to a single dose of 250, 500, or 750 mg/kg ST via gavage at 24 hours after exposure.

A comet assay study revealed significant increases in DNA damage (reported as tail moment) in different types of bone marrow cells obtained from mice exposed to ST via drinking water.[35] Significant increases in tail moment were observed in nonadherent bone marrow cells obtained from mice following exposure to 15 mg/L ST via drinking water for 1, 4, 12, or 16 weeks; 200 mg/L ST via drinking water for 1, 4, or 8 weeks; and 1,000 mg/L ST via drinking water for 4 or 12 weeks. Significant increases in tail moment were also reported for $CD19^{+}$ B cells obtained from the bone marrow of mice exposed to 15 mg/L ST for 1 week or to 15 or 200 mg/L ST for 4 weeks.

Study Rationale

Tungsten was nominated to NTP by the Centers for Disease Control and Prevention to evaluate its potential to cause chronic toxicity and carcinogenicity because of concern about potential human exposure via contaminated drinking water and inadequate data to assess human health implications of elevated exposures. ST was selected for study because tungstate (WO_4^{-2}) is the most naturally occurring form of soluble tungsten, and ST was the most water-soluble form of tungstate.

In the studies described in this Technical Report, drinking water was used as the route of exposure to mimic human exposure.

Materials and Methods

Procurement and Characterization of Sodium Tungstate Dihydrate

Sodium tungstate dihydrate (ST) was procured from Sigma-Aldrich (St. Louis, MO) in two lots (lot 12330JO and lot MKBG9975V). Lot 12330JO was obtained directly from Sigma-Aldrich (St. Louis, MO), whereas lot MKBG9975V was produced by Sigma-Aldrich and obtained from Government Scientific Source, Inc. (Reston, VA). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory and study laboratory at Battelle (Columbus, OH) (Appendix A). Reports on analyses performed in support of the ST studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

Lots 12330JO and MKBG9975V were white solids composed of fine crystals. The 3-month studies used lot 12330JO. For the 2-year studies, the remainder of lot 12330JO was combined with lot MKBG9975V to create lot 07072011.

The identities of the lots were confirmed using infrared spectroscopy. X-ray diffraction patterns were in good agreement with reference standards, and proton-induced x-ray emission spectroscopy yielded expected percent weights of tungsten and sodium. Magnesium (0.7%–0.9%) and aluminum (approximately 0.3%) impurities were identified in both lots. The purities of lots 12330JO and 07072011 were both determined to be approximately 99% using inductively coupled plasma atomic emission spectrometry based on weight percentages of tungsten (55.2%–56.4%) and sodium (13.4%–13.8%). Karl Fisher titration yielded a water content of 9.5% for lot 12330JO and 10.0%–10.3% for lot 07072011, slightly lower than the anticipated 10.9%. Titration of tungstate ion with lead nitrate indicated a purity of 97.6% for lot 12330JO and 98.2% for lot 07072011. Ion chromatography (IC) with a suppressed conductivity detector and liquid chromatography with an inductively coupled plasma-mass spectrometer indicated a purity of 100% for both lots.

Accelerated stability studies were conducted on lot 12330JO and lot MKBG9975V using IC with a suppressed conductivity detector. Stability was confirmed for at least 2 weeks when ST was stored in sealed amber glass bottles at 25°C, 5°C, and −20°C. Therefore, bulk ST was stored in sealed amber glass bottles at 25°C. Periodic analyses of the bulk chemical were conducted during the 3-month and 2-year studies by the study laboratory, and no degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The feed (NIH-07 and NTP-2000) and deionized water used in the 3-month and 2-year studies were analyzed for tungsten and molybdenum concentrations. NIH-07 feed contained approximately 2 ppm tungsten and the concentration in NTP-2000 feed was at the detection limit of the assay (0.80 ppm). Concentrations of tungsten in the deionized water, and the concentration of molybdenum in all feed and water samples, were below the limits of detection of the assay (0.20 to 0.80 ppm).

Stability studies conducted on the 20 mg/L formulation by the analytical chemistry laboratory found that the formulation was stable when sealed and stored in Nalgene bottles for 42 days at

5°C and at room temperature (approximately 25°C). An animal room simulation was conducted using the 20 mg/L formulation stored in a drinking water bottle with aliquots periodically removed to simulate animal drinking. There was no significant loss in tungsten over 7 days at room temperatures.

Dose formulations of ST were prepared monthly (Table A-2). Dose formulations were prepared in deionized water. The 3-month study dose formulations were 0, 125, 250, 500, 1,000, and 2,000 mg/L for both mice and rats. The 2-year mouse study used 0, 500, 1,000 and 2,000 mg/L dose formulations, whereas the 2-year rat study used 0, 250, 500, and 1,000 mg/L dose formulations. Dose formulations were stable for 42 days at room temperature (Appendix A).

Preadministration and postadministration (animal room) analyses of dose formulations were conducted monthly throughout the 3-month studies (Table A-3, Table A-4). During the 2-year studies, preadministration dose formulations were analyzed every 1–3 months, whereas postadministration dose formulations were analyzed every 6–8 months (Table A-5, Table A-6). All preadministration formulations in the 3-month rat and mouse studies were within 10% of the target concentration. In the 3-month mouse study, four postadministration samples were more than 10% below the target concentration, with the largest difference being 12.8% below the target. Three postadministration samples collected from carboys or bottles for the 125, 500, and 2,000 mg/L dose formulations in the 3-month rat study were 10.8% to 12.3% below the corresponding target concentration. In the 2-year studies, all preadministration and postadministration samples were within 10% of the target concentration.

Animal Source

Time-mated (F_0) female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats were obtained from Harlan Inc. (now Envigo, Indianapolis, IN) for the 3-month and 2-year studies. B6C3F1/N mice were obtained from Taconic Biosciences, Inc. (Germantown, NY) for the 3-month and 2-year studies.

Animal Welfare

Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All animal studies were conducted in an animal facility accredited by AAALAC International. Studies were approved by the Battelle (Columbus, OH) Animal Care and Use Committee and conducted in accordance with all relevant National Institutes of Health (NIH) and National Toxicology Program (NTP) animal care and use policies and applicable federal, state, and local regulations and guidelines.

Three-month Studies

Initial Exposure Concentration Selection Rationale

For the perinatal and 3-month study in rats and the 3-month study in adult mice, selection of exposure concentrations of 125, 250, 500, 1,000, and 2,000 mg/L ST was based on available data including a study evaluating the antidiabetic effects of ST in rats following 8 months of exposure via drinking water, which showed no effect on survival and some effect on body weight.[36] The effects from these exposures were then evaluated for selecting exposures for the chronic study, a

study focused on determining the potential chronic toxicity and carcinogenic hazard of sodium tungstate.

Study Design for Rats

F_0 female rats were 11 to 12 weeks old upon receipt. Gestation day (GD) 1 was defined as the first day with evidence of mating. F_0 females were received on GD 1 and held for 5 days. F_0 females were randomly assigned to one of six exposure groups on GD 5 (eight dams/group). Randomization was stratified by body weight that produced similar group mean weights using PATH/TOX SYSTEM software (Xybion Medical Systems Corporation, Cedar Knolls, NJ).

F_0 females were quarantined for 39 days after receipt. Ten nonmated females received in the same shipment as the time-mated dams were designated for disease monitoring and were used for gross necropsies 2 days after arrival; samples were collected for the presence of disease. The health of the F_1 animals was monitored during the study according to the protocols of the NTP Sentinel Animal Program (Appendix C). All test results were negative.

Beginning on GD 6, groups of eight F_0 time-mated females were provided ST in drinking water throughout gestation and lactation at one of five exposure concentrations (125, 250, 500, 1,000, or 2,000 mg/L) or the vehicle control (deionized water). Groups of 10 F_1 rats per sex continued on in the study after weaning and were provided drinking water containing the same respective ST concentration for 3 months.

F_0 female rats were housed individually during gestation and with their respective litters during lactation. Feed and dosed water were available ad libitum. Dam body weights were recorded on GDs 5, 6, 9, 12, 15, 18, and 21 and on lactation days (LDs) 1, 4, 7, 14, and 21. During gestation, water consumption was measured over 3-day intervals from GD 6 through GD 21 (GDs 6–9, 9–12, 12–15, 15–18, and 18–21). The day of parturition was considered to be postnatal (PND) 0. On apparent GD 25, all time-mated females that failed to deliver were euthanized and the uteri were examined and stained for evidence of implantation. Total litter weight and litter weights by sex were collected on PND 1. Individual pup weights were recorded on PNDs 4, 7, 14, and 21. On PND 1, clinical observations, including general appearance were recorded. Pup survival was evaluated and recorded. During lactation, water consumption was measured over 3-day intervals from PND 1 through PND 21 (PNDs 1–4, 4–7, 7–10, 10–14, 14–17, 17–21).

F_1 litters were standardized on PND 4 to eight pups per litter, with at least two pups of each sex and a preference for four males and four females each. Litters that did not meet the minimum of eight pups (or if they had fewer than two pups of either sex) were removed from the study. On the day the last litter reached PND 20, pups were randomly assigned to the 3-month study. For all exposure concentrations, except the 2,000 mg/L group, two pups per sex from five randomly selected litters per exposure group were chosen. For the 2,000 mg/L group, a third male pup was selected from two of the four available litters and a third female pup was selected from the other two litters to obtain the complete number of animals needed for the study. After assignments to the 3-month study were complete, five pups per sex from the remaining vehicle control pups were randomly selected as the end-of-study sentinel animals. On the day the last litter reached PND 21, dams were removed, and the pups were weaned. Weaning marked the beginning of the 3-month study.

After weaning, F_1 rats were housed five per cage. Feed and dosed water were available ad libitum. Water consumption was measured weekly for 3 months. Cages were changed weekly though PND 4, then changed twice weekly. Racks were changed and rotated at least every 2 weeks. Further details of animal maintenance are given in Table 1.

Two diets were used in the rat studies: (1) NIH-07 during the perinatal phase, and (2) NTP-2000 during the postweaning phase. The NIH-07 diet is a higher protein diet that supports reproduction and lactation in rodents, whereas the NTP-2000 diet is a lower protein diet that decreases the incidence of chronic nephropathy in adult rats. Information on feed composition and contaminants for both diets is provided in Appendix B.

Because tungsten is capable of replacing molybdenum (Mo) in Mo-containing enzymes, NTP evaluated the enzyme activity of xanthine oxidase and sulfite oxidase in the liver, kidney, and intestine. Also, because tungsten has been shown to accumulate in femurs of rats and mice after repeated oral gavage administration, urinary calcium and phosphorus concentrations were measured. Other endpoints in the urine, including acetyl glucosaminidase, alkaline phosphatase, and aspartate aminotransferase activities, were also measured.

Urine and blood were also analyzed for tungsten concentrations using validated analytical methods as described in Appendix E.

Study Design for Mice

Male and female B6C3F1/N mice were 4 to 5 weeks old upon receipt and were quarantined for 11 days before study start. Mice were randomly assigned to one of six exposure groups (n = 10 mice/sex/group). Randomization was stratified by body weight that produced similar group mean weights using PATH/TOX SYSTEM software (Xybion Medical Systems Corporation, Cedar Knolls, NJ). Mice were provided ST in drinking water for 3 months at one of five exposure concentrations (125, 250, 500, 1,000, or 2,000 mg/L) or were provided the vehicle control (deionized water).

Five male and five female mice were randomly selected for parasite evaluation and gross observation of disease. The health of the mice was monitored during the study according to the protocols of the NTP Sentinel Animal Program (Appendix C). All test results were negative.

Mice were housed individually (males) or five per cage (females). Feed and dosed water were available ad libitum. Water consumption was measured weekly for 3 months. Cages were changed at least once weekly (males) or twice weekly (females) and rotated every 2 weeks. Racks were changed and rotated every 2 weeks. Further details of animal maintenance are in Table 1. Information on feed composition and contaminants is given in Appendix B.

Clinical Examinations and Pathology

In the 3-month studies in rats and mice, animals were observed twice daily for signs of morbidity and moribundity and were weighed before dosed water administration on day 1, weekly for 3 months, and at study termination. Clinical observations were recorded weekly and at study termination. Water consumption was recorded weekly throughout the study.

At week 12, all F_1 rats were placed in metabolism cages and urine samples were collected during a 16-hour overnight period for urinalysis. Rats were fasted during the collection period and had

access to untreated deionized water while in the metabolism cages. The parameters evaluated are listed in Table 1. Once all urine parameters had been determined, 1 mL of urine from each rat was designated for tungsten analysis, frozen at approximately −20°C, and shipped to Battelle Toxicology Northwest (Richland, WA) for analysis. From the remaining urine, a minimum of 1 mL was frozen at approximately −20°C for xanthine/methionine analysis.

Blood was collected from the retroorbital plexus (rats) or sinus (mice) at the end of the 3-month studies for hematology, clinical chemistry (rats only), erythrocyte micronuclei determination, tungsten determination, serum retention for insulin determination (rats only), and for the comet assay. Animals were anesthetized with a carbon dioxide/oxygen mixture and bled in a random order. Blood was collected into tubes containing ethylenediaminetetraacetic acid (EDTA) (for hematology, erythrocyte micronuclei, and tungsten determination/comet assay) or into serum separator tubes (for clinical chemistry). Hematology parameters were analyzed using an Advia® 120 system (Bayer Diagnostics Division, Tarrytown, NY). Clinical chemistry parameters were analyzed using the Roche cobas c501 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). The parameters measured are listed in Table 1. After evaluation of clinical chemistry parameters, the remaining rat serum was frozen at approximately −80°C and shipped to the NTP Frozen Tissue Bank (Durham, NC) for serum insulin analysis. Samples for erythrocyte micronuclei determination were stored at 2°C–8°C immediately after collection and shipped to Integrated Laboratory Systems, LLC (Durham, NC) for analysis. Samples for the comet assay were transferred to a cryogenic vial, frozen in liquid nitrogen, and stored at −80°C for at least 24 hours before shipment to Integrated Laboratory Systems, LLC (Durham, NC) for analysis. Remaining blood (not used for the comet assay) was designated for tungsten determination, frozen at approximately −80°C, and shipped to Battelle Toxicology Northwest (Richland, WA).

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations from F_1 male and female rats and from male and female mice in the 0, 500, 1,000, and 2,000 mg/L groups. The parameters evaluated are listed in Table 1. Due to inconsistent sample collection and slide staining, an assessment of estrous cyclicity could not be made for F_1 female rats or female mice. Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. After completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was determined microscopically with the aid of a hemocytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemocytometer.

Necropsies were performed on all rats and mice at the end of the 3-month study. Organ weights were recorded for the liver, thymus, right kidney, right testis, heart, and lungs. Tissue samples of 3–5 mm were collected from the left lateral liver lobe, ileum, and a longitudinal section of the left kidney for the comet assay. These samples were transferred to cryogenic vials, frozen in

liquid nitrogen, and stored at −80°C for at least 24 hours before shipment to Integrated Laboratory Systems, LLC (Durham, NC) for analysis. Tissues for microscopic examination were fixed in 10% neutral buffered formalin (except eyes, which were first fixed in Davidson's solution, and testes, vaginal tunics, and epididymides, which were first fixed in modified Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 μm, and stained with hematoxylin and eosin (H&E). Complete histopathological examinations were performed by the study laboratory pathologist on all organs with gross lesions and on all vehicle control and 2,000 mg/L rats and mice. The kidney was identified as a target organ and examined to a no-effect level. Table 1 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment (QA) pathologist, the findings and reviewed slides were submitted to an NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the study laboratory and QA pathologists were resolved by the NTP pathology peer-review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman[37] and Boorman et al.[38]

Two-year Studies

Study Design for Rats

F_0 female rats were 11 to 14 weeks old upon receipt. GD 1 was defined as the first day with evidence of mating. F_0 females were received on GD 2 and held for 4 days. F_0 females were randomly assigned to one of four exposure groups on GD 5. Forty-seven females were assigned to the 0 mg/L group, whereas 41 females were assigned to each of the 250, 500, and 1,000 mg/L groups. Randomization was stratified by body weight that produced similar group mean weights using PATH/TOX SYSTEM software (Xybion Medical Systems Corporation, Cedar Knolls, NJ).

F_0 females were quarantined for 23 days after receipt. Nonmated females designated for disease monitoring and gross necropsies were not received, and therefore 10 undelivered dams were used instead for disease monitoring and gross necropsies on GD 25; samples were collected for the presence of disease or parasites. The health of the F_1 animals was monitored during the study according to the protocols of the NTP Sentinel Animal Program (Appendix C). Pinworms (*Syphacia* spp.) were diagnosed in sentinel animals during routine health monitoring evaluations. Infected animals did not display clinical signs, and no pathological lesions were noted in relation to the presence of the pinworms. Study animals did not receive medication for potential pinworm infection. In accordance with this finding, NTP, in coordination with the testing laboratory, developed and implemented a successful plan of pinworm containment and eradication. NTP required the testing laboratories to actively monitor animals to ensure the continued exclusion of pinworms from all studies going forward. All other test results were negative.

Beginning on GD 6, F_0 females were provided ST in drinking water throughout gestation and lactation at one of four exposure concentrations (0, 250, 500, or 1,000 mg/L); deionized water served as the vehicle control. Groups of 50 F_1 rats/sex/exposure group continued on in the study after weaning and were provided drinking water containing the same respective ST concentration

as their dam for 2 years. An additional 40 F_1 rats/sex/exposure group were used for interim evaluations and provided dosed drinking water for 3, 6, 12, and 18 months.

F_0 female rats were housed individually during gestation and with their respective litters during lactation. Feed and dosed water were available ad libitum. Dam body weights were recorded on GDs 2, 5, 6, 9, 12, 15, 18, and 21 and on LDs 1, 4, 7, 10, 14, 17, and 21. During gestation, water consumption was continuously measured over 3-day intervals from GD 6 through GD 21 (GDs 6–9, 9–12, 12–15, 15–18, and 18–21). The day of parturition was considered to be PND 0. On apparent GD 25, all time-mated females that failed to deliver were euthanized and the uteri were examined and stained for evidence of implantation. Total litter weight and litter weights by sex were collected on PND 1. Individual pup weights were recorded on PNDs 4, 7, 10, 14, and 21. Clinical observations and survival were evaluated throughout lactation. During lactation, water consumption was measured over 3-day intervals from PND 1 through PND 21 (PNDs 1–4, 4–7, 7–10, 10–14, 14–17, 17–21).

F_1 litters were standardized on PND 4 to eight pups per litter, with at least two pups of each sex and a preference for four males and four females each. Litters that did not meet the minimum of eight pups (or if they had fewer than two pups of either sex) were removed from the study. Before weaning, pups (generally two/sex/litter) were randomly assigned to the 2-year study. After assignments to the 2-year study were complete, five pups per sex from the remaining vehicle control pups were randomly selected as the sentinel animals. On the day the last litter reached PND 21, dams were removed, and the pups were weaned. Weaning marked the beginning of the 2-year study.

On the morning of the final PND 21, randomly selected dams (five/exposure group) and one male and one female pup from each selected dam's litter were used for biological sample collection. Blood was collected via cardiac puncture into tubes containing EDTA, the tubes were centrifuged, and the resulting plasma was harvested and stored at −85°C to −60°C until transferred for tungsten analysis.

F_1 rats were housed up to two (males) or five (females) per cage. Feed and dosed water were available ad libitum. Water consumption was measured at the beginning of the study, weekly for 13 weeks, and then at 4-week intervals thereafter. Cages were changed weekly though PND 4, then changed twice weekly. Racks were changed and rotated at least every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix B.

Study Design for Mice

Male and female B6C3F1/N mice were 3 to 4 weeks old upon receipt and were quarantined for 11 days before study start. Mice were randomly assigned to one of four groups (n = 50 mice/sex/exposure group). Mice were provided ST in drinking water for 2 years at one of three exposure concentrations (500, 1,000, or 2,000 mg/L) or were given the vehicle control (deionized water). An additional 40 mice/sex/exposure group were included for interim evaluations at 3, 6, 12, and 18 months. Randomization was stratified by body weight that produced similar group mean weights using PATH/TOX SYSTEM software (Xybion Medical Systems Corporation, Cedar Knolls, NJ).

Before study start, five male and five female mice were randomly selected for parasite evaluation and gross observation of disease. The health of the mice was monitored during the study according to the protocols of the NTP Sentinel Animal Program (Appendix C). All test results were negative.

Mice were housed individually (males) or four (females) per cage. Feed and dosed water were available ad libitum. Water consumption was measured at the beginning of the study, weekly for 13 weeks, and then at 4-week intervals thereafter. Cages were changed at least once weekly (males) or twice weekly (females) and rotated every 2 weeks. Racks were changed and rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is given in Appendix B.

Clinical Examinations, Tungsten Concentrations, and Pathology

In the 2-year studies in rats and mice, animals were observed twice daily for signs of morbidity and moribundity and were weighed before dosed water administration on day 1, weekly for the next 13 weeks, every 4 weeks thereafter, and at study termination. Clinical observations were recorded every 4 weeks beginning on day 36 and at study termination. Water consumption was recorded at the beginning of the study, weekly for 13 weeks, and at 4-week intervals thereafter.

At the 3-, 6-, 12-, and 18-month interim evaluations, urine, feces, blood, and tissues (liver, kidneys, stomach, small intestine, and bone) were collected from up to 10 predesignated F_1 rats/sex/exposure group and up to 10 predesignated mice/sex/exposure group for determination of tungsten concentrations. Organ weights were recorded for selected tissues. Early death animals were not replaced. On the morning of the day before scheduled blood collection, animals were moved to metabolism cages (one animal/cage); while in the metabolism cages, the animals had ad libitum access to feed and their assigned concentration of dosed drinking water. Urine and feces were collected over a 24-hour period, and urine volume, urine creatinine, and fecal weights were recorded. Blood was collected via cardiac puncture into tubes containing K_3 EDTA, centrifuged, and the plasma harvested. Immediately after blood collection, the animals were euthanized and the entire liver, both kidneys, stomach (separated into glandular and non-glandular), small intestine, and both femurs were collected, weighed, and maintained on dry ice until moved into storage. All samples were stored at −85°C to −60°C until transferred for analysis.

Complete necropsies and microscopic examinations were performed on all F_1 rats and all mice at the end of the 2-year studies. At necropsy, all organs and tissues were examined for grossly visible lesions and all major tissues were fixed and preserved in 10% neutral buffered formalin (except eyes, which were first fixed in Davidson's solution, and testes, vaginal tunics, and epididymides, which were first fixed in modified Davidson's solution). Tissues were processed and trimmed, embedded in paraffin, sectioned at a thickness of 4 to 6 μm, and stained with H&E for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. In the original evaluation of the uterus, a transverse section through each uterine horn, approximately 0.5 cm cranial to cervix, was collected for histopathology evaluation. For the residual tissue evaluation of the uterus, all remaining uterine tissue, including the cervix and vaginal tissue, was sectioned longitudinally, processed, and examined histologically. Results from the residual uterine evaluation were combined with those

from the original, transverse section of uterus. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by an independent QA laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a QA pathologist evaluated slides from all neoplasms and all potential target organs, which included the kidney of rats and mice; the liver of male rats and male mice; the uterus of female rats and female mice; the cecum of mice; the adrenal cortex and mandibular lymph node of male rats; the testis and epididymis of male mice; the ovary and nose of female rats; and the spleen and mesenteric lymph node of female mice.

The QA report and the reviewed slides were submitted to the NTP pathologist, who reviewed and addressed any inconsistencies in the diagnoses made by the laboratory and QA pathologist. The QA pathologist, who served as the coordinator of the Pathology Working Group (PWG) presented representative histopathology slides containing examples of lesions related to test agent administration, examples of disagreements in diagnoses between the laboratory and QA pathologist, or lesions of general interest to the PWG for review. The PWG consisted of the NTP pathologist and other pathologists experienced in rodent toxicological pathology. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, QA pathologist, and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman[37] and Boorman et al.[39] or subsequent analyses of the pathology data; the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of Brix et al.[40]

In addition to the routine pathology review, a step section analysis of the kidneys from the chronic mouse study was performed. Paraffin-embedded kidneys were sectioned at 1 mm intervals to obtain three to four additional sections per kidney to allow for the observation of additional renal neoplasms. The evaluation of these additional kidney slides was conducted by a board-certified pathologist other than the QA pathologist. The step section pathologist shared the evaluation findings with the NTP pathologist and the QA pathologist. Representative slides from the kidney step section review were taken to the PWG for the chronic ST mouse study and were examined by the members of that PWG. The final diagnosis of the kidney step section review constitutes a consensus of the kidney step section pathologist, the NTP pathologist, and the PWG participants.

Table 1. Experimental Design and Materials and Methods in the Three-month and Two-year Drinking Water Studies of Sodium Tungstate Dihydrate

Three-month Studies	Two-year Studies
Study Laboratory	
Rats: Battelle (Columbus, OH)	Same as 3-month studies
Mice: Battelle (Columbus, OH)	
Strain and Species	
Rats: Sprague Dawley (Hsd:Sprague Dawley® SD®)	Same as 3-month studies
Mice: B6C3F1/N	
Animal Source	
Rats: Harlan Laboratories, Inc. (now Envigo; Indianapolis, IN)	Same as 3-month studies
Mice: Taconic Biosciences, Inc. (Germantown, NY)	
Time Held Before Studies	
F_0 female rats: 5 days	F_0 female rats: 4 days
Mice: 11 days	Mice: 11 days
Average Age When Studies Began	
F_0 female rats: 12 to 13 weeks	F_0 female rats: 12 to 15 weeks
Mice: 5 to 6 weeks	Mice: 5 to 6 weeks
Date of First Exposure	
F_0 female rats: May 23, 2009 F_1 rats: June 30 (males) or July 1 (females), 2009	F_0 female rats: December 23, 2011 F_1 rats: January 30 (males) or 31 (females), 2012
Mice: June 1 (females) or 2 (males), 2009	Mice: January 16 (females) or 17 (males), 2012
Duration of Exposure	
F_0 female rats: GD 6 to LD 21 F_1 rats: 3 months	F_0 female rats: GD 6 to LD 21 F_1 rats (interim evaluations): 3, 6, 12, and 18 months F_1 rats (2-year study): 105 weeks
Mice: 3 months	Mice (interim evaluations): 3, 6, 12, and 18 months Mice (2-year study): 105 weeks
Date of Last Exposure	
F_0 female rats: June 30, 2009 F_1 rats: September 28 (males) or 29 (females), 2009	F_0 female rats: January 30, 2012 F_1 rats (3-month interim): May 1 (males) or 2 (females), 2012 F_1 rats (6-month interim): August 1 (males) or 2 (females), 2012 F_1 rats (12-month interim): January 30 (males) or 31 (females), 2013 F_1 rats (18-month interim): July 31 (males) or August 1 (females), 2013 F_1 rats (2-year study): January 28 (males) or 31 (females), 2014
Mice: August 31 (females) or September 1 (males), 2009	Mice (3-month interim): April 17 (females) or 18 (males), 2012

Three-month Studies	Two-year Studies
	Mice (6-month interim): July 17 (females) or 18 (males), 2012 Mice (12-month interim): January 15 (females) or 16 (males), 2013 Mice (18-month interim): July 16 (females) or 17 (males), 2013 Mice (2-year study): January 15 (females) or 17 (males), 2014
Necropsy Dates	
F_1 rats: September 28 (males) or 29 (females), 2009	F_1 rats (2-year study): January 27 and 28 (males) or 29 to 31 (females), 2014
Mice: August 31 (females) or September 1 (males), 2009	Mice (2-year study): January 13 to 15 (females) or 15 to 17 (males), 2014
Average Age at Necropsy	
F_1 rats: 15 to 16 weeks	F_1 rats (2-year study): 108 weeks
Mice: 18 to 19 weeks	Mice (2-year study): 109 to 110 weeks
Size of Study Groups	
F_0 female rats: 8 F_1 rats: 10/sex	F_0 female rats: 47 (0 mg/L) or 41 (250, 500, and 1,000 mg/L) F_1 rats (interim evaluations): 40/sex F_1 rats (2-year study): 50/sex
Mice: 10/sex	Mice (interim evaluations): 40/sex Mice (2-year study): 50/sex
Method of Distribution	
Rats: Dams were distributed randomly into groups of approximately equal initial mean body weights. Pups were standardized on each litter's respective PND 4 to a maximum of eight pups per litter. Weaned pups were randomized on PND 20.	Rats: Dams were distributed randomly into groups of approximately equal initial mean body weights. Pups were standardized on each litter's respective PND 4 to a maximum of eight pups per litter. Weaned pups were randomized on PND 19.
Mice: Animals were distributed randomly into groups of approximately equal initial mean body weights.	Mice: Same as 3-month study
Animals per Cage	
F_0 female rats: 1 (with litter) F_1 rats: 5 (males) or 5 (females)	F_0 female rats: 1 (with litter) F_1 rats: up to 2 (males) or up to 5 (females)
Mice: 1 (male) or 5 (females)	Mice: 1 (male) or up to 4 (females)
Method of Animal Identification	
F_0 female rats: Cage card and tail marking with permanent pen F_1 rats: Cage card and tail tattoo	Same as 3-month studies
Mice: Cage card and tail tattoo	
Diet	
Irradiated NIH-07 wafer feed (rats; perinatal phase) or irradiated NTP-2000 wafer feed (rats and mice; 3-month studies) (Zeigler Brothers Inc., Gardners, PA), available ad libitum, changed weekly	Same as 3-month studies

Three-month Studies	Two-year Studies
Water	
Tap water (Columbus municipal supply), deionized, either untreated or containing a formulation of ST via glass bottles (Wheaton Science Products, Millville, NJ [rats and female mice] or Supelco, Bellefonte, PA [male mice]), available ad libitum, changed twice weekly	Tap water (Columbus municipal supply), deionized, either untreated or containing a formulation of ST via glass bottles (Fisher Scientific, Pittsburgh, PA [rats], Qorpak, Bridgeville, PA [female mice], or VWR, West Chester, PA [male mice]), available ad libitum, changed twice weekly (rats and female mice) or once weekly (male mice)
Cages	
Solid polycarbonate (Lab Products, Inc., Seaford, DE) Rats: changed weekly through PND 4, then twice weekly, rotated every 2 weeks Mice: Changed weekly (males) or twice weekly (females), rotated every 2 weeks	Same as 3-month studies
Bedding	
Irradiated Sani-Chips® (P.J. Murphy Forest Products Corporation, Montville, NJ), changed with cage changes	Same as 3-month studies
Rack Filters	
Spun-bonded polyester (Snow Filtration Company, Cincinnati, OH), changed every 2 weeks	Same as 3-month studies
Racks	
Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated every 2 weeks	Same as 3-month studies
Animal Room Environment	
Temperature: 72°F ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Same as 3-month studies
Exposure Concentrations	
0, 125, 250, 500, 1,000, or 2,000 mg/L	Rats: 0, 250, 500, or 1,000 mg/L Mice: 0, 500, 1,000, or 2,000 mg/L
Type and Frequency of Observation	
F_0 female rats: Observed twice daily. Weighed on GDs 5, 6, 9, 12, 15, 18, and 21 and on LDs 1, 4, 7, 14, and 21. Water consumption was measured over 3-day intervals from GD 6 through LD 21.	F_0 female rats: Observed twice daily. Weighed on GDs 2, 5, 6, 9, 12, 15, 18, and 21 and on LDs 1, 4, 7, 10, 14, 17, and 21. Water consumption was measured over 3-day intervals from GD 6 through LD 21.
F_1 rats: Observed twice daily. Litter data (total litter weight, litter weights by sex, and litter observations) were recorded on PND 1. Pup survival was evaluated and recorded. Individual pups were weighed on PNDs 4, 7, 14, and 21, weekly for 3 months, and at the end of the study. Clinical observations were recorded weekly and at the end of the study. Water consumption was recorded weekly for the duration of the study.	F_1 rats: Observed twice daily. Litter data (total litter weight, litter weights by sex, and litter observations) were recorded on PND 1. Pups per litter were recorded on PNDs 2 and 3. Pups were weighed on PNDs 4, 7, 10, 14, and 21, weekly for 3 months, then every 4 weeks, and at the end of the study. Clinical observations were recorded every 4 weeks beginning at week 6 and at the end of the study. Water consumption was recorded

Three-month Studies	Two-year Studies
	initially, weekly for 3 months, and then at 4-week intervals thereafter.
Mice: Observed twice daily. Weighed initially, weekly for 3 months, and at the end of the study. Clinical observations were recorded weekly and at the end of the study. Water consumption was recorded weekly for the duration of the study.	Mice: Observed twice daily. Weighed initially, weekly for 3 months, then every 4 weeks, and at the end of the study. Clinical observations were recorded at week 6 then every 4 weeks and at the end of the study. Water consumption was measured initially, weekly for 3 months, and then at 4-week intervals thereafter.
Method of Euthanasia	
Carbon dioxide	Same as 3-month studies
Necropsy	
Necropsies were performed on all animals. Organs weighed at the end of the study were: liver, thymus, right kidney, right testis, heart, and lungs.	Necropsies were performed on all core animals. Organs collected and weighed at the 3-, 6-, 12-, and 18-month interim evaluations were: liver, left and right kidneys, stomach, small intestine, and bone (femur).
Clinical Pathology	
At the end of the studies, blood was collected from the retroorbital plexus (rats) or sinus (mice) for clinical chemistry (rats only), hematology, and insulin determination (rats only). *Hematology*: erythrocyte count, mean corpuscular volume, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, leukocyte count and differentials, reticulocyte count, and platelet count *Clinical chemistry (rats)*: alanine aminotransferase, albumin, alkaline phosphatase, bile acids, cholesterol, creatinine, creatine kinase, glucose, sorbitol dehydrogenase, total protein, triglycerides, and urea nitrogen	None
Histopathology	
Complete histopathology was performed on all F_1 rats and all mice in the vehicle control and 2,000 mg/L groups. The kidney was identified as a target organ and examined to a no-effect level. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart, kidney, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), larynx, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pharynx, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spinal cord, spleen, sternum, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, vagina, and Zymbal's gland.	Complete histopathology was performed on all core F_1 rats and all core mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart, kidney, large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, uterus (including cervix), and vagina.

Three-month Studies	Two-year Studies
Sperm Motility	
At the end of the studies, sperm samples were collected from F_1 male rats and male mice in the vehicle control, 500, 1,000, and 2,000 mg/L groups for sperm evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed.	None
Urinalysis	
During week 12, urine samples were collected from F_1 rats in metabolism cages for urinalysis.	At the 3-, 6-, 12-, and 18-month interim evaluations, urine was collected from up to 10 predesignated rats/sex/exposure group in metabolism cages for urinalysis.
Parameters Evaluated (rats): specific gravity, volume, sediment exam, protein, glucose, creatinine, calcium, phosphorous, N-acetyl-β-glucosaminidase, alkaline phosphatase, and aspartate aminotransferase	*Parameters Evaluated (rats)*: volume and creatinine
Xanthine and Methionine Analysis	
At the end of the studies, urine samples were collected from all F_1 rats for xanthine/methionine determination.	None
Internal Dose Assessment	
At the end of the studies, urine (F_1 rats) and blood (F_1 rats, mice) samples were collected for tungsten determinations.	At the 3-, 6-, 12-, and 18-month interim evaluations, urine, plasma, and kidney were collected from up to 10 predesignated animals/sex/exposure group for tungsten determination. Tungsten concentrations were determined using validated analytical methods (Appendix E). On PND 21, tungsten concentrations were determined in plasma from dams (five/exposure group) and their pups (one male and one female from each selected dam's litter).

GD = gestation day; LD = lactation day; PND = postnatal day.

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier[41] and is presented graphically. Animals surviving to the end of the observation period are treated as censored observations, as are animals dying from unnatural causes within the observation period. Animals dying from natural causes are included in analyses and are treated as uncensored observations. For the 2-year mouse study, exposure concentration-related trends are identified with Tarone's life-table test,[42] and pairwise exposure concentration-related effects are assessed using Cox's method.[43] For the rat perinatal study, exposure concentration-related trends and pairwise exposure concentration-related effects on survival are assessed using a Cox proportional hazards model[43] with a random litter effect. All reported p values for the survival analyses are two-sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented as the numbers of animals bearing such lesions at a specific anatomical site. For calculation of incidence rates, the denominator for most neoplasms and all nonneoplastic lesions is the number of animals where the site was examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, the denominator consists of the number of animals that had a gross abnormality. When neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominator consists of the number of animals on which a necropsy was performed. Additional study data also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal euthanasia.

Analysis of Neoplasm and Nonneoplastic Lesion Incidence

Statistical analyses of neoplasm and nonneoplastic lesion incidence considered two features of the data. Some animals did not survive the entire 2 years of the study, so survival differences between groups had to be considered. Also, for the rat study, up to two animals per sex were randomly selected from each rat litter to participate in the study. The statistical analysis of lesion incidence used the Poly-3 test to account for survival differences, with a Rao-Scott adjustment for litter effects as needed. This analysis is described below.

The Poly-k test[44-46] was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to account for survival differences. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is 1 if the animal had a lesion at that site or if it survived until terminal euthanasia; if the animal died before terminal euthanasia and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only on the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time.[44] Unless otherwise specified, a value of k = 3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier[44] following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control Fischer 344 rats and B6C3F1 mice.[47] Bailer and Portier[44] showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams.[48] Poly-3 tests used the continuity correction described by Nam.[49]

Littermates tend to be more like each other than fetuses/pups in other litters. Failure to account for correlation within litters leads to underestimates of variance in statistical tests, resulting in higher probabilities of Type I errors ("false positives"). Because up to two pups/sex/litter were

present in the core rat study, the Poly-3 test was modified to accommodate litter effects using the Rao-Scott approach.[50] The Rao-Scott approach accounts for litter effects by estimating the ratio of the variance in the presence of litter effects to the variance in the absence of litter effects. This ratio is then used to adjust the sample size downward to yield the estimated variance in the presence of litter effects. The Rao-Scott approach was implemented in the Poly-3 test as recommended by Fung et al.,[51] formula $\overline{T}_{RS2}$.

Tests of significance included pairwise comparisons of each exposure group with control groups and a test for an overall exposure concentration-related trend. Continuity-corrected Rao-Scott-adjusted Poly-3 tests were used in the analysis of lesion incidence and reported p values are one-sided. The significance of a lower incidence or negative trend in lesions is approximated as 1−p with the letter N added (e.g., p = 0.99 is presented as p = 0.01N). For neoplasms and nonneoplastic lesions observed without litter structure (e.g., for the mouse studies), Poly-3 tests that included the continuity correction, but without adjustment for potential litter effects, were used for trend and pairwise comparisons to the control group.

To evaluate incidence rates by litter, the proportions of litters affected by each lesion type were tested among groups. Cochran-Armitage trend tests and Fisher's exact tests[52] were used to test for trends and pairwise differences from the control group, respectively.

Analysis of Continuous Variables

Before statistical analysis, extreme values identified by the outlier test of Dixon and Massey[53] for small samples ($n < 20$) and Tukey's outer fences method[54] for large samples ($n \geq 20$) were examined by NTP personnel, and implausible values were eliminated from the analysis.

For the perinatal and 2-year study in rats and the 3-month and 2-year studies in mice, litter effects were not considered in the analysis of the continuous data. Organ and body weight measurements, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett[55] and Williams.[56; 57] Dam gestational and lactational feed consumption, litter sizes, pup survival, implantations, number of resorptions, and proportions of male pups per litter for all rat studies were analyzed using the nonparametric multiple comparison methods of Shirley[58] (as modified by Williams[59] and Dunn[60]) given that these endpoints typically have skewed distributions. For all quantitative endpoints unaffected by litter structure, the Jonckheere test[61] was used to assess the significance of the exposure concentration-related trends and to determine, at the 0.01 level of significance, whether a trend-sensitive test (the Williams or Shirley test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic exposure concentration-related trend (the Dunnett or Dunn test).

For the perinatal and 3-month study in rats, there were two or more littermates in each exposure group analyzed. Consequently, organ and body weight endpoints were analyzed using linear mixed models, with litter as a random effect. To adjust for multiple comparisons, a Dunnett-Hsu adjustment was used.[62] For other endpoints, for which normality was not assumed, the trend across exposure groups was analyzed by a permutation test based on the Jonckheere trend test implemented by randomly permuting whole litters across exposure groups and bootstrapping within the litters (see, for example, Davison and Hinckley[63]). Pairwise comparisons were made

by using a modified Wilcoxon test that incorporated litter effects.[64] The Hommel procedure was used to adjust for multiple comparisons.[65]

Postweaning body weights were measured on two pups/sex/litter in the perinatal and 2-year and perinatal and 3-month rat studies; more than two pups/sex/litter were possible in preweaning body weight measurements. The analyses of pup body weights and body weights adjusted for litter size (described below) took litter effects into account using a mixed model with litter as random effect. To adjust for multiple comparisons, a Dunnett-Hsu adjustment was used.[62] Dam body weights during gestation and lactation were analyzed with the parametric multiple comparison procedures of Dunnett[55] or Williams,[56; 57] depending on whether the Jonckheere test indicated the use of a trend-sensitive test. P values for these analyses are two-sided.

Analysis of Gestational and Fertility Indices

Cochran-Armitage trend tests were used to test the significance of trends in gestational and fertility data across exposure groups. Fisher's exact test was used to conduct pairwise comparisons of each exposure group with the control group. P values for these analyses are two-sided.

Body Weight Adjustments

To adjust preweaning pup body weights for live litter size, a linear model was fit to body weights as a function of exposure concentration and litter size. The estimated coefficient of litter size was then used to adjust each pup body weight based on the difference between its litter size and the mean litter size. Prestandardization PND 4 body weights were adjusted for PND 1 litter size, and body weights measured between PND 4 poststandardization and PND 21 were adjusted for PND 4 poststandardization litter size. After adjustment, body weights were analyzed with a linear mixed model with a random litter effect.

Historical Control Data

The concurrent control group is the most valid comparison to the exposed groups and is the only control group analyzed statistically in NTP bioassays. Historical control data are often helpful in interpreting potential exposure-related effects, however, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical control data must be generally similar. Significant factors affecting the background incidence of neoplasms at a variety of sites are diet, sex, strain/stock, and route of exposure. The NTP historical control database contains all 2-year studies for each species, sex, and strain/stock with histopathological findings in control animals completed within the most recent 5-year period[66-68] including the concurrent control for comparison across multiple technical reports. In general, the historical control data for a given study includes studies using the same route of administration, and the overall incidence of neoplasms in control groups for all routes of administration are included for comparison, including the current study.

Quality Assurance Methods

The 3-month and 2-year studies were conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice Regulations.[69] In addition, the 3-month and 2-year study reports were audited retrospectively by an independent QA contractor against study

records submitted to the NTP Archives. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The genetic toxicity of ST was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli*, to increase the frequency of micronucleated erythrocytes in rat and mouse peripheral blood, and to increase DNA damage in cells from liver, kidney, ileum, and peripheral blood from rats and mice. The protocols and results for these studies are given in Appendix D.

The genetic toxicity studies are an outcome of an earlier effort by NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on the results from several in vitro and in vivo short-term tests measuring functionally distinct genotoxicity endpoints. The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage on the basis of the relationship between electrophilicity and mutagenicity[70] and the somatic mutation theory of cancer.[71; 72] It should be noted, however, that not all cancers arise through genotoxic mechanisms.

Bacterial Mutagenicity

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites.[73] A positive response in the *Salmonella* test was shown to be the most predictive in vitro indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens).[74; 75] Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone; however, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation. The protocol for these studies and the results are given in Appendix D.

Peripheral Blood Micronucleus Test

Micronuclei are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division.[76; 77] The predictivity for carcinogenicity of a positive response in acute in vivo bone marrow chromosome aberration or micronucleus tests appears to be less than that of the *Salmonella* test.[78; 79] However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies.[80] Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of in vivo genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical. The protocol for these studies and the results are given in Appendix D.

Comet Assay

The alkaline (pH > 13) comet assay[81] (also known as the single cell gel electrophoresis assay) detects DNA damage in any of a variety of eukaryotic cell types[82-85]; cell division is not required. The type of DNA damage detected includes nicks, adducts, strand breaks, and abasic sites that are converted to DNA strand breaks after treatment of cells in an alkaline (pH > 13) solution. Transient DNA strand breaks generated by the process of DNA excision repair might also be detected. DNA damage caused by crosslinking agents has been detected as a reduction of DNA migration.[86; 87] The fate of the DNA damage detected by the comet assay is varied; most of the damage is rapidly repaired and results in no sustained effect on the tissue, but some might result in cell death or be incorrectly processed by repair proteins and lead to a fixed mutation or chromosomal alteration. The protocol for these studies and the results are given in Appendix D.

In the rat study, ileum (male rats) and kidney (male and female rats) samples were tested in the comet assay, but due to inconsistencies of the results, the assay was deemed an invalid test for these samples.

Results

Data Availability

The National Toxicology Program (NTP) evaluated all study data. Data relevant for evaluating toxicological findings are presented here. All study data are available in the NTP Chemical Effects in Biological Systems (CEBS) database: https://doi.org/10.22427/NTP-DATA-TR-599.[88]

Rats

Three-month Study (Perinatal Phase)

No significant effects related to sodium tungstate dihydrate (ST) exposure were observed on pregnancy status, maternal survival, or the number of dams that littered (Table 2). There were no clinical observations in dams (Appendix G). One dam in the 250 mg/L group was euthanized on gestation day (GD) 25 due to moribundity associated with incomplete labor, and one 2,000 mg/L dam (and her pups) was euthanized moribund on lactation day (LD) 6.

No significant effects on dam mean body weight during gestation were observed, but mean body weights were significantly decreased in the 1,000 and 2,000 mg/L groups starting at LD 14. By the end of lactation (LD 21), the 1,000 and 2,000 mg/L dam groups showed significant decreases in group mean body weight of approximately 10% and 18%, respectively, when compared to the vehicle control group (Table 3).

Table 2. Summary of the Disposition of F_0 Female Rats during Perinatal Exposure in the Perinatal and Three-month Drinking Water Study of Sodium Tungstate Dihydrate

	0 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Reproductive Performance						
Time-mated Females (GD 6)	8	8	8	8	8	8
Females Pregnant (%)[a]	7 (87.5)	8 (100.0)	8 (100.0)	6 (75.0)	6 (75.0)	6 (75.0)
Females Not Pregnant (%)	1 (12.5)	0	0	2 (25.0)	2 (25.0)	2 (25.0)
Dams Not Delivering with Evidence of Pregnancy (%)	0	0	1 (12.5)[b]	0	0	0
Dams with Litters on LD 0 (%)	7 (100)	8 (100.0)	7 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)
Litters Poststandardization (LD 4)[c]	5	7	7	6	5	5

GD = gestation day; LD = lactation day.
[a]Statistical analysis performed by the Cochran-Armitage (trend) and Fisher's exact (pairwise) tests.
[b]Dam died before littering.
[c]Standardization to eight pups/litter (four pups/sex). Only litters with at least two pups/sex and at least eight pups total/litter were retained to continue on study.

Table 3. Summary of Mean Body Weights and Body Weight Gains of F_0 Female Rats during Gestation and Lactation in the Perinatal and Three-month Drinking Water Study of Sodium Tungstate Dihydrate

Parameter[a,b]	0 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Gestation Day						
6	229.8 ± 3.1 (7)	220.5 ± 8.8 (8)	224.5 ± 6.2 (8)	228.1 ± 5.7 (6)	225.5 ± 4.6 (6)	228.9 ± 4.6 (6)
9	243.3 ± 2.7 (7)	241.4 ± 5.3 (8)	245.6 ± 4.3 (8)	239.1 ± 5.4 (6)	237.2 ± 5.1 (6)	242.1 ± 5.8 (6)
12	259.1 ± 3.6 (7)	257.4 ± 4.9 (8)	261.4 ± 4.4 (8)	255.5 ± 5.8 (6)	254.5 ± 4.8 (6)	258.3 ± 6.6 (6)
15	275.5 ± 6.0 (7)	279.9 ± 4.9 (8)	281.5 ± 5.1 (8)	276.2 ± 5.0 (6)	269.3 ± 4.3 (6)	277.9 ± 8.6 (6)
18	304.9 ± 10.9 (7)	317.6 ± 5.6 (8)	313.4 ± 7.8 (8)	309.3 ± 5.8 (6)	301.2 ± 4.5 (6)	306.1 ± 16.0 (6)
21	345.0 ± 19.2 (7)	367.4 ± 7.2 (8)	353.9 ± 11.9 (8)	352.0 ± 6.0 (6)	338.7 ± 8.4 (6)	339.3 ± 26.0 (6)
Gestation Weight Change						
Gestation Day Interval						
6–9	13.5 ± 0.9 (7)	20.8 ± 4.3 (8)	21.1 ± 5.5 (8)	11.0 ± 0.7 (6)	11.7 ± 1.2 (6)	13.2 ± 1.6 (6)
9–12	15.8 ± 1.2 (7)	16.1 ± 0.9 (8)	15.8 ± 0.8 (8)	16.3 ± 1.0 (6)	17.3 ± 0.9 (6)	16.1 ± 1.5 (6)
12–15	16.4 ± 3.3 (7)	22.5 ± 1.3 (8)	20.0 ± 1.7 (8)	20.8 ± 1.2 (6)	14.8 ± 1.0 (6)	19.6 ± 2.9 (6)
15–18	29.3 ± 5.6 (7)	37.6 ± 1.4 (8)	32.0 ± 3.5 (8)	33.0 ± 1.5 (6)	31.8 ± 4.9 (6)	28.2 ± 8.7 (6)
18–21	40.1 ± 8.4 (7)	49.9 ± 3.0 (8)	40.5 ± 4.4 (8)	42.7 ± 1.8 (6)	37.5 ± 5.1 (6)	33.1 ± 11.5 (6)
6–21	115.2 ± 18.0 (7)	146.9 ± 6.4 (8)	129.4 ± 12.7 (8)	123.9 ± 2.2 (6)	113.3 ± 10.6 (6)	110.3 ± 22.9 (6)
Lactation Day						
1	275.0 ± 4.7 (6)	270.6 ± 3.7 (8)	277.2 ± 7.4 (7)	268.3 ± 4.7(6)	264.3 ± 5.3 (6)	257.2 ± 10.9 (6)
4	290.1 ± 5.4 (5)	287.2 ± 5.1 (7)	285.8 ± 7.2 (7)	279.9 ± 2.9(6)	271.1 ± 3.9 (5)	280.3 ± 8.2 (5)
7	300.2 ± 5.3* (5)	289.4 ± 4.5 (7)	299.4 ± 6.2 (7)	289.0 ± 2.7(6)	279.9 ± 5.1 (5)	281.9 ± 6.0 (4)[c]
14	325.4 ± 5.7** (5)	331.8 ± 6.7 (7)	326.1 ± 6.6 (7)	313.3 ± 6.3(6)	303.0 ± 3.7* (5)	288.6 ± 6.0** (4)
21	313.4 ± 5.5** (5)	315.2 ± 8.1 (7)	308.3 ± 8.3 (7)	306.4 ± 5.7(6)	283.4 ± 3.4* (5)	256.1 ± 8.7** (4)
Lactation Weight Change						
Lactation Day Interval						
1–4	12.7 ± 3.8 (5)	15.8 ± 3.2 (7)	8.5 ± 3.2 (7)	11.7 ± 2.1 (6)	11.3 ± 1.7 (5)	13.2 ± 3.4 (5)
4–7	10.1 ± 2.6 (5)	2.3 ± 2.0 (7)	13.6 ± 2.1 (7)	9.1 ± 2.4 (6)	8.8 ± 4.4 (5)	2.8 ± 6.0 (4)[c]
7–14	25.1 ± 2.7** (5)	42.4 ± 3.3 (7)	26.7 ± 4.3 (7)	24.3 ± 4.5 (6)	23.1 ± 4.8 (5)	6.7 ± 3.4** (4)
14–21	−12.0 ± 8.7 (5)	−16.6 ± 3.7 (7)	−17.8 ± 6.4 (7)	−7.0 ± 5.2 (6)	−19.6 ± 2.5 (5)	−32.5 ± 7.3 (4)
1–21	36.0 ± 8.6** (5)	43.8 ± 4.7 (7)	31.0 ± 9.7 (7)	38.1 ± 5.6 (6)	23.6 ± 4.5 (5)	−9.6 ± 6.0** (4)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Each exposure group was compared to the vehicle control group with the Williams test when a trend was present ($p \leq 0.01$ from the Jonckheere trend test) or with the Dunnett test when no trend was present.
[b]Data presented as mean ± standard error (number of dams). Body weight data are presented in grams.
[c]One dam and her pups in the 2,000 mg/L group were euthanized on PND 6 due to moribundity.

There were no significant changes in water consumption of exposed dams compared to vehicle control dams during gestation; however, during most of the lactation period, water consumption was significantly decreased for the 2,000 mg/L dams, with the largest difference (approximately 27%) occurring during the end of lactation (LD 17–21) (Table 4). Water consumption by the 500 mg/L group was up to 19% lower (LD 4–7) than that of the vehicle control group; however, consumption by the 1,000 mg/L group was no more than 8% lower (LD 17–21) than that of the vehicle control group (Table 4). Daily ST consumption for the 125, 250, 500, 1,000, and 2,000 mg/L groups averaged approximately 17, 33, 58, 132, and 247 mg ST/kg body weight/day (mg/kg/day), respectively, during GD 6–21 and approximately 25, 56, 96, 220, and 374 mg/kg/day, respectively, during LD 1–14 (Table 4).

Table 4. Summary of Water and Sodium Tungstate Dihydrate Consumption of F_0 Female Rats during Gestation and Lactation in the Perinatal and Three-month Drinking Water Study

Parameter[a]	0 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Gestation Day Interval[b,c]						
6–9	28.9 ± 1.7 (7)	30.5 ± 1.2 (8)	30.9 ± 1.6 (8)	26.4 ± 2.0 (6)	28.1 ± 1.5 (6)	27.5 ± 1.7 (6)
9–12	30.7 ± 1.8 (7)	30.6 ± 1.7 (8)	30.7 ± 1.0 (8)	27.9 ± 1.7 (6)	36.3 ± 7.1 (6)	27.7 ± 1.2 (6)
12–15	33.1 ± 2.3 (7)	36.0 ± 1.2 (8)	36.2 ± 1.3 (8)	31.4 ± 0.8 (6)	33.5 ± 2.1 (6)	33.9 ± 1.6 (6)
15–18	40.6 ± 3.4 (7)	45.8 ± 2.9 (8)	40.4 ± 1.7 (8)	37.5 ± 1.2 (6)	39.7 ± 3.5 (6)	42.0 ± 1.8 (6)
18–21	41.4 ± 3.7* (7)	44.4 ± 2.9 (8)	42.1 ± 1.2 (8)	37.3 ± 1.3 (6)	39.6 ± 4.1 (6)	37.8 ± 3.0 (6)
6–21	34.9 ± 2.4* (7)	37.4 ± 1.5 (8)	36.1 ± 1.2 (8)	32.1 ± 1.2 (6)	35.5 ± 3.1 (6)	33.8 ± 1.0 (6)
Lactation Day Interval[b,c]						
1–4	49.25 ± 2.66 (6)	51.10 ± 2.24 (8)	51.37 ± 2.67 (7)	44.20 ± 1.07 (6)	57.63 ± 7.59 (6)	45.00 ± 1.99 (6)
4–7	53.62 ± 2.43 (5)	45.77 ± 1.25 (7)	61.27 ± 5.07 (7)	43.50 ± 1.85* (6)	50.56 ± 2.06 (5)	45.08 ± 1.94 (4)[d]
7–10	68.78 ± 3.43** (5)	64.20 ± 1.51 (7)	71.41 ± 5.17 (7)	58.77 ± 2.59 (6)	64.94 ± 1.46 (5)	50.20 ± 1.25** (4)
10–14	85.46 ± 3.83** (5)	78.13 ± 2.49 (7)	84.93 ± 2.65 (7)	74.73 ± 1.33 (6)	78.56 ± 3.16 (5)	66.05 ± 2.66** (4)
14–17	90.48 ± 4.39* (5)	85.83 ± 4.03 (7)	89.60 ± 2.63 (7)	82.78 ± 2.94 (6)	87.48 ± 2.65 (5)	64.08 ± 0.54* (4)
17–21	108.6 ± 2.86** (5)	104.5 ± 2.59 (7)	107.3 ± 2.20 (6)	96.67 ± 3.50* (6)	99.96 ± 0.71* (5)	79.68 ± 2.02** (4)
1–14	66.42 ± 2.70** (5)	61.25 ± 1.70 (7)	68.61 ± 2.21 (7)	56.79 ± 1.14 (6)	62.48 ± 1.61 (5)	52.79 ± 1.31** (4)
Chemical Intake (mg/kg/day)[e,f]						
GD 6–21	0.00 ± 0.00 (7)	16.80 ± 0.50 (8)	32.50 ± 1.11 (8)	58.33 ± 1.35 (6)	131.9 ± 11.20 (6)	246.6 ± 8.10 (6)
LD 1–14	0.00 ± 0.00 (5)	25.35 ± 0.69 (7)	56.46 ± 1.76 (7)	96.43 ± 1.61 (6)	219.5 ± 4.80 (5)	374.3 ± 6.22 (4)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
GD = gestation day; LD = lactation day.
[a]Data presented as mean ± standard error (number of dams).
[b]Water consumption data are presented as grams/animal/day.
[c]Each exposure group was compared to the vehicle control group with the Shirley test when a trend was present ($p \leq 0.01$ from the Jonckheere trend test) or with the Dunn test when no trend was present.
[d]One dam and her pups in the 2,000 mg/L group were euthanized on PND 6 due to moribundity.
[e]Chemical intake calculated as: ([exposure concentration × water consumption]/[average body weight of day range]).
[f]No statistical analysis was performed on the chemical intake data.

There were no exposure-related differences between the vehicle control group and the ST-exposed groups in the number of litters, total litter weight, or litter size of males and females

on PND 1 or PND 4 (Table 5). The litter size did not change appreciably between PND 1 and litter standardization on PND 4.

Two pups in the 125 mg/L group and one pup in the 1,000 mg/L group were found dead on PND 1; one pup in the 1,000 mg/L group was missing on PND 2; and one pup in the 2,000 mg/L group was found dead on PND 3. There was no pup mortality after PND 6. There were no exposure-related clinical observations in dams or pups (Appendix G).

When adjusted for litter size, the mean body weight of male and female pups in the 2,000 mg/L group on PND 21 was significantly decreased by approximately 16% and 11%, respectively, compared to the corresponding vehicle control groups. For male and female pups in the 2,000 mg/L group combined, the mean body weight on PND 21 was significantly decreased by approximately 14% relative to the vehicle control male and female pups combined (Table 6).

Table 5. Summary of Mean Litter Size and Survival Ratio of F_1 Male and Female Rats during Lactation in the Perinatal and Three-month Drinking Water Study of Sodium Tungstate Dihydrate

Parameter	0 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
PND 1[a,b]						
Total	11.0 ± 1.2 (6)	13.0 ± 0.7 (8)	12.0 ± 0.7 (7)	12.0 ± 0.7 (6)	10.8 ± 2.1 (6)	12.2 ± 1.7 (6)
Live	11.0 ± 1.2 (6)	12.8 ± 0.7 (8)	12.0 ± 0.7 (7)	12.0 ± 0.7 (6)	10.7 ± 2.0 (6)	12.2 ± 1.7 (6)
% Male per Litter[c]	42.3 ± 8.2 (4)	44.3 ± 6.1 (5)	48.4 ± 4.9 (5)	53.7 ± 9.2 (4)	39.2 ± 10.7 (5)	54.7 ± 6.1 (5)
% Male[c,d,e]	39 (38)	44 (63)	49 (61)	52 (48)	48 (50)	54 (56)
Male[a,b]						
PND 1[c]	3.8 ± 0.3 (4)	5.6 ± 1.0 (5)	6.0 ± 0.9 (5)	6.3 ± 0.8 (4)	4.8 ± 1.3 (5)	6.0 ± 1.0 (5)
PND 4 Prestandardization[c]	2.8 ± 0.9 (4)	5.0 ± 1.4 (5)	6.0 ± 0.9 (5)	6.3 ± 0.8 (4)	4.8 ± 1.3 (5)	5.4 ± 1.5 (5)
PND 4 Poststandardization	3.8 ± 0.2* (5)	4.0 ± 0.0 (7)	4.0 ± 0.0 (7)	4.7 ± 0.4 (6)	4.0 ± 0.0 (5)	4.4 ± 0.4 (5)
Female[a,b]						
PND 1[c]	5.8 ± 1.3 (4)	7.0 ± 0.9 (5)	6.2 ± 0.5 (5)	5.8 ± 1.3 (4)	5.2 ± 1.2 (5)	5.2 ± 1.2 (5)
PND 4 Prestandardization[c]	5.3 ± 1.8 (4)	5.0 ± 1.3 (5)	6.2 ± 0.5 (5)	5.8 ± 1.3 (4)	5.0 ± 1.4 (5)	4.6 ± 1.5 (5)
PND 4 Poststandardization	4.2 ± 0.2* (5)	4.0 ± 0.0 (7)	4.0 ± 0.0 (7)	3.3 ± 0.4 (6)	4.0 ± 0.0 (5)	3.6 ± 0.4 (5)
Male and Female[a,b]						
PND 4 Prestandardization	11.0 ± 1.2 (6)	12.8 ± 0.7 (8)	12.0 ± 0.7 (7)	12.0 ± 0.7 (6)	10.5 ± 1.9 (6)	12.0 ± 1.7 (6)
PND 4 Poststandardization	8.0 ± 0.0 (5)	8.0 ± 0.0 (7)	8.0 ± 0.0 (7)	8.0 ± 0.0 (6)	8.0 ± 0.0 (5)	8.0 ± 0.0 (5)
PND 21	8.0 ± 0.0 (5)	8.0 ± 0.0 (7)	8.0 ± 0.0 (7)	8.0 ± 0.0 (6)	8.0 ± 0.0 (5)	6.4 ± 1.6 (5)
Survival per Litter						
Total Dead: PND 1–4[e,f]	0 (6)	2 (8)[g]	0 (7)	0 (6)	2 (6)[g]	1 (6)
Total Dead: PND 4–21[e,f]	0 (5)	0 (7)	0 (7)	0 (6)	0 (5)	8 (5)[h]
Dead: PND 1–4[a,b,i]	0.0 ± 0.0 (6)	0.3 ± 0.2 (8)	0.0 ± 0.0 (7)	0.0 ± 0.0 (6)	0.3 ± 0.3 (6)	0.2 ± 0.2 (6)
Dead: PND 4–21[a,b,i]	0.0 ± 0.0 (5)	0.0 ± 0.0 (7)	0.0 ± 0.0 (7)	0.0 ± 0.0 (6)	0.0 ± 0.0 (5)	1.6 ± 1.6 (5)
Survival Ratio: PND 1–4[a,b,j]	1.00 ± 0.00 (6)	1.00 ± 0.00 (8)	1.00 ± 0.00 (7)	1.00 ± 0.00 (6)	0.99 ± 0.01 (6)	0.99 ± 0.01 (6)
Survival Ratio: PND 4–21[a,b,k]	1.00 ± 0.00 (5)	1.00 ± 0.00 (7)	1.00 ± 0.00 (7)	1.00 ± 0.00 (6)	1.00 ± 0.00 (5)	0.80 ± 0.20 (5)

Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$.
PND = postnatal day.
[a]Each exposure group was compared to the vehicle control group with the Shirley test when a trend was present ($p \leq 0.01$ from the Jonckheere trend test) or with the Dunn test when no trend was present.
[b]Data presented as mean ± standard error (number of litters).
[c]Litters where the male/female pup counts were inconsistent between PND 1 and PND 4 were excluded from the male/female-specific endpoints.
[d]100 × [number of live males in exposure group]/[number of live males and females in dietary exposure group] (number of pups).
[e]No statistics done on this endpoint.
[f]Total dead in exposure group (number of litters).
[g]Two pups in the 125 mg/L group and one pup in the 1,000 mg/L group were found dead on PND 1.
[h]One dam and her pups in the 2,000 mg/L group were euthanized on PND 6 due to moribundity.
[i]Number dead/litter.
[j]Survival per litter: number of pups prestandardization on PND 4/total live pups on PND 1.
[k]Survival per litter: number of live pups on PND 21/number of live pups poststandardization on PND 4.

Table 6. Summary of Preweaning F_1 Male and Female Rat Pup Mean Body Weights Following Perinatal Exposure to Sodium Tungstate Dihydrate

Parameter	0 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Male (g)						
PND 1[a,b,c]	7.8 ± 0.4** (4)	7.8 ± 0.2 (5)	7.8 ± 0.4 (5)	7.3 ± 0.3 (4)	7.2 ± 0.2 (4)	6.7 ± 0.3* (5)
PND 4[d,e,f]	10.84 ± 0.46* (19/5)	10.49 ± 0.28 (28/7)	10.88 ± 0.52 (28/7)	9.90 ± 0.34 (28/6)	10.49 ± 0.39 (20/5)	9.34 ± 0.48 (22/5)
PND 7[d,e,g]	16.94 ± 0.73* (19/5)	15.96 ± 0.41 (28/7)	16.67 ± 0.80 (28/7)	14.65 ± 0.43 (28/6)	15.61 ± 0.43 (20/5)	14.70 ± 0.81 (18/4)
PND 14[d,e,g]	32.98 ± 0.87** (19/5)	32.16 ± 0.56 (28/7)	33.13 ± 1.15 (28/7)	30.40 ± 1.04 (28/6)	31.41 ± 0.73 (20/5)	29.22 ± 1.09 (18/4)
PND 21[d,e,g]	55.56 ± 1.59** (19/5)	54.74 ± 0.58 (28/7)	55.05 ± 1.75 (28/7)	50.77 ± 1.92 (28/6)	51.89 ± 0.89 (20/5)	46.53 ± 1.44** (18/4)
Female (g)						
PND 1[a,b,c]	7.1 ± 0.2 (4)	7.2 ± 0.1 (5)	7.6 ± 0.3 (5)	7.0 ± 0.3 (4)	7.1 ± 0.3 (5)	6.6 ± 0.5 (5)
PND 4[d,e,f]	10.16 ± 0.49 (21/5)	9.69 ± 0.22 (28/7)	10.43 ± 0.47 (28/7)	9.19 ± 0.50 (20/6)	9.95 ± 0.25 (20/5)	9.19 ± 0.60 (18/5)
PND 7[d,e,g]	15.91 ± 0.76 (21/5)	14.95 ± 0.27 (28/7)	16.29 ± 0.71 (28/7)	13.64 ± 0.74 (20/6)	15.00 ± 0.24 (20/5)	14.45 ± 1.16 (14/4)
PND 14[d,e,g]	31.80 ± 0.99 (21/5)	30.48 ± 0.52 (28/7)	32.44 ± 1.22 (28/7)	28.04 ± 1.74 (20/6)	30.44 ± 0.53 (20/5)	28.63 ± 1.33 (14/4)
PND 21[d,e,g]	51.71 ± 1.31** (21/5)	51.21 ± 0.39 (28/7)	53.09 ± 1.58 (28/7)	46.06 ± 2.53 (20/6)	49.40 ± 0.88 (20/5)	45.79 ± 2.04* (14/4)
Male and Female (g)						
PND 1[a,b,c]	7.4 ± 0.2** (6)	7.4 ± 0.1 (8)	7.5 ± 0.2 (7)	7.2 ± 0.2 (6)	7.1 ± 0.3 (6)	6.6 ± 0.3* (6)
PND 4[d,e,f]	10.49 ± 0.45* (40/5)	10.09 ± 0.23 (56/7)	10.65 ± 0.49 (56/7)	9.69 ± 0.32 (48/6)	10.22 ± 0.32 (40/5)	9.24 ± 0.51 (40/5)
PND 7[d,e,g]	16.41 ± 0.72 (40/5)	15.46 ± 0.31 (56/7)	16.48 ± 0.75 (56/7)	14.37 ± 0.36 (48/6)	15.31 ± 0.32 (40/5)	14.52 ± 0.93 (32/4)
PND 14[d,e,g]	32.37 ± 0.88** (40/5)	31.32 ± 0.44 (56/7)	32.79 ± 1.19 (56/7)	29.76 ± 0.84 (48/6)	30.92 ± 0.56 (40/5)	28.91 ± 1.19 (32/4)
PND 21[d,e,g]	53.53 ± 1.29** (40/5)	52.98 ± 0.38 (56/7)	54.07 ± 1.65 (56/7)	49.33 ± 1.55 (48/6)	50.65 ± 0.76 (40/5)	46.08 ± 1.65** (32/4)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
PND = postnatal day.
[a]Data presented as mean ± standard error (number of litters).
[b]Each exposure group was compared to the vehicle control group with the Williams test when a trend was present ($p \leq 0.01$ from the Jonckheere trend test) or with the Dunnett test when no trend was present.
[c]Litter weights of live pups at PND 1 were divided by live litter size on PND 1 to obtain a pup mean weight/litter. Mean values were then adjusted using live litter size on PND 1 as a covariate.
[d]Statistical analysis was performed using mixed models with random litter effect for both trend and pairwise tests, using the Dunnett-Hsu adjustment for multiple comparisons.
[e]Data presented as mean of litter means ± standard error (number of pups/number of litters).
[f]PND 4 post-standardization.
[g]Individual pup weights first adjusted for live litter size on PND 4 poststandardization.

Three-month Study (Postweaning Phase)

There were no early deaths during the 3-month study; all F_1 rats survived until study termination (Table 7, Table 8). There were no clinical observations related to exposure, and all exposed animals were similar in overt behavior and general appearance to the vehicle control animals (Appendix G). Initial mean body weights were 9% and 16% below those of the vehicle control group for the 1,000 and 2,000 mg/L males, respectively; and 14%, 11%, and 13% below those of the vehicle control group for the 500, 1,000, and 2,000 mg/L females, respectively (Table 7, Table 8; Figure 2). Final mean body weights were lower for the 1,000 and 2,000 mg/L males and females, with the 2,000 mg/L males weighing approximately 29% less than the vehicle control group and the 2,000 mg/L females weighing approximately 18% less than the vehicle control group.

Water consumption was lower for the 1,000 and 2,000 mg/L males and females, with overall reductions of 27% and 42% for males and females, respectively, in the 2,000 mg/L groups compared to the respective vehicle control groups (Table 9; Appendix G). Drinking water concentrations of 125, 250, 500, 1,000, and 2,000 mg/L resulted in average daily ST doses of approximately 11.8, 24.3, 48.9, 91.8, and 157.2 mg/kg/day for males and 14.0, 26.1, 54.4, 101.4, and 160.5 mg/kg/day for females.

Table 7. Summary of Survival and Mean Body Weights of Male Rats in the Perinatal and Three-month Drinking Water Study of Sodium Tungstate Dihydrate

Study Day[a]	0 mg/L		125 mg/L			250 mg/L			500 mg/L			1,000 mg/L			2,000 mg/L		
	Av. Wt. (g)[b]	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters
1	57.4	5	57.8	100.7	5	57.3	99.9	5	55.2	96.2	5	52.2	91.1	5	48.4	84.3	4
7	82.1	5	81.4	99.2	5	81.5	99.2	5	76.4	93.1	5	71.4	87.0	5	60.0	73.2	4
14	108.1	5	119.0	110.1	5	112.0	103.6	5	109.1	100.9	5	98.3	90.9	5	74.7	69.1	4
21	171.9	5	175.5	102.1	5	172.8	100.6	5	159.8	93.0	5	136.4	79.3	5	91.2	53.1	4
28	191.1	5	193.0	101.0	5	198.1	103.6	5	181.4	94.9	5	168.2	88.0	5	112.4	58.8	4
35	261.7	5	257.7	98.4	5	249.2	95.2	5	245.1	93.6	5	200.0	76.4	5	132.9	50.8	4
42	300.6	5	294.1	97.9	5	298.5	99.3	5	284.8	94.7	5	243.0	80.9	5	160.7	53.5	4
49	319.6	5	322.7	101.0	5	324.6	101.6	5	300.8	94.1	5	267.4	83.7	5	188.6	59.0	4
56	352.5	5	355.1	100.8	5	352.3	99.9	5	339.4	96.3	5	301.5	85.5	5	212.6	60.3	4
63	345.9	5	345.4	99.9	5	341.5	98.7	5	335.2	96.9	5	310.5	89.8	5	222.4	64.3	4
70	384.2	5	383.9	99.9	5	379.5	98.8	5	368.0	95.8	5	339.7	88.4	5	250.1	65.1	4
77	398.1	5	398.3	100.1	5	389.5	97.8	5	385.5	96.8	5	350.4	88.0	5	270.6	68.0	4
84	414.0	5	412.3	99.6	5	407.9	98.5	5	400.0	96.6	5	365.2	88.2	5	285.0	68.8	4
EOS	**422.7**	**5**	**425.7**	**100.7**	**5**	**419.7**	**99.3**	**5**	**412.9**	**97.7**	**5**	**379.3**	**89.7**	**5**	**300.6**	**71.1**	**4**

EOS = end of study; No. of litters = number of litters represented in weight average.
[a]Study day 1 is the day animals were placed on study after pups were weaned.
[b]Average weights shown are means of litter means.

Table 8. Summary of Survival and Mean Body Weights of Female Rats in the Perinatal and Three-month Drinking Water Study of Sodium Tungstate Dihydrate

Study Day[a]	0 mg/L		125 mg/L			250 mg/L			500 mg/L			1,000 mg/L			2,000 mg/L		
	Av. Wt. (g)[b]	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters
1	54.4	5	57.5	105.8	5	56.1	103.1	5	46.7	85.9	5	48.5	89.2	5	47.5	87.4	4
7	79.7	5	81.1	101.8	5	80.7	101.3	5	69.2	86.8	5	70.8	88.8	5	60.5	76.0	4
14	104.1	5	116.0	111.4	5	111.1	106.7	5	100.8	96.8	5	98.4	94.5	5	74.5	71.6	4
21	142.4	5	147.0	103.3	5	150.5	105.7	5	129.6	91.0	5	121.7	85.5	5	86.9	61.1	4
28	165.1	5	173.5	105.1	5	167.2	101.3	5	153.6	93.1	5	141.9	85.9	5	102.2	61.9	4
35	179.2	5	188.9	105.4	5	191.1	106.6	5	175.2	97.8	5	163.7	91.3	5	114.7	64.0	4
42	198.3	5	207.0	104.4	5	208.9	105.4	5	191.0	96.3	5	179.1	90.3	5	132.7	66.9	4
49	210.8	5	223.4	106.0	5	216.3	102.6	5	202.4	96.0	5	191.8	91.0	5	150.7	71.5	4
56	215.5	5	225.4	104.6	5	230.3	106.9	5	217.9	101.1	5	203.8	94.6	5	166.0	77.1	4
63	230.9	5	239.3	103.6	5	243.6	105.5	5	230.0	99.6	5	214.6	92.9	5	177.3	76.8	4
70	238.6	5	255.5	107.1	5	252.8	105.9	5	241.1	101.0	5	222.2	93.1	5	190.0	79.6	4
77	243.4	5	255.8	105.1	5	254.1	104.4	5	244.4	100.4	5	228.6	93.9	5	194.8	80.0	4
84	250.4	5	260.4	104.0	5	260.8	104.2	5	251.7	100.5	5	236.0	94.3	5	201.3	80.4	4
EOS	**259.5**	**5**	**268.6**	**103.5**	**5**	**271.2**	**104.5**	**5**	**257.9**	**99.4**	**5**	**242.8**	**93.6**	**5**	**213.1**	**82.1**	**4**

EOS = end of study; No. of litters = number of litters represented in weight average.
[a]Study day 1 is the day animals were placed on study after pups were weaned.
[b]Average weights shown are means of litter means.

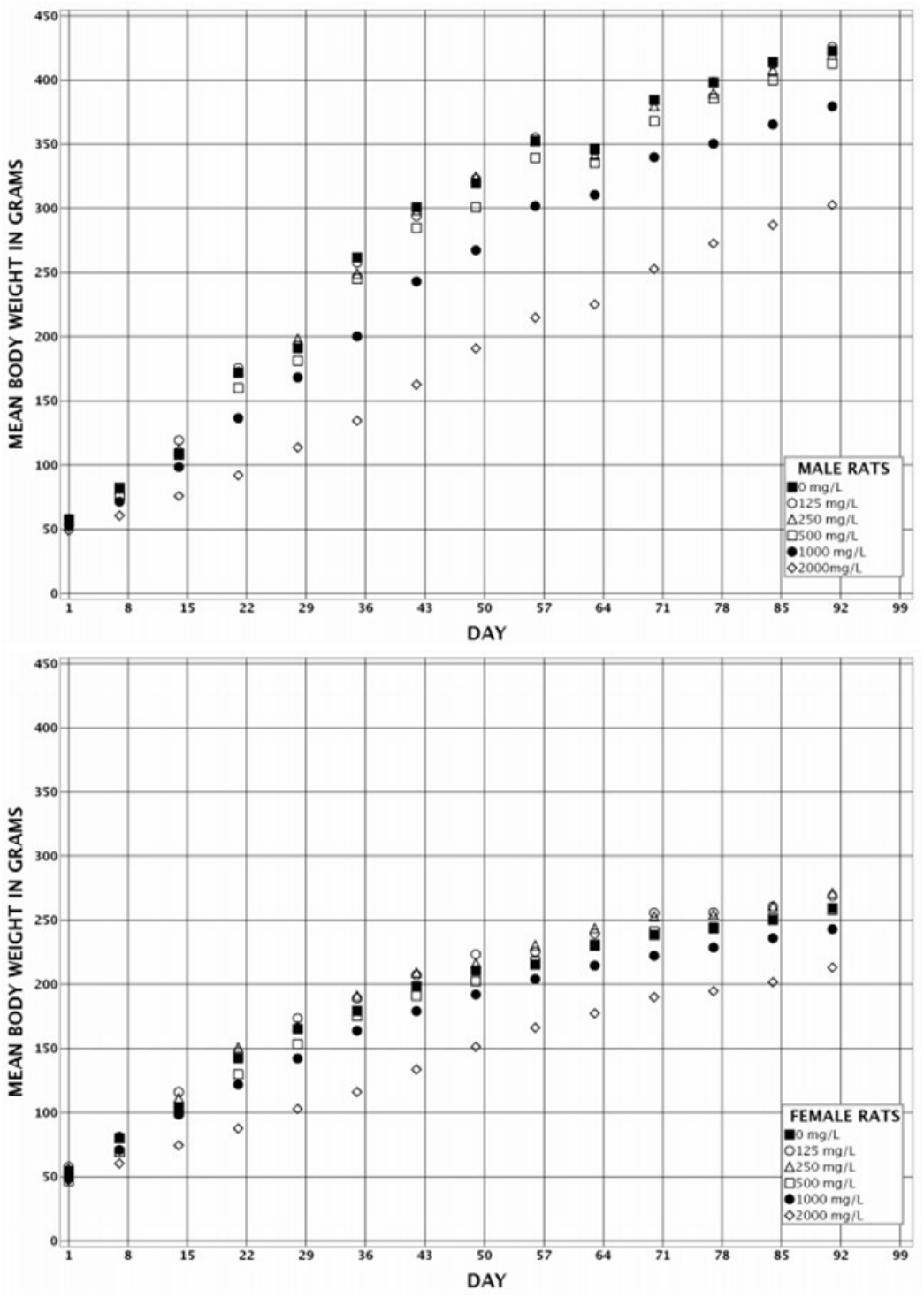


Figure 2. Growth Curves for Rats Exposed to Sodium Tungstate Dihydrate in Drinking Water for Three Months

Table 9. Summary of Water and Sodium Tungstate Dihydrate Consumption of Male and Female Rats in the Perinatal and Three-month Drinking Water Study

Week	0 mg/L	125 mg/L		250 mg/L		500 mg/L		1,000 mg/L		2,000 mg/L	
	Water (g/day)[a]	Water (g/day)	Dose (mg/kg/day)[b]	Water (g/day)	Dose (mg/kg/day)	Water (g/day)	Dose (mg/kg/day)	Water (g/day)	Dose (mg/kg/day)	Water (g/day)	Dose (mg/kg/day)
Male											
1	11.5	10.2	18.3	11.1	40.0	9.5	72.2	8.8	142.4	6.6	241.3
4	23.4	22.3	14.4	23.8	30.0	22.9	63.1	19.9	118.3	10.0	176.3
12	17.2	17.0	5.2	17.3	10.6	16.6	20.8	14.2	38.9	10.8	75.3
Female											
1	11.1	11.0	19.8	10.9	39.8	10.0	86.3	9.6	160.9	6.5	241.6
4	20.9	21.3	15.3	19.2	28.7	19.2	62.5	16.6	117.0	8.8	171.3
12	20.8	22.2	10.7	19.9	19.1	18.4	36.5	16.9	71.6	12.5	124.1

[a]Grams of water consumed/animal/day.
[b]Milligrams of sodium tungstate dihydrate consumed/kilogram body weight/day.

Blood was collected from up to 10 animals per group (originating from 4 or 5 litters) on the morning of day 91. Urine was collected overnight (for approximately 16 hours) during week 12 of the study from up to five animals per group; during collection, animals had access to untreated water. Total blood and urine tungsten concentrations were determined using validated analytical methods (Appendix E) and corresponding data are presented in Table 10. In both males and females, the total tungsten concentration in blood increased proportionally to the exposure concentration with no observed sex difference. The blood tungsten concentration in vehicle control animals was below the limit of detection (LOD; 0.0016 µg/g) of the assay. The urine tungsten concentration is presented as both µg/g of urine and after correcting for urinary creatinine concentrations (µg/mg creatinine) (Table 10). Low concentrations of tungsten were detected in urine from vehicle control male and female groups. The concentrations of creatinine-corrected tungsten in urine increased proportionally to the exposure concentration in both males and females and were significantly increased in all exposed groups compared to the corresponding vehicle control groups. As with blood, there was no observed sex difference in urinary tungsten concentrations.

Table 10. Summary of Blood and Urine Tungsten Concentration Data for Male and Female Rats in the Perinatal and Three-month Drinking Water Study of Sodium Tungstate Dihydrate[a]

	0 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
n	5	5	5	5	5	4
Male						
Blood (µg/g)	BD	0.49 ± 0.07	0.99 ± 0.10	1.93 ± 0.21	4.67 ± 0.45	10.66 ± 0.93
Urine[b,c]						
Urine (µg/g urine)	0.04 ± 0.00**	9.78 ± 3.02**	24.21 ± 5.08**	67.11 ± 22.66**	61.91 ± 11.79**	184.98 ± 30.14**
Urine (µg/mg creatinine)	0.06 ± 0.01**	11.84 ± 1.43**	33.68 ± 4.97**	42.40 ± 4.64**	86.68 ± 5.67**	291.52 ± 25.65**
Female						
Blood (µg/g)	BD	0.59 ± 0.06	1.19 ± 0.13	2.83 ± 0.29	5.67 ± 0.35	11.54 ± 1.03
Urine[b,c]						
Urine (µg/g urine)	0.03 ± 0.00**	9.10 ± 1.54**	26.40 ± 8.95**	27.65 ± 6.43**	98.98 ± 14.40**	182.81 ± 33.44**
Urine (µg/mg creatinine)	0.07 ± 0.01**	18.88 ± 1.40**	33.55 ± 3.82**	45.59 ± 4.34**	142.92 ± 21.99**	280.85 ± 46.16**

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
**Statistically significant at $p \leq 0.01$.
BD = below detection; group did not have more than 20% of its values above the limit of detection (LOD).
[a]Data presented as mean ± standard error of the litter means, where n = the number of litters.
[b]Values below the LOD (0.0054 µg/g) were substituted with 1/2 the LOD value. If 80% or more of the values in the vehicle control group were below the LOD, no mean or standard error were calculated, and no statistical analysis was performed.
[c]Statistical analysis performed using a bootstrapped Jonckheere test for trend and a Datta-Satten modified Wilcoxon test with Hommel adjustment for pairwise comparisons.

In female rats, there was a mild (<10%) significant decrease in the erythron characterized by a significant decrease in the hemoglobin concentration in the 2,000 mg/L group and a significant negative trend in the hematocrit concentration, hemoglobin concentration, and erythrocyte count with increasing exposure (Table 11). Although there were no significant pairwise changes observed in the male erythron, there were significant negative trends in hematocrit concentration, hemoglobin concentration, and erythrocyte count with increasing exposure concentration. The

reticulocyte count was unchanged in both males and females. These mild erythron changes were most likely due to the stress of exposure,[89] which is supported by the lower mean body weights observed in the 2,000 mg/L groups.

In male rats, blood urea nitrogen (BUN) was significantly increased, and the total protein, globulin concentrations, and insulin concentrations were significantly decreased in the 2,000 mg/L group (Table 11). The BUN was likely increased due to the lower water consumption values in that exposure group. The toxicological relevance of the observed decreases in the total protein and globulins is uncertain; these changes could be a secondary effect of exposure.

The urine xanthine/creatinine ratios were significantly increased in all male and female exposed groups relative to the vehicle control groups (Table 12).

Table 11. Summary of Select Clinical Pathology Data for Male and Female Rats in the Perinatal and Three-month Drinking Water Study of Sodium Tungstate Dihydrate[a,b]

	0 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
n	5	5	5	5	5	4
Male						
Hematocrit (%)	50.0 ± 1.2**	48.2 ± 0.9	49.3 ± 0.5	48.5 ± 0.6	46.8 ± 0.8	45.7 ± 1.1
Hemoglobin (g/dL)	15.2 ± 0.3**	14.9 ± 0.2	15.3 ± 0.1	15.2 ± 0.1	14.6 ± 0.3	14.3 ± 0.3
Erythrocytes (10^6/µL)	8.69 ±0.18*	8.45 ± 0.10	8.72 ± 0.10	8.54 ± 0.13	8.23 ± 0.15	8.13 ± 0.22
Urea Nitrogen (mg/dL)	18.1 ± 0.5**	14.8 ± 0.5*	17.3 ± 0.3	17.6 ± 0.7	19.2 ± 0.4	24.4 ± 1.8*
Total Protein (g/dL)	6.64 ± 0.04	6.67 ± 0.06	6.76 ± 0.09	6.75 ± 0.10	6.73 ± 0.07	6.04 ± 0.07*
Globulin (g/dL)	2.46 ± 0.05*	2.37 ± 0.03	2.48 ± 0.07	2.48 ± 0.05	2.37 ± 0.05	1.86 ± 0.04*
Insulin (ng/mL)	4.36 ± 0.29**	3.36 ± 0.33	3.09 ± 0.40	3.36 ± 0.42	2.52 ± 0.33	1.94 ± 0.34*
Female						
Hematocrit (%)	44.7 ± 0.6*	45.5 ± 0.9	44.4 ± 0.6	44.6 ± 0.7	44.5 ± 0.8	42.3 ± 0.2
Hemoglobin (g/dL)	14.2 ± 0.1*	14.3 ± 0.3	14.1 ± 0.2	14.2 ± 0.1	14.0 ± 0.2	13.4 ± 0.1*
Erythrocytes (10^6/uL)	7.80 ± 0.07*	7.87 ± 0.14	7.78 ± 0.05	7.70 ± 0.08	7.73 ± 0.07	7.38 ± 0.12

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data presented as mean ± standard error of the litter means, where n = the number of litters.
[b]Statistical analysis performed using a bootstrapped Jonckheere test for trend and a Datta-Satten modified Wilcoxon test with Hommel adjustment for pairwise comparisons.

Table 12. Summary of Select Urinalysis Data for Male and Female Rats in the Perinatal and Three-month Drinking Water Study of Sodium Tungstate Dihydrate[a,b]

	0 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
n	5	5	5	5	5	4
Male						
Creatinine (mg/dL)	77.9 ± 10.2	80.5 ± 22.6	76.2 ± 11.3	154.5 ± 44.5	73.9 ± 15.1	73.5 ± 11.8
Xanthine (µg/mL)	1.20 ± 0.16**	1.51 ± 0.44	1.58 ± 0.24	4.48 ± 1.75	3.20 ± 0.52*	5.45 ± 0.88*
Xanthine/Creatinine (µg/mg)	1.55 ± 0.09**	1.83 ± 0.04*	2.13 ± 0.08*	2.79 ± 0.37*	4.63 ± 0.27*	7.65 ± 0.26*
Female						
Creatinine (mg/dL)	43.7 ± 6.1*	47.8 ± 4.6	77.1 ± 22.9	58.9 ± 10.2	66.7 ± 4.1	82.3 ± 22.3
Xanthine (µg/mL)	0.43 ± 0.07**	0.74 ± 0.12	1.77 ± 0.54*	2.00 ± 0.35*	3.30 ± 0.21*	3.72 ± 0.75
Xanthine/Creatinine (µg/mg)	1.02 ± 0.09**	1.51 ± 0.14*	2.27 ± 0.27**	3.39 ± 0.18**	5.21 ± 0.38**	7.28 ± 0.86*

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data presented as mean ± standard error of the litter means, where n = the number of litters.
[b]Statistical analysis performed using a bootstrapped Jonckheere test for trend and a Datta-Satten modified Wilcoxon test with Hommel adjustment for pairwise comparisons.

Absolute kidney weights were reduced in males in all exposed groups, relative to the vehicle control group, with a significant decrease observed in the 2,000 mg/L group (approximately 21%). Relative kidney weights were higher in 1,000 mg/L females and significantly increased in the 2,000 mg/L males and females, relative to the vehicle control group (Table 13). Although the kidney was a target tissue, it is unlikely that the lesions observed were responsible for the differences in kidney weights; it is more likely that these organ weight differences are an effect of body weight differences.

When compared to vehicle control groups, significant differences were also observed in other organ weights, including decreased absolute heart and lung weights in males and females; decreased absolute liver weights in males and increased relative liver weights in females; decreased absolute thymus weights in males and increased relative testis weights (Appendix G). These changes were considered secondary to body weight reductions. Rats administered 2,000 mg/L ST exhibited significantly decreased left cauda epididymis (14%) and epididymis (13%) weights, and lower testis weights (8%) compared to the vehicle control group (Appendix G). Although these were significant (cauda and epididymis) and/or displayed a significant negative trend with increasing exposure concentration (right testis), rats in the 2,000 mg/L group displayed mean body weights that were 28% lower than the vehicle control group. There were no changes in reproductive parameters or alterations in contralateral testis and epididymis or in histopathology (Appendix G). Given the magnitude of the body weight effect and the absence of changes in other endpoints, the lower reproductive organ weights are likely secondary to effects on body weight.

Although the weights of the left epididymis and the left cauda were significantly decreased in the 2,000 mg/L males, there were no corresponding changes in sperm parameters, including number

of sperm/mg cauda epididymis, total number of sperm/cauda, sperm motility, number of homogenization-resistant spermatids/mg testis, or total number of spermatids (Appendix G).

No exposure-related gross lesions were recorded. Exposure-related histological lesions were found in the kidneys (Table 13). Renal tubule regeneration was increased in the male and female 1,000 and 2,000 mg/L groups; the increases in the 2,000 mg/L groups were significant relative to the vehicle control groups (Table 13). The lesion was characterized by hyperplasia of proximal convoluted tubular epithelial cells that manifested as cytoplasmic basophilia, nuclear crowding, and occasional mitotic figures. Renal tubule regeneration occurs as a response to previous degeneration or necrosis and is one of the most common exposure-related lesions in the kidney.[90] Degeneration and necrosis were not present in this study, perhaps due to the fact that by the time of necropsy, the response of the kidney had progressed from degeneration to regeneration. Renal tubule regeneration differed from chronic progressive nephropathy (CPN) by the lack of thickened basement membranes, associated inflammatory cells, proteinaceous casts, and cytoplasmic pigment—all features typically seen with CPN. The incidences and severities of CPN were not increased in exposed groups of animals (Appendix G).

Table 13. Summary of Renal Findings for Male and Female Rats in the Perinatal and Three-month Drinking Water Study of Sodium Tungstate Dihydrate

	0 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
n	5	5	5	5	5	4
Male						
Necropsy Body Wt. (g)[a]	422.7 ± 5.2**	425.7 ± 3.9	419.7 ± 9.0	412.9 ± 10.2	379.3 ± 6.7**	300.6 ± 17.9**
R. Kidney Weight[a]						
Absolute (g)	1.35 ± 0.04**	1.32 ± 0.03	1.25 ± 0.05	1.26 ± 0.04	1.22 ± 0.02	1.06 ± 0.06**
Relative (mg/g)[b]	3.20 ± 0.08**	3.11 ± 0.06	2.99 ± 0.10	3.06 ± 0.03	3.21 ± 0.01	3.56 ± 0.09**
Histological Findings						
Kidney[c]	10	10	10	10	10	10
Renal tubule, regeneration[d]	0**	0	0	0	3 (1.0)[e]	10** (2.0)
Female						
Necropsy Body Wt. (g)	259.5 ± 3.8**	268.6 ± 9.1	271.2 ± 7.0	257.9 ± 6.3	242.8 ± 0.8	213.1 ± 4.1**
R. Kidney Weight						
Absolute (g)	0.84 ± 0.02	0.87 ± 0.03	0.84 ± 0.02	0.81 ± 0.02	0.84 ± 0.02	0.85 ± 0.03
Relative (mg/g)	3.25 ± 0.04**	3.25 ± 0.03	3.08 ± 0.05	3.13 ± 0.05	3.47 ± 0.07	3.98 ± 0.15**
Histological Findings						
Kidney	10	10	10	10	10	10
Renal tubule, regeneration	0**	0	0	0	3 (1.0)	10** (2.0)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
Statistical analysis for organ weight data performed using mixed models, with litter as a random effect and a Dunnett-Hsu adjustment for multiple comparisons. Statistical analysis for histological findings performed by the Rao-Scott test.
**Statistically significant at $p \leq 0.01$.
[a]Data presented as mean ± standard error of the litter means.
[b]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.
[c]Number of animals examined microscopically.
[d]Number of animals with lesion.
[e]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

Exposure Concentration Selection Rationale for the Perinatal and Two-year Study in Rats

A >10% reduction in final mean body weight in males and females (Table 7, Table 8) and up to a 65% reduction in water consumption for males (at week 3), and a 60% reduction in water consumption for females (at week 3) (Table 9) in the 3-month studies coupled with lesions noted in the kidney (Table 13), led to the assessment that 2,000 mg/L was too high for the 2-year studies. The 1,000 mg/L concentration was considered an adequate top exposure concentration to challenge the animals based on an approximate 10% reduction in final mean body weight in males, an approximate 20% reduction in water consumption in females, and minimal to mild renal tubule regeneration in the 3-month studies. Because there was no overlap in tungstate blood concentrations between the 1,000 mg/L and 2,000 mg/L groups, and no significant effects were noted at 500 mg/L or lower, the exposure concentrations were spaced by half. Hence, exposure concentrations selected for the chronic studies were 0, 250, 500 and 1,000 mg/L. Additionally, tissue tungsten concentrations were evaluated in the kidney, plasma, and urine at 3, 6, 12, and 18 months in an additional group of animals to determine systemic exposure and to help identify species differences given the findings from the 3-month studies showing the kidney as a target organ of toxicity.

Two-year Study (Perinatal Phase)

No significant clinical observations were noted in dams and no significant effects were noted on reproductive performance, including the percentage of mated females producing pups (Table 14). Gestational mean body weights of dams in the 1,000 mg/L group were less than that of the vehicle control group by up to 5% by GD 21 (Table 15). During lactation, mean body weights of dams in the 1,000 mg/L group were significantly decreased by 3%, 3%, and 4% relative to the vehicle control group at LD 1, LD 17, and LD 21, respectively (Table 15).

Table 14. Summary of the Disposition of F_0 Female Rats during Perinatal Exposure in the Perinatal and Two-year Drinking Water Study of Sodium Tungstate Dihydrate

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Reproductive Performance				
Time-mated Females (GD 6)	47	41	41	41
Females Pregnant (%)[a]	41 (87.2)	34 (82.9)	36 (87.8)	36 (87.8)
Females Not Pregnant (%)	6 (12.8)	7 (17.1)	5 (12.2)	5 (12.2)
Dams Not Delivering with Evidence of Pregnancy (%)	0	0	1 (2.8)[b]	2 (5.6)
Dams with Litters on LD 0 (%)	41 (100.0)	34 (100.0)	35 (100.0)	34 (94.4)
Litters Poststandardization (LD 4)[c]	37	33	33	32

GD = gestation day; LD = lactation day.
[a]Statistical analysis performed by the Cochran-Armitage (trend) and Fisher's exact (pairwise) tests.
[b]Dam died before littering.
[c]Standardization to eight pups/litter (four pups/sex). Only litters with at least two pups/sex and at least eight pups total/litter were retained to continue on study.

Table 15. Summary of Mean Body Weights and Body Weight Gains of F_0 Female Rats during Gestation and Lactation in the Perinatal and Two-year Drinking Water Study of Sodium Tungstate Dihydrate

Parameter[a,b]	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Gestation Day				
6	233.4 ± 1.87 (41)	236.6 ± 2.01 (34)	236.2 ± 1.71 (36)	233.4 ± 1.82 (36)
9	251.4 ± 2.07 (41)	252.9 ± 2.10 (34)	250.6 ± 1.93 (36)	248.2 ± 1.61 (36)
12	265.7 ± 1.81 (41)	267.1 ± 2.36 (34)	264.5 ± 1.90 (36)	260.7 ± 1.73 (36)
15	288.2 ± 2.21** (41)	289.0 ± 2.45 (34)	286.3 ± 2.05 (36)	279.2 ± 1.90** (36)
18	325.7 ± 2.75** (41)	326.9 ± 2.85 (34)	323.8 ± 2.56 (36)	312.6 ± 2.96** (36)
21	373.7 ± 4.07** (41)	377.0 ± 3.44 (34)	371.0 ± 3.45 (36)	354.3 ± 4.76** (36)
Gestation Weight Change				
Gestation Day Interval				
6–9	17.9 ± 0.8** (41)	16.3 ± 0.5 (34)	14.3 ± 0.6** (36)	14.8 ± 0.9** (36)
9–12	14.3 ± 1.0 (41)	14.2 ± 0.7 (34)	14.0 ± 0.5 (36)	12.5 ± 0.6 (36)
12–15	22.5 ± 0.7** (41)	21.9 ± 0.6 (34)	21.8 ± 0.6 (36)	18.5 ± 0.8** (36)
15–18	37.5 ± 1.2 (41)	37.9 ± 0.9 (34)	37.5 ± 0.9 (36)	33.4 ± 1.6* (36)
18–21	48.0 ± 1.6** (41)	50.1 ± 1.2 (34)	47.2 ± 1.3 (36)	41.7 ± 2.1** (36)
6–21	140.3 ± 3.6** (41)	140.4 ± 2.1 (34)	134.8 ± 2.6 (36)	120.9 ± 4.8** (36)
Lactation Day				
1	282.4 ± 2.04** (41)	282.7 ± 2.49 (34)	276.9 ± 2.01 (35)	273.8 ± 2.16** (34)
4	295.1 ± 2.15* (41)	295.7 ± 2.58 (34)	291.8 ± 2.42 (35)	288.7 ± 2.48 (34)
7	302.6 ± 2.04 (37)	303.8 ± 2.65 (33)	297.0 ± 2.74 (33)	298.4 ± 2.07 (32)
10	312.8 ± 2.4* (37)	310.4 ± 3.0 (32)	308.6 ± 2.7 (32)	305.0 ± 2.4 (32)
14	321.2 ± 2.45 (37)	321.5 ± 2.66 (32)	320.0 ± 2.66 (32)	316.6 ± 2.48 (32)
17	320.5 ± 2.35* (37)	321.0 ± 3.12 (32)	321.9 ± 3.20 (32)	309.9 ± 2.13* (32)
21	301.6 ± 2.70* (37)	306.4 ± 3.45 (32)	299.8 ± 3.84 (32)	289.2 ± 4.05* (32)
Lactation Weight Change				
Lactation Day Interval				
1–4	12.7 ± 1.2 (41)	12.9 ± 1.5 (34)	15.0 ± 1.2 (35)	15.0 ± 2.2 (34)
4–7	7.5 ± 1.2 (37)	8.6 ± 1.1 (33)	4.8 ± 1.5 (33)	8.7 ± 1.9 (32)
7–10	10.2 ± 1.4 (37)	6.4 ± 1.4 (32)	10.9 ± 1.2 (32)	6.5 ± 1.6 (32)
10–14	8.4 ± 1.6 (37)	11.0 ± 2.0 (32)	11.4 ± 1.6 (32)	11.6 ± 2.0 (32)
14–17	−0.7 ± 2.1 (37)	−0.5 ± 1.8 (32)	1.8 ± 1.7 (32)	−6.7 ± 2.0 (32)
17–21	−18.9 ± 2.6 (37)	−14.5 ± 2.8 (32)	−22.1 ± 3.3 (32)	−20.7 ± 3.5 (32)
1–21	19.5 ± 2.9 (37)	24.2 ± 2.5 (32)	22.5 ± 3.4 (32)	15.0 ± 4.1 (32)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Each exposure group was compared to the vehicle control group with the Williams test when a trend was present ($p \leq 0.01$ from the Jonckheere trend test) or with the Dunnett test when no trend was present.
[b]Data presented as mean ± standard error (number of dams). Body weight data are presented in grams.

Summaries of water and ST consumption are presented in Table 16. Water consumption trends were similar during gestation and lactation. During gestation, water consumption of all ST-exposed groups was within 9% of the vehicle control group (ranging from 99% to 109%). During lactation, water consumption of all ST-exposed groups was within 8% of the vehicle control group (ranging from 92% to 100%) (Table 16). Daily ST consumption for the 250, 500, and 1,000 mg/L groups averaged approximately 32, 65, and 143 mg/kg/day, respectively, during GD 6–21, and approximately 48, 98, and 197 mg/kg/day, respectively, during LD 1–14 (Table 16).

Table 16. Summary of Water and Sodium Tungstate Dihydrate Consumption by F_0 Female Rats during Gestation and Lactation in the Perinatal and Two-year Drinking Water Study

Parameter[a]	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Gestation Day Interval[b,c]				
6–9	29.72 ± 0.83* (39)	29.89 ± 0.77 (34)	30.21 ± 0.91 (35)	33.29 ± 1.03 (36)
9–12	31.55 ± 0.96 (41)	32.01 ± 0.93 (34)	31.92 ± 0.82 (36)	34.46 ± 1.04 (36)
12–15	36.94 ± 0.79 (41)	36.47 ± 0.89 (34)	35.33 ± 0.91 (36)	38.93 ± 1.06 (36)
15–18	43.39 ± 1.00* (41)	44.50 ± 1.23 (34)	43.54 ± 1.09 (36)	47.06 ± 1.38* (36)
18–21	45.27 ± 1.16 (41)	45.61 ± 1.25 (34)	44.43 ± 1.13 (36)	46.40 ± 1.54 (36)
6–21	37.31 ± 0.83* (39)	37.70 ± 0.91 (34)	37.19 ± 0.89 (35)	40.03 ± 1.08 (36)
Lactation Day Interval[b,c]				
1–4	47.08 ± 1.08 (41)	46.17 ± 1.07 (34)	46.03 ± 0.93 (35)	46.36 ± 1.22 (34)
4–7	50.35 ± 1.05 (37)	49.85 ± 1.09 (33)	47.90 ± 0.92 (33)	48.68 ± 0.81 (32)
7–10	63.61 ± 1.51 (37)	60.39 ± 1.27 (32)	60.49 ± 1.20 (32)	60.80 ± 1.36 (32)
10–14	74.39 ± 1.27 (35)	74.75 ± 1.18 (31)	76.51 ± 1.43 (32)	74.59 ± 1.49 (32)
14–17	86.82 ± 1.69* (37)	82.54 ± 1.50 (32)	85.16 ± 1.93 (32)	80.28 ± 1.85 (32)
17–21	96.58 ± 1.57 (37)	91.38 ± 1.92 (32)	90.17 ± 2.46 (32)	90.51 ± 2.68 (31)
1–14	59.71 ± 1.04 (35)	58.86 ± 0.92 (31)	59.17 ± 0.92 (32)	59.10 ± 1.04 (32)
Chemical Intake (mg/kg/day)[d,e]				
GD 6–21	0.00 ± 0.00 (39)	32.49 ± 0.68 (34)	64.77 ± 1.52 (35)	143.2 ± 3.70 (36)
LD 1–14	0.00 ± 0.00 (35)	48.14 ± 0.72 (31)	97.92 ± 1.53 (32)	197.3 ± 3.44 (32)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$.
GD = gestation day; LD = lactation day.
[a]Data presented as mean ± standard error (number of dams).
[b]Water consumption data are presented as grams/animal/day.
[c]Each exposure group was compared to the vehicle control group with the Shirley test when a trend was present ($p \leq 0.01$ from the Jonckheere trend test) or with the Dunn test when no trend was present.
[d]Chemical intake calculated as: ([exposure concentration × water consumption]/[average body weight of day range]).
[e]No statistical analysis was performed on the chemical intake data.

No significant clinical observations were noted in pups (Appendix G). No exposure-related differences were noted between the vehicle control groups and the ST-exposed groups in the number of litters, litter size, mean litter weights, sex ratio, or the pup mean weights of males and

females on PND 1 or PND 4 (Table 17, Table 18). Litter size did not change appreciably between PND 1 and litter standardization on PND 4. Pups were weaned on PND 21, and this was considered day 1 of the 2-year exposure period.

Table 17. Summary of Mean Litter Size and Survival Ratio of F_1 Male and Female Rats during Lactation in the Perinatal and Two-year Drinking Water Study of Sodium Tungstate Dihydrate

Parameter	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
PND 1[a,b]				
Total	12.83 ± 0.45 (41)	13.15 ± 0.31 (34)	13.03 ± 0.36 (35)	11.82 ± 0.49 (34)
Live	12.51 ± 0.49 (41)	12.82 ± 0.33 (34)	12.69 ± 0.37 (35)	11.68 ± 0.51 (34)
% Male per Litter	51.83 ± 2.36 (41)	49.27 ± 3.10 (34)	47.95 ± 2.53 (35)	51.42 ± 2.04 (34)
% Male[c,d]	52 (513)	50 (436)	48 (444)	52 (397)
Male[a,b]				
PND 1	6.54 ± 0.42 (41)	6.35 ± 0.44 (34)	6.11 ± 0.38 (35)	6.03 ± 0.37 (34)
PND 4 Prestandardization	6.51 ± 0.42 (41)	6.29 ± 0.44 (34)	6.11 ± 0.38 (35)	6.03 ± 0.37 (34)
PND 4 Poststandardization	4.05 ± 0.12 (37)	4.00 ± 0.13 (33)	3.97 ± 0.08 (33)	3.97 ± 0.07 (32)
Female[a,b]				
PND 1	5.98 ± 0.33 (41)	6.47 ± 0.37 (34)	6.57 ± 0.33 (35)	5.65 ± 0.34 (34)
PND 4 Prestandardization	5.85 ± 0.34 (41)	6.38 ± 0.39 (34)	6.49 ± 0.34 (35)	5.56 ± 0.33 (34)
PND 4 Poststandardization	3.95 ± 0.12 (37)	4.00 ± 0.13 (33)	4.03 ± 0.08 (33)	4.03 ± 0.07 (32)
Male and Female[a,b]				
PND 4 Prestandardization	12.37 ± 0.49 (41)	12.68 ± 0.34 (34)	12.60 ± 0.39 (35)	11.59 ± 0.51 (34)
PND 4 Poststandardization	8.00 ± 0.00 (37)	8.00 ± 0.00 (33)	8.00 ± 0.00 (33)	8.00 ± 0.00 (32)
PND 21	7.92 ± 0.05 (37)	7.94 ± 0.04 (32)	7.94 ± 0.04 (32)	7.81 ± 0.13 (32)
Survival per Litter				
Total Dead: PND 1–4[d,e]	19 (41)	16 (34)	15 (35)	8 (34)
Total Dead: PND 5–21[d,e]	3 (37)	2 (32)	2 (32)	6 (32)
Dead: PND 1–4[a,b,f]	0.463 ± 0.140 (41)	0.471 ± 0.185 (34)	0.429 ± 0.170 (35)	0.235 ± 0.085 (34)
Dead: PND 4–21[a,b,f]	0.081 ± 0.045 (37)	0.063 ± 0.043 (32)	0.063 ± 0.043 (32)	0.188 ± 0.130 (32)
Survival Ratio: PND 1–4[a,b,g]	0.988 ± 0.007 (41)	0.988 ± 0.007 (34)	0.991 ± 0.005 (35)	0.993 ± 0.004 (34)
Survival Ratio: PND 4–21[a,b,h]	0.990 ± 0.006 (37)	0.992 ± 0.005 (32)	0.992 ± 0.005 (32)	0.977 ± 0.016 (32)

PND = postnatal day.
[a]Each exposure group was compared to the vehicle control group with the Shirley test when a trend was present ($p \leq 0.01$ from the Jonckheere trend test) or with the Dunn test when no trend was present.
[b]Data presented as mean ± standard error (number of litters).
[c]100 × [number of live males in exposure group]/[number of live males and females in exposure group].
[d]No statistics done on this endpoint.
[e]Total dead in exposure group (number of litters).
[f]Number dead/litter.
[g]Survival per litter: number of pups prestandardization on PND 4/total live pups on PND 1.
[h]Survival per litter: number of live pups on PND 21/number of live pups poststandardization on PND 4.

Table 18. Summary of Preweaning F_1 Male and Female Rat Pup Mean Body Weights Following Perinatal Exposure to Sodium Tungstate Dihydrate

Parameter	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male (g)				
PND 1[a,b,c]	6.82 ± 0.07 (41)	6.80 ± 0.08 (34)	6.74 ± 0.07 (35)	6.69 ± 0.11 (34)
PND 4[d,e,f]	9.80 ± 0.15 (267/41)	9.30 ± 0.22 (214/34)	9.75 ± 0.15 (214/35)	9.18 ± 0.21* (205/34)
PND 7[d,e,g]	14.97 ± 0.35 (150/37)	14.19 ± 0.37 (131/33)	14.70 ± 0.35 (131/33)	14.35 ± 0.34 (125/32)
PND 14[d,e,g]	30.97 ± 0.65 (148/37)	30.12 ± 0.48 (126/32)	30.26 ± 0.55 (126/32)	29.84 ± 0.55 (125/32)
PND 21[d,e,g]	51.32 ± 1.05 (148/37)	49.48 ± 0.86 (126/32)	49.77 ± 0.94 (126/32)	48.96 ± 1.08 (125/32)
Female (g)				
PND 1[a,b,c]	6.43 ± 0.08 (41)	6.50 ± 0.09 (34)	6.46 ± 0.07 (35)	6.34 ± 0.09 (34)
PND 4[d,e,f]	9.31 ± 0.17 (240/41)	8.98 ± 0.22 (217/34)	9.43 ± 0.15 (226/35)	8.86 ± 0.18 (189/34)
PND 7[d,e,g]	14.19 ± 0.35 (146/37)	13.67 ± 0.41 (130/33)	14.21 ± 0.36 (131/33)	13.56 ± 0.34 (128/32)
PND 14[d,e,g]	29.71 ± 0.65 (145/37)	29.17 ± 0.52 (128/32)	29.55 ± 0.63 (128/32)	28.39 ± 0.69 (125/32)
PND 21[d,e,g]	48.27 ± 1.03 (145/37)	47.00 ± 0.87 (128/32)	47.59 ± 0.92 (128/32)	46.26 ± 1.10 (125/32)
Male and Female (g)				
PND 1[a,b,c]	6.63 ± 0.07 (41)	6.65 ± 0.09 (34)	6.60 ± 0.07 (35)	6.52 ± 0.10 (34)
PND 4[d,e,f]	9.58 ± 0.16 (507/41)	9.14 ± 0.22 (431/34)	9.59 ± 0.15 (440/35)	9.05 ± 0.19 (394/34)
PND 7[d,e,g]	14.60 ± 0.34 (296/37)	13.94 ± 0.37 (261/33)	14.47 ± 0.36 (262/33)	13.95 ± 0.34 (253/32)
PND 14[d,e,g]	30.37 ± 0.63 (293/37)	29.64 ± 0.49 (254/32)	29.91 ± 0.58 (254/32)	29.18 ± 0.54 (250/32)
PND 21[d,e,g]	49.84 ± 1.01 (293/37)	48.23 ± 0.85 (254/32)	48.71 ± 0.91 (254/32)	47.64 ± 1.01 (250/32)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
*Statistically significant at $p \leq 0.05$.
PND = postnatal day.
[a]Data presented as mean ± standard error (number of litters).
[b]Each exposure group was compared to the vehicle control group with the Williams test when a trend was present ($p \leq 0.01$ from the Jonckheere trend test) or with the Dunnett test when no trend was present.
[c]Litter weights of live pups at PND 1 were divided by live litter size on PND 1 to obtain a pup mean weight/litter. Mean values were then adjusted using live litter size on PND 1 as a covariate.
[d]Statistical analysis was performed using mixed models with random litter effect for both trend and pairwise tests, using the Dunnett-Hsu adjustment for multiple comparisons.
[e]Data presented as the mean of litter means ± standard error (number of pups/number of litters).
[f]PND 4 prestandardization.
[g]Individual pup weights first adjusted for live litter size on PND 4 poststandardization.

Plasma from up to five dams per exposure group along with plasma from one pup/sex/dam were collected on PND 21. Total tungsten concentrations were determined using a validated analytical method (Appendix E). In dams, tungsten concentrations increased with the exposure concentration (Table 19). Tungsten was also detected in male and female pups with concentrations slightly lower than in dams, suggesting significant exposure of pups to tungsten via lactation and/or from direct consumption of dosed drinking water. There was no apparent sex difference in tungsten concentrations in the pups.

Table 19. Summary of Internal Dose Data for F_0 Female Rats and Pups in the Perinatal and Two-year Drinking Water Study of Sodium Tungstate Dihydrate[a]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
n	5	5	5	5
Tungsten Concentration (µg/mL)				
Postnatal Day 21				
Dam plasma	BD	1.54 ± 0.17	2.35 ± 0.10	3.82 ± 0.84
Male pup plasma	BD	0.84 ± 0.17	1.48 ± 0.30	3.68 ± 0.89
Female pup plasma	BD	0.76 ± 0.26	1.35 ± 0.18	2.58 ± 0.56[b]

BD = below detection; group did not have over 20% of its values above the limit of detection (LOD; 0.013 µg/mL).
[a]Data are presented as mean ± standard error.
[b]n = 4.

Two-year Study (Interim Evaluations – 3, 6, 12, and 18 Months)

Ten predesignated rats/sex from each exposure group were evaluated at 3, 6, 12, or 18 months for organ weights and tungsten concentrations. Mean body weights of the 500 mg/L males were significantly decreased by approximately 12% relative to the vehicle control group at the 12-month time point (Table 20). Mean body weights of all other groups, both males and females, were within 10% of their respective vehicle control group at all interim evaluations (Table 20, Table 21).

The mean relative kidney weights were significantly increased at 3 months in the 1,000 mg/L males relative to the vehicle control group (Table 20); in females, there were no significant differences in kidney weights between exposed groups and the vehicle control group (Table 21). At 6 months, there were no significant differences in the kidney weights of males (Table 20), but in females, the mean absolute kidney weights were significantly increased in the 500 mg/L and 1,000 mg/L groups by approximately 6% and 7%, respectively, compared to the vehicle control group, and the mean relative kidney weight was significantly increased in the 1,000 mg/L group (Table 21). By 12 months, the mean absolute kidney weight was significantly decreased by approximately 13% in the 500 mg/L males compared to the vehicle control males (Table 20), and in females, the mean relative kidney weights were significantly increased in the 500 and 1,000 mg/L groups, relative to the vehicle control group (Table 21). At 18 months, mean absolute kidney weights were significantly decreased in the 500 and 1,000 mg/L males, by approximately 16% and 28%, respectively, relative to the vehicle control group (Table 20), whereas in females, there were no significant differences in kidney weights (Table 21).

At 3 months, the mean absolute liver weights were significantly decreased relative to the vehicle control group by approximately 12% in the 250 mg/L males. By 12 months, the mean absolute liver weights were significantly decreased relative to the vehicle control group in all male exposed groups by approximately 11%, 16%, and 17% in the 250, 500 and 1,000 mg/L males, respectively (Table 20). At 18 months, the mean absolute liver weight was significantly decreased relative to the vehicle control group by approximately 18% in the 1,000 mg/L males (Table 20). In females, the trend was significant for decreased absolute liver weight with increasing exposure concentration at 18 months (Table 21). The biological importance of these changes in liver weights is unknown; no liver effects were observed histologically in the 2-year study.

Other sporadic differences in organ weights were considered isolated changes of no toxicological significance (Appendix G).

Table 20. Summary of Select Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Rats Exposed to Sodium Tungstate Dihydrate in Drinking Water for 3, 6, 12, and 18 Months[a,b]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Three Months[c]	10	10	10	10
Necropsy Body Wt. (g)	420.7 ± 8.3*	387.7 ± 8.9*	407.8 ± 11.0*	384.0 ± 6.3*
Kidneys				
Absolute (g)	2.82 ± 0.07	2.72 ± 0.07	2.81 ± 0.11	2.76 ± 0.08
Relative (mg/g)[d]	6.71 ± 0.10*	7.02 ± 0.12	6.87 ± 0.14	7.17 ± 0.14*
Liver				
Absolute (g)	16.95 ± 0.55	14.84 ± 0.51*	16.30 ± 0.74	15.48 ± 0.55
Relative (mg/g)	40.23 ± 0.84	38.21 ± 0.65	39.85 ± 1.09	40.24 ± 0.96
Six Months	10	10	10	10
Necropsy Body Wt. (g)	481.4 ± 14.1	473.9 ± 9.9	467.3 ± 5.7	452.6 ± 12.0
Kidneys				
Absolute (g)	2.96 ± 0.12	2.87 ± 0.08	2.84 ± 0.07	2.75 ± 0.05
Relative (mg/g)	6.14 ± 0.11	6.05 ± 0.10	6.08 ± 0.14	6.09 ± 0.11
Liver				
Absolute (g)	15.55 ± 0.75	14.96 ± 0.59	15.03 ± 0.41	14.02 ± 0.50
Relative (mg/g)	32.16 ± 0.79	31.48 ± 0.72	32.19 ± 0.85	30.95 ± 0.63
Twelve Months	8	10	10	10
Necropsy Body Wt. (g)	586.4 ± 18.8*	571.9 ± 16.3	515.3 ± 15.8*	524.8 ± 18.5
Kidneys				
Absolute (g)	3.57 ± 0.09	3.45 ± 0.10	3.10 ± 0.11*	3.31 ± 0.14
Relative (mg/g)	6.11 ± 0.19	6.04 ± 0.15	6.03 ± 0.19	6.32 ± 0.20
Liver				
Absolute (g)	21.84 ± 0.93**	19.46 ± 0.44*	18.26 ± 0.73**	18.09 ± 0.91**
Relative (mg/g)	37.25 ± 1.08	34.20 ± 0.95	35.40 ± 0.81	34.38 ± 0.79
Eighteen Months	8	9	8	9
Necropsy Body Wt. (g)	604.9 ± 24.1*	609.5 ± 14.2	594.2 ± 15.6	500.0 ± 33.6*
Kidneys				
Absolute (g)	4.57 ± 0.36**	4.04 ± 0.19	3.84 ± 0.11*	3.28 ± 0.18**
Relative (mg/g)	7.72 ± 0.81	6.64 ± 0.34	6.48 ± 0.23	6.67 ± 0.32
Liver				
Absolute (g)	21.01 ± 0.64**	21.98 ± 0.78	19.91 ± 1.10	17.28 ± 1.31*
Relative (mg/g)	34.90 ± 0.96	36.03 ± 0.75	33.46 ± 1.46	34.47 ± 0.82

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and the Williams or Dunnett (pairwise) tests.
[c]Number of animals examined at each time point.
[d]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

Table 21. Summary of Select Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats Exposed to Sodium Tungstate Dihydrate in Drinking Water for 3, 6, 12, and 18 Months[a,b]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Three Months[c]	10	10	10	10
Necropsy Body Wt. (g)	251.6 ± 3.9	257.2 ± 6.9	245.6 ± 5.9	236.9 ± 6.5
Kidneys				
Absolute (g)	1.67 ± 0.03	1.66 ± 0.05	1.66 ± 0.06	1.67 ± 0.05
Relative (mg/g)[d]	6.64 ± 0.09	6.45 ± 0.14	6.74 ± 0.20	7.05 ± 0.13
Liver				
Absolute (g)	9.01 ± 0.14	9.25 ± 0.46	9.09 ± 0.31	8.59 ± 0.31
Relative (mg/g)	35.82 ± 0.53	35.83 ± 1.04	36.94 ± 0.55	36.23 ± 0.74
Six Months	10	10	10	10
Necropsy Body Wt. (g)	278.8 ± 4.0	277.3 ± 6.7	287.5 ± 6.4	275.6 ± 7.4
Kidneys				
Absolute (g)	1.71 ± 0.03**	1.67 ± 0.04	1.82 ± 0.05*	1.83 ± 0.03*
Relative (mg/g)	6.12 ± 0.04**	6.04 ± 0.08	6.32 ± 0.11	6.67 ± 0.17**
Liver				
Absolute (g)	8.66 ± 0.37	8.69 ± 0.35	9.09 ± 0.34	8.45 ± 0.27
Relative (mg/g)	31.01 ± 1.14	31.23 ± 0.64	31.59 ± 0.91	30.73 ± 0.92
Twelve Months	9	10	10	10
Necropsy Body Wt. (g)	313.9 ± 16.0	336.9 ± 15.7	310.0 ± 9.6	290.9 ± 10.5
Kidneys				
Absolute (g)	1.87 ± 0.07	1.95 ± 0.05	2.06 ± 0.06	1.93 ± 0.06
Relative (mg/g)	6.02 ± 0.21**	5.84 ± 0.16	6.68 ± 0.22*	6.67 ± 0.23*
Liver				
Absolute (g)	9.61 ± 0.44	10.81 ± 0.53	10.03 ± 0.49	8.73 ± 0.36
Relative (mg/g)	30.74 ± 0.84	32.40 ± 1.64	32.33 ± 1.12	30.06 ± 0.99
Eighteen Months	10	8	10	8
Necropsy Body Wt. (g)	359.5 ± 11.5*	372.9 ± 25.5	353.8 ± 11.5	313.6 ± 16.4
Kidneys				
Absolute (g)	2.20 ± 0.06	2.12 ± 0.05	2.12 ± 0.08	2.05 ± 0.06
Relative (mg/g)	6.16 ± 0.16	5.91 ± 0.49	6.02 ± 0.26	6.63 ± 0.28
Liver				
Absolute (g)	10.97 ± 0.52*	11.58 ± 0.64	10.54 ± 0.58	9.49 ± 0.50
Relative (mg/g)	30.67 ± 1.52	31.98 ± 2.75	29.83 ± 1.47	30.29 ± 0.62

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and the Williams or Dunnett (pairwise) tests.
[c]Number of animals examined at each time point.
[d]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

Plasma, kidney, and urine from up to 10 animals per exposure group were collected from interim animals at 3, 6, 12, and 18 months. Total tungsten concentrations in all matrices were determined using validated analytical methods (Appendix E). In male rats, plasma tungsten concentrations increased proportionally with exposure concentration at all time points (except at 18 months for the 500 mg/L group) with no observed differences between time points (Table 22; Figure 3A). In female rats, plasma tungsten concentrations increased proportionally with exposure concentration up to 500 mg/L at all time points (except at 18 months for the 250 mg/L group); however, at 1,000 mg/L, the trend was toward a more-than-proportional increase in tungsten concentrations with increasing exposure concentration (Table 23; Figure 3A). Low tungsten concentrations were observed in some vehicle control groups; however, tungsten concentrations in exposed groups were significantly higher than those in corresponding vehicle control groups. There was no observed sex difference in plasma tungsten concentrations in rats (Table 22, Table 23; Figure 3A).

In male rats, at 3 months, tungsten concentrations in the kidney increased proportionally with exposure concentration (Table 22; Figure 3B); however, the trend was toward a less-than-proportional increase in tungsten concentrations with increasing exposure concentration at 6, 12, and 18 months in males (except at 18 months for the 1,000 mg/L group) and at all time points in females (Table 22, Table 23; Figure 3B). Kidney tungsten concentrations increased with increasing exposure duration and concentration in both males and females, with the kidney/plasma ratio >1 (Table 22, Table 23). Taken collectively, these data demonstrate that tungsten is retained in the kidney, and the retention increases with the exposure duration (Figure 3B). Low tungsten concentrations were observed in some vehicle control groups; however, tungsten concentrations in exposed groups were significantly higher than those in corresponding vehicle control groups. There were no observed sex differences in kidney tungsten concentrations in rats (Table 22, Table 23; Figure 3B).

Tungsten concentrations in urine are presented as both µg/mL of urine and µg/mg creatinine. Creatinine-corrected tungsten concentrations in urine increased with exposure concentration in both males and females (Figure 3C). The trend was toward a less-than-proportional increase in tungsten concentration with increasing exposure concentration and an increase in tungsten concentration with exposure duration, both of which were more evident in females than in males. Depending on the exposure concentration and duration, females excreted 1.2- to 4-fold more tungsten in urine compared to males (Table 22, Table 23). Low tungsten concentrations were observed in some vehicle control groups; however, the tungsten concentrations in exposed groups were significantly higher than those in the corresponding vehicle control groups.

Table 22. Summary of Plasma, Kidney, and Urine Tungsten Concentration Data for Male Rats Exposed to Sodium Tungstate Dihydrate in Drinking Water for 3, 6, 12, and 18 Months[a]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Three Months				
Kidney (µg/g)	BD	2.79 ± 0.24 (10)	5.30 ± 0.50 (10)	10.50 ± 2.94 (10)
Plasma (µg/mL)[b,c]	0.01 ± 0.00** (10)	1.34 ± 0.20** (10)	2.91 ± 0.27** (10)	5.87 ± 0.54** (10)
Kidney/Plasma Ratio[d]	BD	2.51 ± 0.42 (10)	1.87 ± 0.16 (10)	1.83 ± 0.51 (10)
Urine[b,c]				
Urine (µg/mL urine)	0.04 ± 0.01** (10)	135.36 ± 16.14** (9)	321.33 ± 21.53** (9)	862.80 ± 58.27** (10)

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Urine (µg/mg creatinine)	0.02 ± 0.00** (10)	101.00 ± 12.63** (9)	268.52 ± 30.82** (9)	663.17 ± 41.98** (10)
Six Months				
Kidney (µg/g)	BD	6.77 ± 2.13 (10)	13.40 ± 1.86 (10)	14.11 ± 1.05 (10)
Plasma (µg/mL)[b,c]	0.02 ± 0.00** (10)	1.37 ± 0.14** (10)	4.89 ± 1.02** (10)	6.48 ± 0.78** (10)
Kidney/Plasma Ratio	BD	5.55 ± 2.04 (10)	3.39 ± 0.64 (10)	2.45 ± 0.33 (10)
Urine[b,c]				
Urine (µg/mL urine)	0.04 ± 0.00** (10)	143.37 ± 16.83** (10)	258.30 ± 27.17** (10)	572.10 ± 59.06** (10)
Urine (µg/mg creatinine)	0.03 ± 0.00** (10)	107.36 ± 10.22** (10)	216.91 ± 30.97** (10)	338.59 ± 41.61** (10)
Twelve Months				
Kidney (µg/g)	BD	14.84 ± 2.09 (10)	20.42 ± 3.85 (10)	27.98 ± 2.71 (10)
Plasma (µg/mL)[b,c]	0.01 ± 0.00** (8)	2.22 ± 0.25** (10)	3.85 ± 0.62** (10)	7.05 ± 0.55** (10)
Kidney/Plasma Ratio	BD	7.50 ± 1.09 (10)	6.22 ± 1.26 (10)	4.11 ± 0.45 (10)
Urine[b,c]				
Urine (µg/mL urine)	1.29 ± 0.95** (8)	161.92 ± 15.97** (10)	368.70 ± 52.38** (10)	654.23 ± 125.91** (10)
Urine (µg/mg creatinine)	2.05 ± 1.76** (8)	94.50 ± 14.54** (10)	284.48 ± 66.96** (10)	435.73 ± 65.39** (10)
Eighteen Months				
Kidney (µg/g)[b,c]	0.05 ± 0.01** (6)	25.74 ± 3.34** (9)	30.27 ± 5.21** (8)	74.90 ± 20.04** (9)
Plasma (µg/mL)	BD	2.64 ± 0.35 (9)	2.34 ± 0.95 (8)	7.59 ± 2.66 (9)
Kidney/Plasma Ratio	BD	12.63 ± 3.18 (9)	24.26 ± 8.14 (8)	16.69 ± 4.27 (9)
Urine[b,c]				
Urine (µg/mL urine)	0.03 ± 0.01** (9)	121.77 ± 9.17** (9)	159.93 ± 28.11** (8)	390.11 ± 38.03** (9)
Urine (µg/mg creatinine)	0.03 ± 0.01** (8)	104.37 ± 7.08** (9)	156.40 ± 27.17** (7)	436.76 ± 109.49** (9)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
**Statistically significant at $p \leq 0.01$.
BD = below detection; group did not have more than 20% of its values above the limit of detection (LOD).
[a]Data presented as mean ± standard error (n).
[b]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.
[c]Values below the LOD (0.013 µg/mL) were substituted with 1/2 the LOD value. If 80% or more of the values in the vehicle control group were below the LOD, no mean or standard error were calculated, and no statistical analysis was performed.
[d]For the kidney/plasma ratio calculation, a plasma density of 1 g/mL was assumed.

Table 23. Summary of Plasma, Kidney, and Urine Tungsten Concentration Data for Female Rats Exposed to Sodium Tungstate Dihydrate in Drinking Water for 3, 6, 12, and 18 Months[a]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Three Months				
Kidney (µg/g)	BD	3.92 ± 0.24 (10)	6.39 ± 0.52 (10)	11.93 ± 0.71 (10)
Plasma (µg/mL)	BD	1.92 ± 0.13 (10)	3.67 ± 0.58 (10)	10.99 ± 1.40 (10)
Kidney/Plasma Ratio[b]	BD	2.06 ± 0.08 (10)	1.92 ± 0.18 (10)	1.17 ± 0.09 (10)
Urine[c,d]				
Urine (µg/mL urine)	0.04 ± 0.01** (10)	167.30 ± 9.11** (10)	355.50 ± 26.36** (8)	512.20 ± 43.84** (10)
Urine (µg/mg creatinine)	0.05 ± 0.01** (10)	247.12 ± 14.01** (10)	484.15 ± 56.46** (8)	809.98 ± 103.55** (10)

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Six Months				
Kidney (µg/g)	BD	6.65 ± 0.92 (10)	9.89 ± 0.67 (10)	17.11 ± 1.81 (10)
Plasma (µg/mL)[c,d]	0.05 ± 0.01** (10)	2.41 ± 0.43** (10)	4.01 ± 0.39** (10)	10.44 ± 1.38** (10)
Kidney/Plasma Ratio	BD	2.96 ± 0.32 (10)	2.56 ± 0.13 (10)	1.70 ± 0.11 (10)
Urine[c,d]				
Urine (µg/mL urine)	0.04 ± 0.01** (10)	174.10 ± 10.74** (10)	413.30 ± 20.05** (10)	619.20 ± 50.46** (10)
Urine (µg/mg creatinine)	0.05 ± 0.00** (10)	228.49 ± 20.07** (10)	467.46 ± 40.93** (10)	856.85 ± 58.29** (10)
Twelve Months				
Kidney (µg/g)	BD	10.62 ± 0.53 (10)	19.26 ± 1.89 (10)	31.65 ± 2.20 (10)
Plasma (µg/mL)[c,d]	0.03 ± 0.01** (8)[e]	2.47 ± 0.29** (10)	4.55 ± 0.33** (10)	12.06 ± 1.31** (10)
Kidney/Plasma Ratio	4.57 ± 0.85** (4)	4.76 ± 0.50 (10)	4.36 ± 0.42 (10)	2.96 ± 0.48 (10)
Urine[c,d]				
Urine (µg/mL urine)	0.07 ± 0.02** (9)	158.00 ± 8.45** (10)	291.60 ± 17.88** (10)	672.30 ± 51.25** (10)
Urine (µg/mg creatinine)	0.10 ± 0.02** (8)	298.03 ± 26.20** (10)	567.24 ± 42.20** (10)	1,036.16 ± 57.95** (10)
Eighteen Months				
Kidney (µg/g)[c,d]	0.09 ± 0.01** (3)	25.89 ± 7.69* (8)	27.96 ± 2.79** (10)	43.29 ± 2.90** (8)
Plasma (µg/mL)[c,d]	0.02 ± 0.00** (2)	5.45 ± 3.00* (8)	5.71 ± 0.59* (10)	11.78 ± 1.51** (8)
Kidney/Plasma Ratio	NR[f]	7.11 ± 1.17 (8)	5.47 ± 0.79 (10)	3.99 ± 0.42 (8)
Urine[c,d]				
Urine (µg/mL urine)	0.03 ± 0.00** (8)	162.00 ± 17.50** (8)	328.11 ± 37.85** (9)	612.38 ± 47.87** (8)
Urine (µg/mg creatinine)	0.05 ± 0.01** (8)	349.42 ± 34.89** (8)	628.91 ± 105.62** (9)	1,089.91 ± 100.25** (8)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
BD = below detection; group did not have more than 20% of its values above the limit of detection (LOD).
[a]Data presented as mean ± standard error (n).
[b]For the kidney/plasma ratio calculation, a plasma density of 1 g/mL was assumed.
[c]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.
[d]Values below the LOD (0.013 µg/mL) were substituted with 1/2 the LOD value. If 80% or more of the values in the vehicle control group were below the LOD, no mean or standard error were calculated, and no statistical analysis was performed.
[e]The plasma concentration value for one female in the 0 mg/L group at 12 months was excluded from the analysis as an implausible value.
[f]The kidney/plasma ratio could not be calculated for females in the 0 mg/L group at 18 months because no animals in the group had concentration measures for both kidney and plasma.

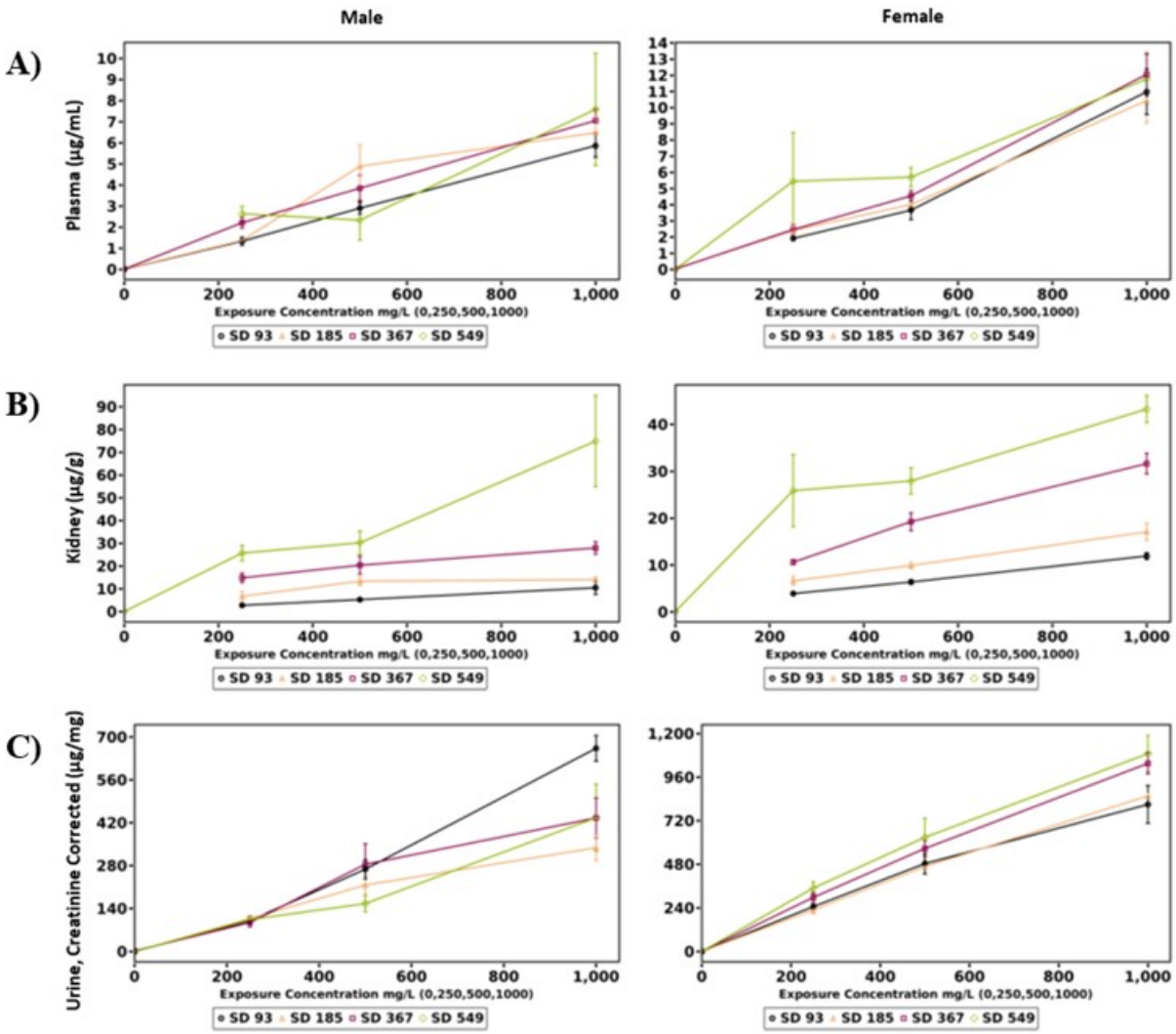


Figure 3. Tungsten Concentrations in Plasma, Kidney, and Urine in Rats Exposed to Sodium Tungstate Dihydrate in Drinking Water for 3, 6, 12, and 18 Months

Study day (SD) 93 = 3 months; SD 185 = 9 months; SD 367 = 12 months; SD 549 = 18 months.

Two-year Study (Postweaning Phase)

Survival

Survival to study termination was significantly increased in the groups of exposed male rats compared to the vehicle control males (Table 24; Figure 4). The survival of the vehicle control males was lower than that typically seen in groups of control male Sprague Dawley (Hsd:Sprague Dawley® SD®) rats in previous 2-year NTP studies. Early deaths in the vehicle control males were attributed at necropsy primarily to CPN (22 animals). Pituitary gland adenomas were listed as the cause of death at necropsy for six vehicle control males, compared to two, two, and one animal in the 250, 500, and 1,000 mg/L males, respectively. The large number of vehicle control males with CPN as a cause of death at necropsy corresponded to an

increased severity of nephropathy observed histologically in that group. There were no exposure-related differences in the survival of female groups.

Table 24. Summary of Survival of Male and Female Rats in the Perinatal and Two-year Drinking Water Study of Sodium Tungstate Dihydrate

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male				
Animals Initially in Study	50	50	50	50
Moribund	17	14	10	8
Natural Deaths	21	10	16	13
Animals Surviving to Study Termination	12	26	24[a]	29
Percent Probability of Survival at End of Study[b]	24.0%	52.0%	48.0%	58.0%
Mean Survival (Days)[c]	620.7 ± 17.4	656.6 ± 20.3	670.6 ± 13.7	657.4 ± 17.6
Survival Analysis[d]	p = 0.008N	p = 0.028N	p = 0.018N	p = 0.004N
Female				
Animals Initially in Study	50	50	50	50
Moribund	13	10	13	9
Natural Deaths	7	7	6	10
Animals Surviving to Study Termination	30	33	31	31[e]
Percent Probability of Survival at End of Study	60.0%	66.0%	62.0%	62.0%
Mean Survival (Days)	667.0 ± 15.1	686.7 ± 12.3	661.8 ± 18.0	674.7 ± 14.8
Survival Analysis	p = 0.976N	p = 0.467N	p = 0.964	p = 0.794

[a]Includes one animal that died naturally during the last week of the study.
[b]Kaplan-Meier determinations.
[c]Mean of litter means of all deaths (uncensored, censored, and study termination) ± standard error.
[d]The result of the Cox proportional hazards trend test with random litter effects is in the vehicle control group column, and the results of the proportional hazards pairwise comparisons to the vehicle control group with random litter effects are in the exposed group columns. A negative trend or lower mortality in an exposure group is indicated by N.
[e]Includes one animal that was euthanized moribund during the last week of the study.

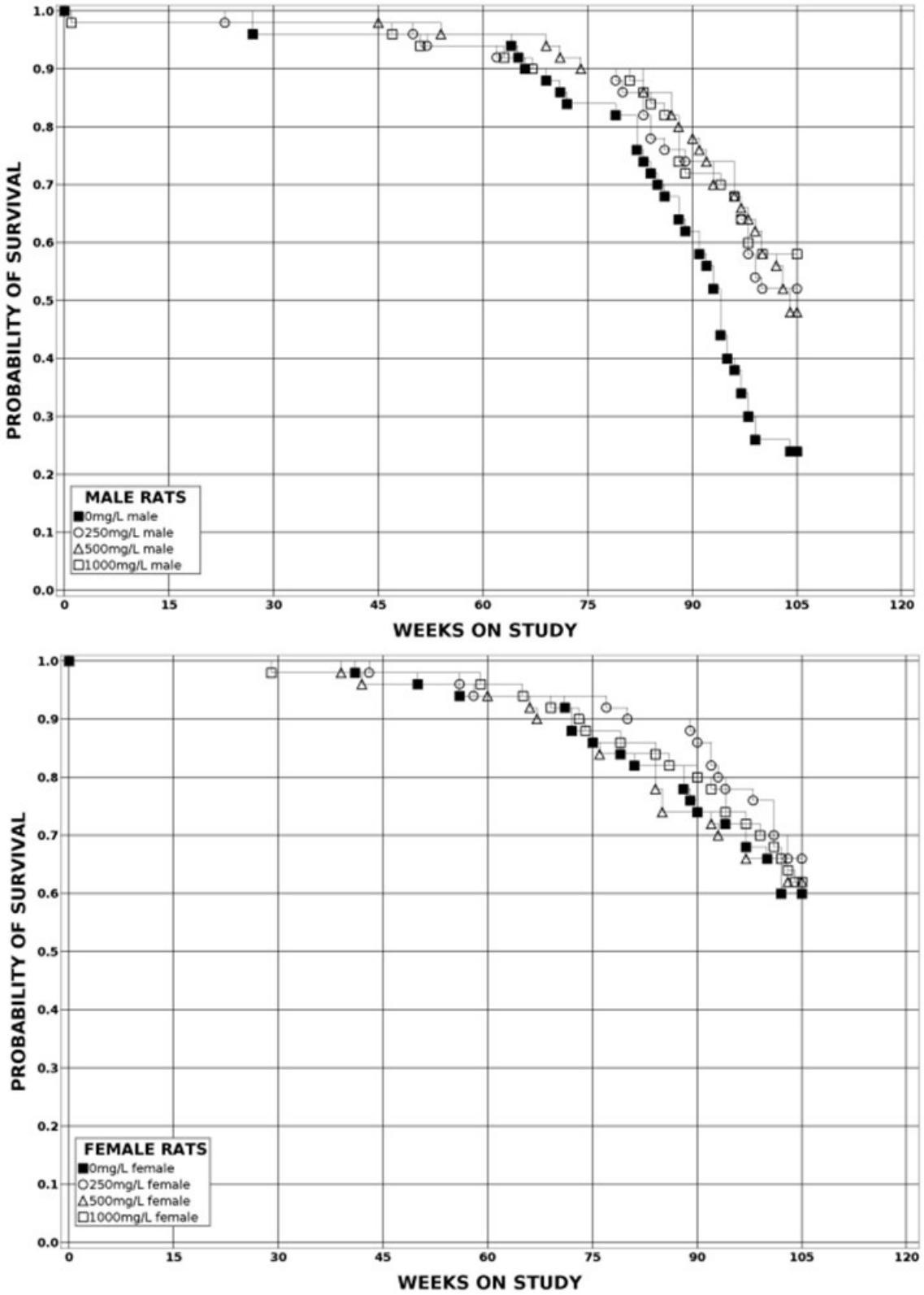


Figure 4. Kaplan-Meier Survival Curves for Rats Exposed to Sodium Tungstate Dihydrate in Drinking Water for Two Years

Body Weights, Water and Compound Consumption, and Clinical Observations

At study termination, mean body weights of all groups of exposed males were within 10% of the vehicle control group (Table 25; Figure 5). In females, mean body weights of the 500 mg/L and 1,000 mg/L groups at study termination were 88.9% and 78.4% of the vehicle control group, respectively (Table 26; Figure 5).

Over the course of the chronic study, the respective mean water consumption for the 250, 500, and 1,000 mg/L groups averaged 93%, 99%, and 84% of the vehicle control males and averaged 95%, 100%, and 91% of the vehicle control females (Table 27, Table 28). The daily ST consumption averaged 14.2, 30.4, and 54.5 mg/kg/day for males, and 18.2, 39.3, and 74.3 mg/kg/day for females, respectively (Appendix G). Consumption at weeks 1, 13, 54, and 102 is presented in Table 27 and Table 28. Overall, the consumed dose of ST was of similar proportionality (ranging from 1.9- to 2.1-fold increase from one dose to the next) to the increase in ST concentration in drinking water (2-fold increases from one concentration to the next). Dose proportionality was consistent for both male and female rats (Table 27, Table 28). No clinical observations were considered exposure related (Appendix G).

Table 25. Summary of Survival and Mean Body Weights of Male Rats in the Perinatal and Two-year Drinking Water Study of Sodium Tungstate Dihydrate

Study Day[a]	0 mg/L		250 mg/L			500 mg/L			1,000 mg/L		
	Av. Wt. (g)[a]	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters
1	53.2	25	52.2	98.2	25	52.6	98.9	25	52.6	98.9	25
8	82.2	25	81.1	98.6	25	82.3	100.1	25	80.7	98.2	25
15	127.5	25	125.7	98.6	25	127.4	100.0	25	118.0	92.5	25
22	178.3	25	174.4	97.8	25	177.1	99.3	25	156.0	87.5	25
29	226.9	25	222.1	97.9	25	225.2	99.2	25	194.0	85.5	25
36	271.3	25	267.6	98.6	25	269.1	99.2	25	235.4	86.8	25
43	304.9	25	302.9	99.3	25	301.3	98.8	25	270.5	88.7	25
50	326.8	25	324.7	99.3	25	325.7	99.7	25	299.4	91.6	25
57	352.9	25	349.8	99.1	25	350.5	99.3	25	325.6	92.3	25
64	371.4	25	363.8	97.9	25	366.8	98.8	25	345.9	93.1	25
71	386.9	25	380.7	98.4	25	378.1	97.7	25	357.1	92.3	25
78	399.7	25	394.7	98.7	25	392.3	98.1	25	374.5	93.7	25
85	411.8	25	405.7	98.5	25	402.3	97.7	25	385.6	93.6	25
92	419.1	25	415.4	99.1	25	408.9	97.6	25	395.5	94.4	25
120	443.5	25	431.7	97.4	25	429.7	96.9	25	420.0	94.7	25
148	466.5	25	459.1	98.4	25	448.9	96.2	25	442.1	94.8	25
176	489.9	25	478.8	97.7	25	471.6	96.3	25	460.6	94.0	25
204	505.2	25	494.7	97.9	25	487.0	96.4	25	476.7	94.4	25
232	521.0	25	508.6	97.6	25	500.1	96.0	25	491.5	94.3	25
260	537.7	25	523.7	97.4	25	517.0	96.1	25	505.3	94.0	25
288	549.3	25	533.1	97.0	25	528.4	96.2	25	516.2	94.0	25
316	560.4	25	547.1	97.6	25	540.4	96.4	25	527.5	94.1	25
344	573.4	25	555.6	96.9	25	553.2	96.5	25	537.9	93.8	25
372	585.5	25	570.5	97.4	25	561.7	95.9	25	547.6	93.5	25
400	593.9	25	580.4	97.7	25	572.1	96.3	25	555.5	93.5	25
428	598.3	25	589.3	98.5	25	579.9	96.9	25	561.8	93.9	25
456	601.3	25	589.9	98.1	25	584.7	97.2	25	566.2	94.2	25
484	603.4	25	595.5	98.7	25	589.1	97.6	25	568.4	94.2	25
512	606.9	25	597.1	98.4	25	589.5	97.1	25	566.7	93.4	25
540	602.0	25	596.8	99.1	25	587.0	97.5	25	564.0	93.7	25
568	595.3	25	594.7	99.9	23	578.2	97.1	25	558.6	93.8	25
596	593.0	23	588.5	99.2	22	576.8	97.3	24	552.7	93.2	25
624	586.2	21	589.2	100.5	21	566.4	96.6	24	557.9	95.2	24
652	580.7	18	572.2	98.5	21	576.3	99.3	22	551.3	94.9	23
680	582.7	12	551.4	94.6	19	551.0	94.6	22	545.5	93.6	23
708	601.8	9	559.8	93.0	15	550.9	91.5	19	548.3	91.1	21
EOS	**587.2**	**9**	**545.7**	**92.9**	**15**	**551.8**	**94.0**	**16**	**545.1**	**92.8**	**21**

EOS = end of study; No. of litters = number of litters represented in weight average.
[a]Study day 1 is the day animals were placed on study after pups were weaned.
[b]Average weights shown are means of litter means.

Table 26. Summary of Survival and Mean Body Weights of Female Rats in the Perinatal and Two-year Drinking Water Study of Sodium Tungstate Dihydrate

Study Day[a]	0 mg/L		250 mg/L			500 mg/L			1,000 mg/L		
	Av. Wt. (g)[b]	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters
1	51.4	25	49.7	96.6	25	49.8	96.9	25	52.1	101.2	25
8	78.6	25	76.9	97.9	25	77.6	98.7	25	76.0	96.7	25
15	113.7	25	109.1	95.9	25	110.6	97.2	25	100.6	88.5	25
22	139.1	25	136.0	97.8	25	135.6	97.5	25	122.9	88.3	25
29	166.2	25	162.9	98.0	25	163.3	98.3	25	144.9	87.2	25
36	180.9	25	181.7	100.5	25	181.3	100.3	25	164.5	90.9	25
43	198.4	25	199.3	100.4	25	194.8	98.2	25	185.6	93.5	25
50	211.8	25	212.5	100.3	25	207.6	98.0	25	201.8	95.3	25
57	222.7	25	225.0	101.1	25	220.3	99.0	25	215.0	96.6	25
64	227.2	25	224.8	99.0	25	223.1	98.2	25	215.1	94.7	25
71	233.4	25	229.9	98.5	25	227.5	97.5	25	226.5	97.0	25
78	241.6	25	238.9	98.9	25	239.7	99.2	25	235.8	97.6	25
85	247.4	25	245.0	99.0	25	243.5	98.4	25	241.0	97.4	25
92	254.4	25	249.5	98.1	25	249.1	97.9	25	246.5	96.9	25
120	266.2	25	259.3	97.4	25	261.2	98.1	25	258.9	97.3	25
148	275.0	25	273.4	99.4	25	269.7	98.0	25	266.4	96.9	25
176	286.4	25	283.1	98.9	25	278.6	97.3	25	275.7	96.3	25
204	290.4	25	289.3	99.6	25	280.2	96.5	25	284.4	97.9	25
232	295.5	25	297.6	100.7	25	289.2	97.9	25	291.9	98.8	25
260	303.1	25	302.8	99.9	25	293.0	96.7	25	297.7	98.2	25
288	307.0	25	311.6	101.5	25	299.8	97.7	25	301.7	98.3	25
316	317.7	25	320.1	100.7	25	305.5	96.1	25	307.1	96.6	25
344	324.4	25	324.8	100.1	25	309.8	95.5	25	312.8	96.4	25
372	332.0	25	329.6	99.3	25	314.9	94.8	25	316.9	95.4	25
400	343.4	25	338.1	98.5	25	321.6	93.6	25	323.3	94.2	25
428	348.4	25	342.8	98.4	25	325.4	93.4	25	325.7	93.5	25
456	354.2	25	353.0	99.7	25	337.2	95.2	24	327.5	92.5	25
484	364.9	25	359.6	98.6	25	345.8	94.8	24	331.8	90.9	25
512	365.5	25	363.0	99.3	25	355.2	97.2	24	333.2	91.2	25
540	381.5	25	371.1	97.3	25	365.8	95.9	24	342.6	89.8	25
568	377.9	24	377.3	99.8	25	376.4	99.6	24	339.0	89.7	25
596	385.4	24	384.3	99.7	25	363.6	94.3	22	345.3	89.6	25
624	393.9	24	388.1	98.5	25	370.7	94.1	22	342.5	86.9	24
652	392.7	24	390.5	99.4	25	381.9	97.3	21	335.4	85.4	23
680	384.1	24	381.9	99.4	25	366.2	95.3	21	330.1	85.9	23
708	386.1	22	384.4	99.6	24	372.8	96.5	21	326.9	84.7	22
EOS	**414.9**	**20**	**399.2**	**96.2**	**23**	**368.9**	**88.9**	**20**	**325.5**	**78.4**	**20**

EOS = end of study; No. of litters = number of litters represented in weight average.
[a]Study day 1 is the day animals were placed on study after pups were weaned.
[b]Average weights shown are means of litter means.

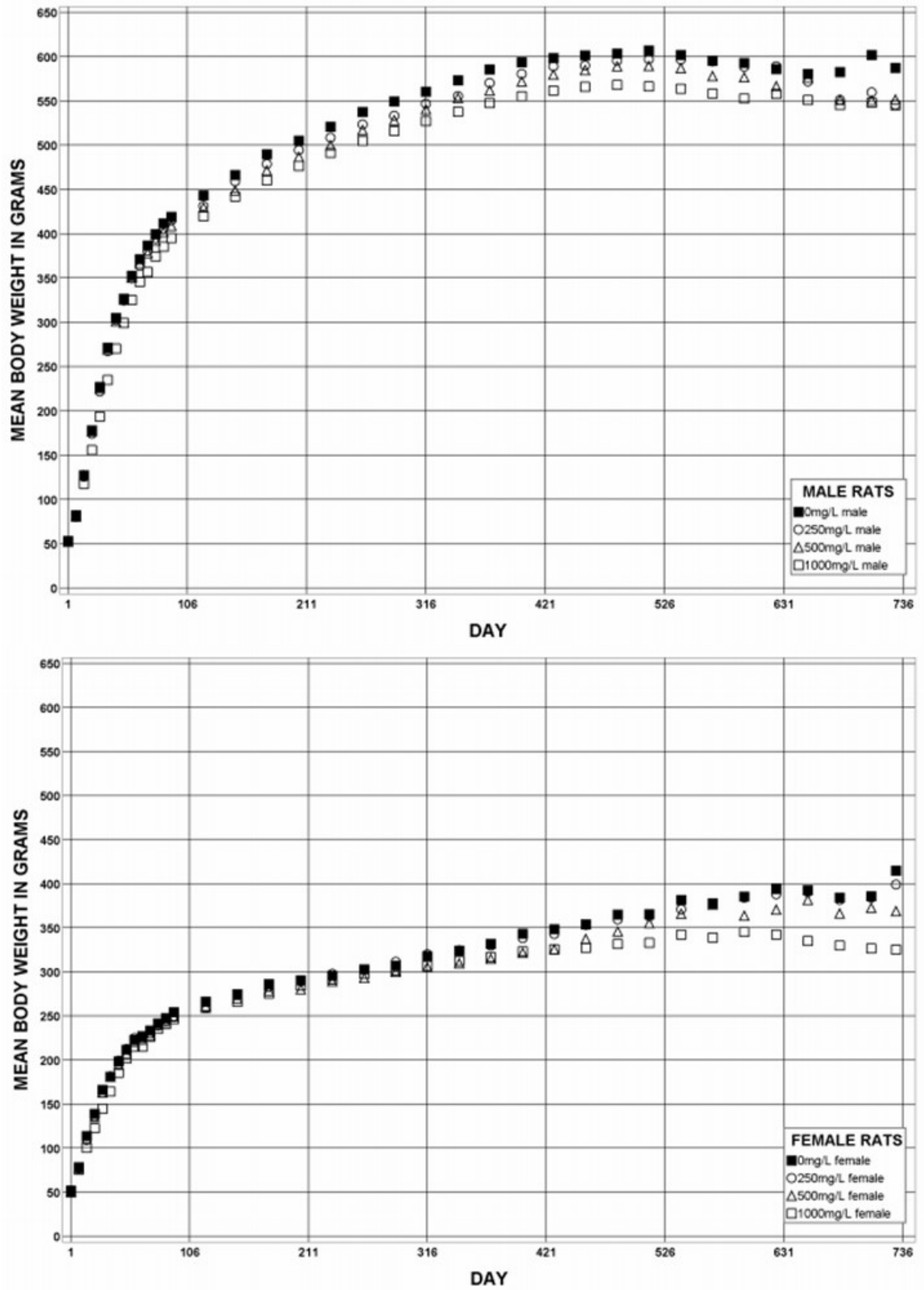


Figure 5. Growth Curves for Rats Exposed to Sodium Tungstate Dihydrate in Drinking Water for Two Years

Table 27. Summary of Water and Sodium Tungstate Dihydrate Consumption of Male Rats in the Perinatal and Two-year Drinking Water Study

Week	0 mg/L	250 mg/L		500 mg/L		1,000 mg/L	
	Water (g/day)[a]	Water (g/day)	Dose (mg/kg/day)[b]	Water (g/day)	Dose (mg/kg/day)	Water (g/day)	Dose (mg/kg/day)
1	8.9	8.8	42.1	8.3	78.9	9.6	182.5
13	24.9	23.4	14.4	24.8	30.8	23.3	60.3
54	26.5	24.9	10.9	25.4	22.6	22.9	41.7
102	42.4	35.9	16.0	42.2	38.0	27.7	49.8

[a]Grams of water consumed/animal/day.
[b]Milligrams of sodium tungstate dihydrate consumed/kilogram body weight/day.

Table 28. Summary of Water and Sodium Tungstate Dihydrate Consumption of Female Rats in the Perinatal and Two-year Drinking Water Study of Sodium Tungstate Dihydrate

Week	0 mg/L	250 mg/L		500 mg/L		1,000 mg/L	
	Water (g/day)[a]	Water (g/day)	Dose (mg/kg/day)[b]	Water (g/day)	Dose (mg/kg/day)	Water (g/day)	Dose (mg/kg/day)
1	10.2	9.9	49.8	10.3	103.3	9.9	190.1
13	20.0	18.2	18.6	18.9	38.8	17.6	73.0
54	22.7	21.0	15.8	22.3	35.5	19.9	62.7
102	27.0	24.7	16.1	29.0	39.6	28.1	87.4

[a]Grams of water consumed per animal/day.
[b]Milligrams of sodium tungstate dihydrate consumed/kilogram body weight/day.

Histopathology

This section describes the significant or biologically noteworthy changes in the incidence of neoplasms and nonneoplastic lesions of the thyroid gland, kidney, and uterus. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms are presented as supplemental data in Appendix G.

Thyroid Gland: There was a significant increase in the incidence of thyroid gland C-cell adenomas in the 500 mg/L females relative to the vehicle control group (Table 29). There was no significant increase in C-cell carcinomas, although the 1,000 mg/L females had a slightly higher incidence than the other exposure groups (Table 29). There were no significant differences in the incidences of C-cell adenoma or carcinoma (combined) in any exposed group compared to the vehicle control group; however, the incidences in the 250 and 500 mg/L groups were outside of the historical control range (Table 29). The incidences of C-cell hyperplasia were not significantly increased in any exposed group when compared to the vehicle control group (Table 29). In males, the incidence of C-cell neoplasms was greater in the 500 and 1,000 mg/L groups than in the control group, but the differences were not significant, and the incidences in all the groups were within the historical control range. C-cell adenomas consisted of a discrete proliferation of C-cells that was larger than five follicles in diameter and caused compression of surrounding follicles (Figure 6). C-cell carcinomas tended to be large neoplasms that replaced the normal architecture of the thyroid gland and were diagnosed when there was evidence of invasion into the thyroid gland capsule or surrounding tissue (Figure 7).

Table 29. Incidences of Neoplastic and Nonneoplastic Lesions of the Thyroid Gland in Female Rats in the Perinatal and Two-year Drinking Water Study of Sodium Tungstate Dihydrate

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
n[a]	50	50	49	50
C-cell, Hyperplasia[b]	14 (2.5)[c]	13 (1.8)	9 (2.0)	12 (1.8)
C-cell Adenoma[d]				
Overall rate[e]	5/50 (10%)	13/50 (26%)	13/49 (27%)	8/50 (16%)
Rate per litters[f]	4/25 (16%)	12/25 (48%)	10/25 (40%)	8/25 (32%)
Adjusted rate[g]	12.1%	29.7%	31.9%	18.5%
Terminal rate[h]	2/30 (7%)	12/33 (36%)	8/31 (26%)	5/31 (16%)
First incidence (days)	676	701	585	583
Rao-Scott-adjusted Poly-3 test[i]	p = 0.451	p = 0.057	p = 0.040	p = 0.321
C-cell Carcinoma[j]				
Overall rate	2/50 (4%)	2/50 (4%)	2/49 (4%)	4/50 (8%)
Rate per litters	2/25 (8%)	2/25 (8%)	2/25 (8%)	4/25 (16%)
Adjusted rate	4.9%	4.6%	5%	9.5%
Terminal rate	1/30 (3%)	1/33 (3%)	1/31 (3%)	4/31 (13%)
First incidence (days)	708	630	716	730 (T)
Rao-Scott-adjusted Poly-3 test	p = 0.228	p = 0.651N	p = 0.659	p = 0.344
C-cell Adenoma or Carcinoma (Combined)[k]				
Overall rate	7/50 (14%)	15/50 (30%)	14/49 (29%)	11/50 (22%)
Rate per litters	6/25 (24%)	13/25 (52%)	11/25 (44%)	11/25 (44%)
Adjusted rate	16.9%	34%	34.4%	25.4%
Terminal rate	3/30 (10%)	13/33 (39%)	9/31 (29%)	8/31 (26%)
First incidence (days)	676	630	585	583
Rao-Scott-adjusted Poly-3 test	p = 0.369	p = 0.073	p = 0.072	p = 0.260

[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[d]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 70/488 (15.05% ± 7.65%); range: 4% to 24%.
[e]Number of animals with neoplasm/number of animals necropsied.
[f]Number of litters with neoplasm-bearing animals/number of litters examined at site.
[g]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[h]Observed incidence at terminal euthanasia.
[i]Beneath the control incidence is the p value associated with the trend test. Beneath the exposed group incidences are the p values corresponding to pairwise comparisons between the vehicle control group and that exposed group. The Rao-Scott test adjusts the Poly-3 test (which accounts for differential mortality in animals that do not reach terminal euthanasia) for within-litter correlation. A negative trend or a lower incidence in an exposure group is indicated by N.
[j]Historical control incidence: 7/488 (1.56% ± 1.67%); range: 0% to 4%.
[k]Historical control incidence: 76/488 (16.38% ± 8.21%); range: 4% to 28%.

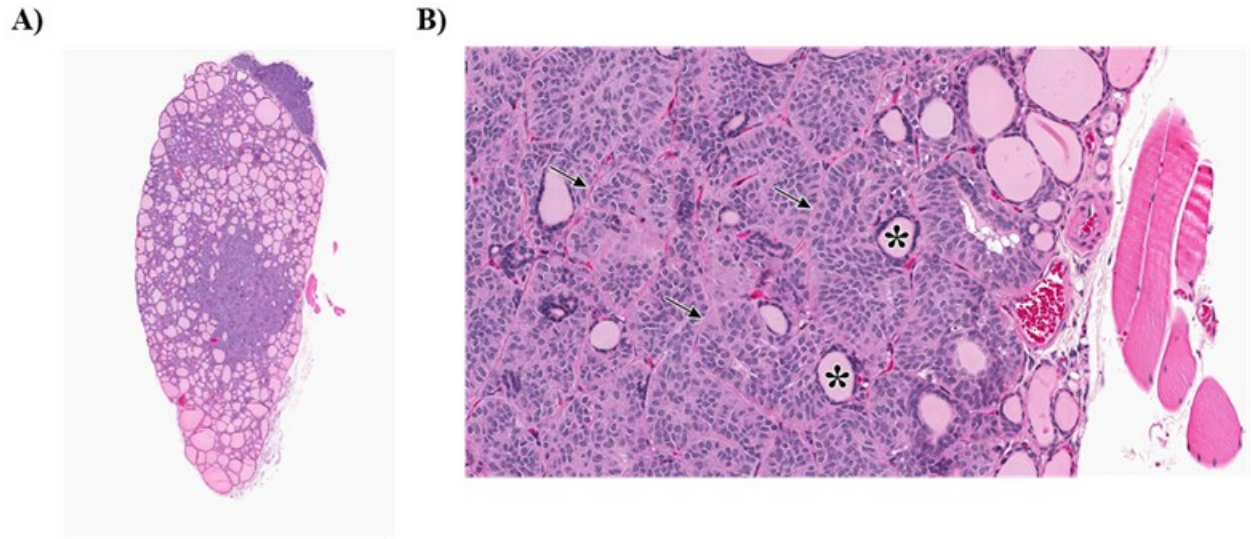


Figure 6. C-cell Adenoma in the Thyroid Gland of a Female Sprague Dawley Rat Exposed to 1,000 mg/L Sodium Tungstate Dihydrate for Two Years (H&E)

A) The C-cell adenoma is a mass of several nests, or clusters, of cells separated by a delicate fibrovascular stroma. B) Higher magnification of the C-cell adenoma in panel A; at this magnification, the fibrovascular stroma that separates the nests of C-cells is more apparent (arrows). The cells that make up the adenoma have oval nuclei and pale eosinophilic cytoplasm. A few follicles are entrapped (asterisks).

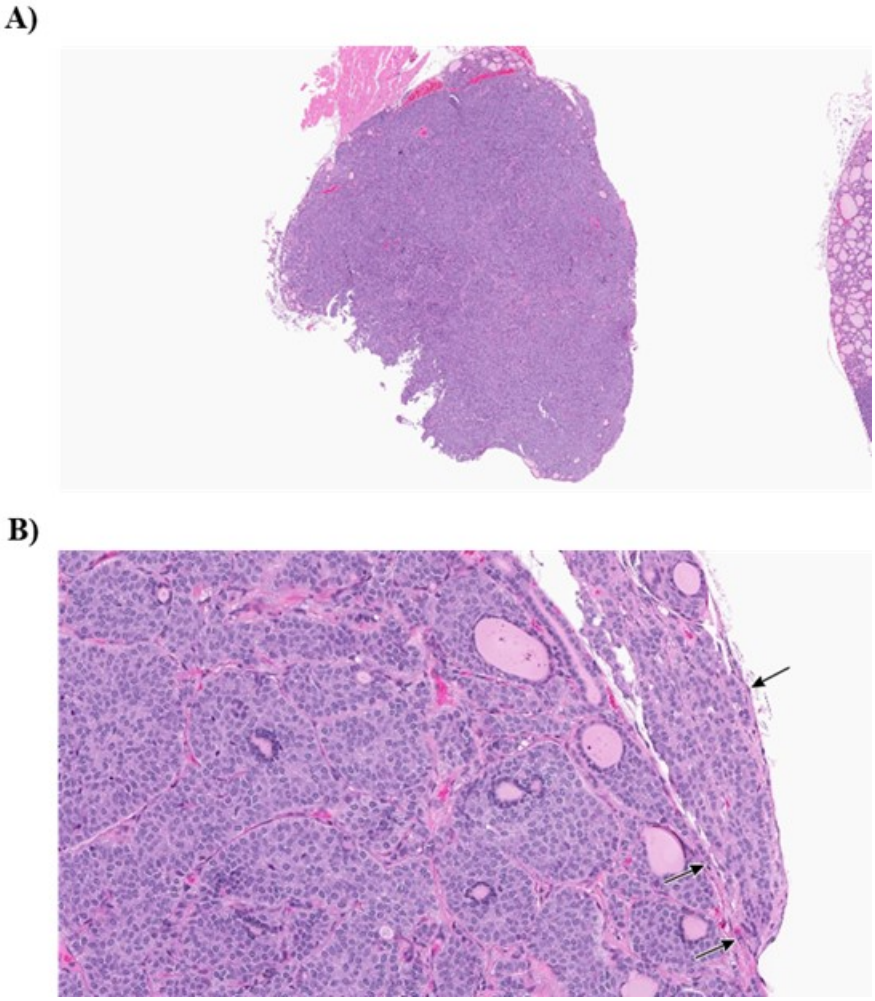


Figure 7. C-cell Carcinoma in the Thyroid Gland of a Female Sprague Dawley Rat Exposed to 1,000 mg/L Sodium Tungstate Dihydrate for Two Years (H&E)

A) The C-cell carcinoma completely obliterates the normal architecture of the thyroid gland. B) Higher magnification of the C-cell carcinoma in panel A; the cells tend to have slightly less cytoplasm than those of the C-cell adenoma (Figure 6), but the main feature of C-cell carcinomas is invasion through the capsule (arrows).

Kidney: The incidences of suppurative inflammation of the renal tubules were significantly increased in the 1,000 mg/L males and females, and the incidence of renal tubule regeneration was significantly increased in the 1,000 mg/L females, relative to the respective vehicle control groups (Table 30). Renal tubule suppurative inflammation consisted of dilated renal tubules filled with neutrophils and necrotic debris (Figure 8). One to five affected renal tubules in a section of kidney was graded as minimal; 6 to 15 affected tubules in a section of kidney was graded as mild; and more than 15 affected tubules in a section of kidney was graded as moderate. Renal tubule regeneration was characterized by cytoplasmic basophilia, karyomegaly, hypertrophy and hyperplasia of the renal tubule epithelium (Table 30; Figure 9). Occasional mitotic figures were also present. Severity grading was based on the amount of renal cortex involved, with minimal regeneration involving <10% of the cortex; mild regeneration involving approximately 10%–25% of the cortex; moderate or marked regeneration was not observed. In

males and some females, regeneration could not be distinguished as a separate morphologic finding when more advanced CPN was present (Table 30).

CPN was recorded in almost every male rat in the study; however, the mean severity score was the highest in the vehicle control group, and lowest in the 1,000 mg/L males (Table 30; Figure 10). Although no statistical comparison was conducted on the mean severity scores, the scores did parallel the incidences of several other lesions that are considered secondary to kidney failure associated with CPN. These lesions, which occurred at lower incidences in higher exposure concentration groups than in the vehicle control group, include diffuse hyperplasia of the parathyroid and mineral of the kidney, blood vessel, heart, large intestine (cecum), and glandular stomach (Appendix G).

Table 30. Incidences of Nonneoplastic Lesions of the Kidney in Male and Female Rats in the Perinatal and Two-year Drinking Water Study of Sodium Tungstate Dihydrate

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
n[a]	50	50	50	50
Male				
Renal Tubule, Regeneration[b]	0	1 (2.0)[c]	0	0
Nephropathy, Chronic Progressive	50 (3.4)	50 (3.2)	50 (3.2)	49 (2.5)
Renal Tubule, Inflammation, Suppurative	25** (1.2)	33 (1.3)	35 (1.3)	41** (1.6)
Female				
Renal Tubule, Regeneration	0**	0	0	18** (1.8)
Nephropathy, Chronic Progressive	49 (1.8)	49 (1.4)	48 (1.4)	47 (1.7)
Renal Tubule, Inflammation, Suppurative	8** (1.0)	9 (1.0)	6 (1.0)	19* (1.1)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Rao-Scott test; **$p \leq 0.01$.
[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

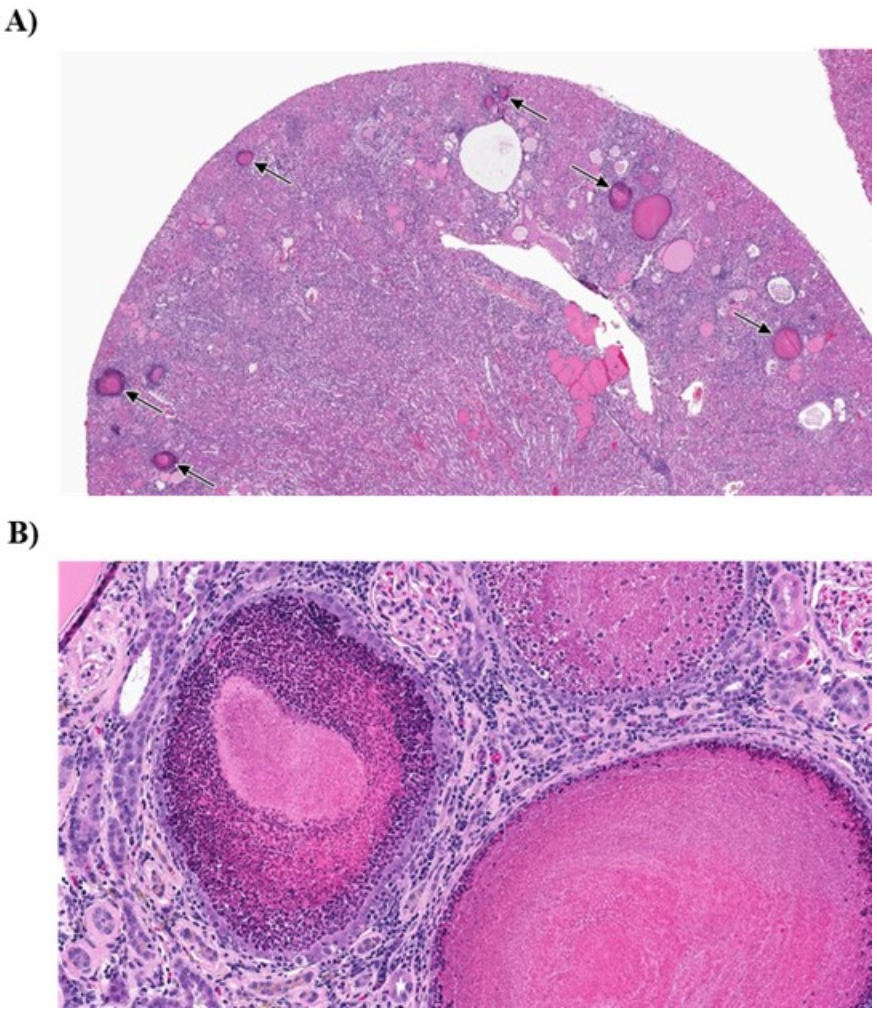


Figure 8. Suppurative Inflammation in the Renal Tubules of the Kidney from a Male Sprague Dawley Rat Exposed to 1,000 mg/L Sodium Tungstate Dihydrate for Two Years (H&E)

A) Numerous dilated tubules in the kidney are filled with neutrophils and necrotic debris (arrows). B) Higher magnification of the renal tubule suppurative inflammation shown in panel A; the tubules are filled with neutrophils and cell debris, and there is a mixed inflammatory response in the interstitium.

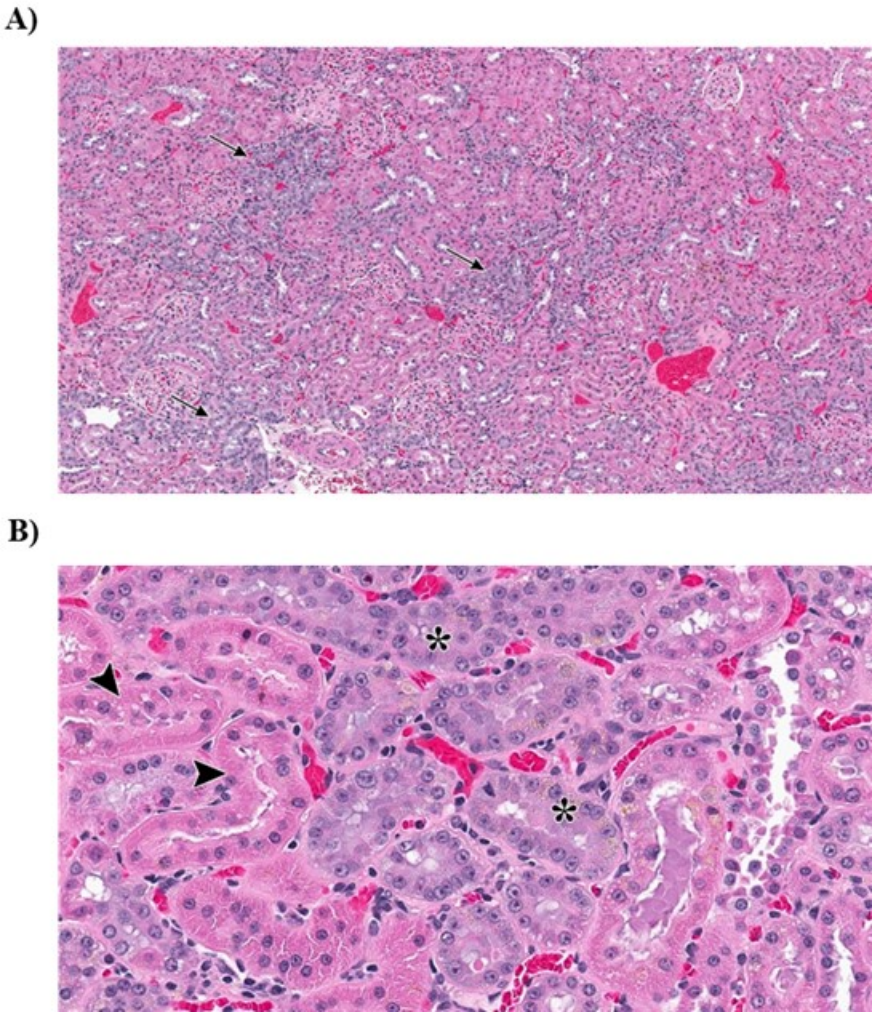


Figure 9. Renal Tubule Regeneration in the Kidney of a Female Sprague Dawley Rat Exposed to 1,000 mg/L Sodium Tungstate Dihydrate for Two Years (H&E)

A) At this magnification, the regenerative tubules are evidenced by basophilic tubules (arrows). B) Higher magnification of the renal tubule regeneration shown in panel A in which the affected tubules (asterisks) are basophilic, and there is slight crowding of the nuclei compared with the cells in the normal, more eosinophilic tubules (arrowheads). No thickening of the basement membrane is observed, as is seen in chronic progressive nephropathy; compare these tubules with those seen in Figure 10.

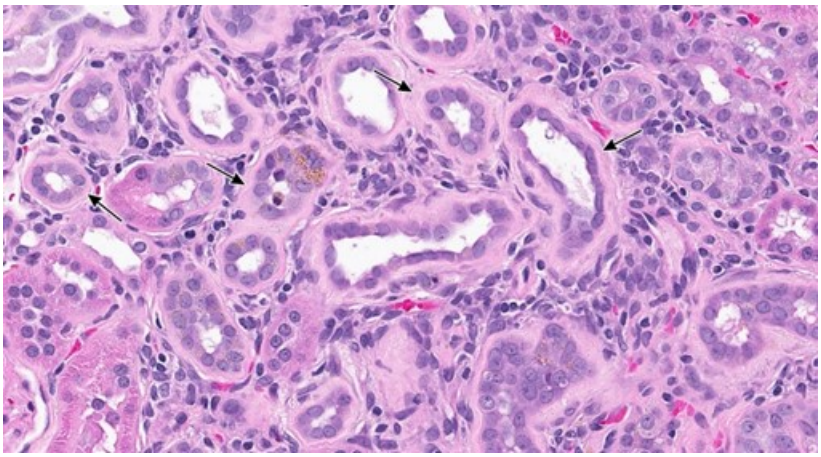


Figure 10. Chronic Progressive Nephropathy in the Kidney of a Female Sprague Dawley Rat Exposed to 1,000 mg/L Sodium Tungstate Dihydrate for Two Years (H&E)

Compared with renal tubule regeneration (Figure 9), thickened basement membranes are visible surrounding the renal tubules (arrows).

Uterus: A significant increase in the incidence of atypical hyperplasia of the uterus, relative to the vehicle control group, occurred in the 500 mg/L females (Table 31). Atypical hyperplasia is considered a preneoplastic lesion,[91] which can progress to uterine adenomas and then uterine adenocarcinoma, or directly to adenocarcinomas. There were no significant differences for the few adenocarcinomas that occurred in this study and the group incidences were within the historical control range.

Atypical hyperplasia involved both the endometrial glands and the surface epithelium (Figure 11). Affected endometrial glands were characterized by clusters of enlarged glands lined by disorganized, large epithelial cells that were crowded and piled up on each other to form multiple layers; this often resulted in blebbing (small protrusions of cells in the glandular lumen). The involved cells displayed pleomorphism and anisokaryosis, and an occasional mitotic figure was present.

Atypical hyperplasia affecting the surface epithelium consisted of branching, frond-like projections of epithelial cells on a fibrovascular stalk that extended into the uterine lumen. These thickened papillary projections were composed of cells that often contained clear vacuoles.

The severity (minimal, mild, moderate, marked) of atypical hyperplasia was graded based on the amount of uterus that was involved in the lesion, as well as on the extent of the proliferation and cellular atypia. Minimal lesions were small and focal, confined to one small area of the uterus, and involved only one or a few glands or a small area of surface endometrium. Although the cells might display some atypical characteristics (such as having differently sized nuclei or abundant cytoplasm), the cells were not so anaplastic that they resembled those of an adenocarcinoma. Blebbing of the cytoplasm was infrequent, and although the cells might have been crowded, they did not typically form multiple layers. Increasing severity grades reflected the involvement of a larger area of the uterus and a greater degree of cellular pleomorphism, atypia, and proliferation, resulting in frequently vacuolated cells lining frond-like extensions from the surface endometrium and crowded glands lined by multiple layers of abnormal looking epithelial cells.

Table 31. Incidences of Neoplastic and Nonneoplastic Lesions of the Uterus in Female Rats in the Perinatal and Two-year Drinking Water Study of Sodium Tungstate Dihydrate

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
n[a]	50	50	50	50
Hyperplasia, Atypical[b]	4 (2.3)[c]	7 (1.4)	19** (1.7)	8 (2.3)
Adenocarcinoma[d]	3	0	2	5
Adenoma[e]	0	0	0	1

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
**Statistically significant at $p \leq 0.01$ by the Rao-Scott test.
[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[d]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 20/350 (5.71% ± 3.35%); range: 2% to 10%.
[e]Historical control incidence: 1/350 (0.29% ± 0.76%); range: 0% to 2%.

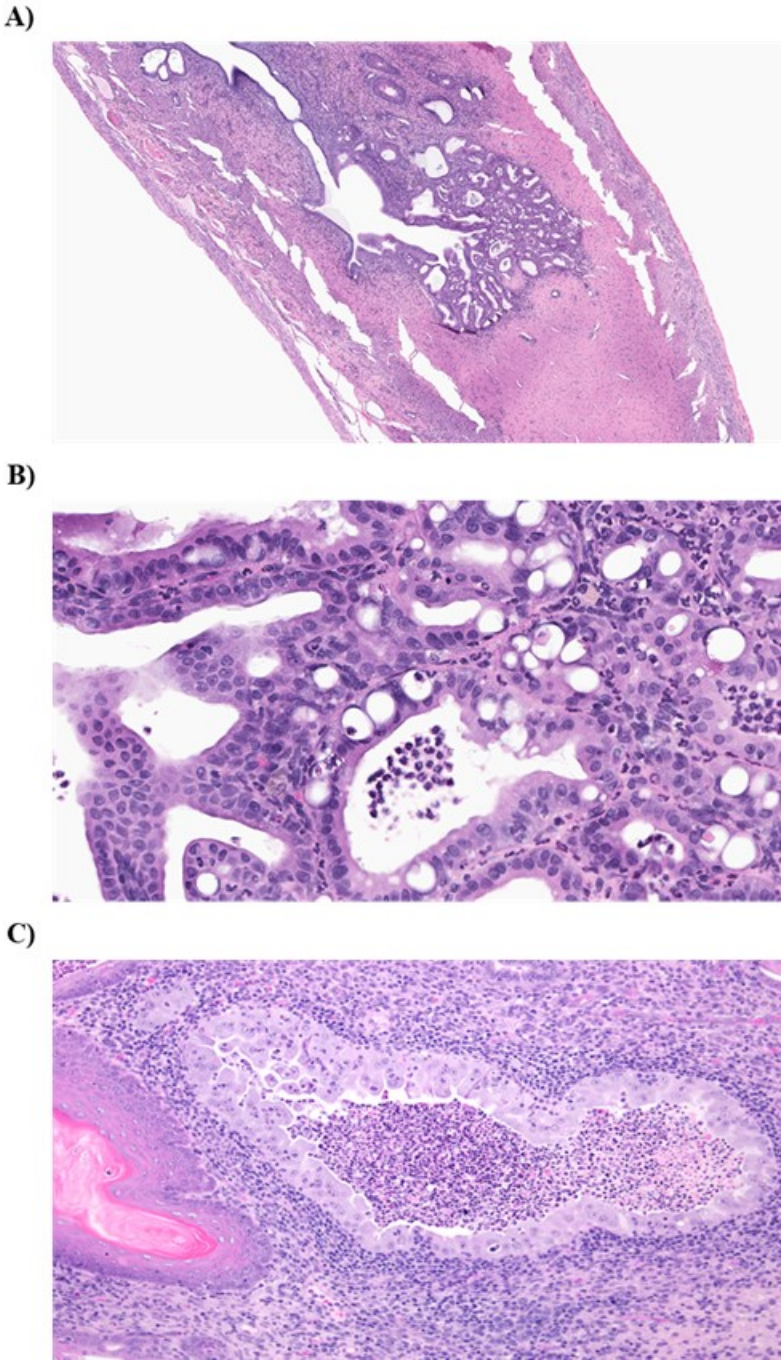


Figure 11. Atypical Hyperplasia in the Uterus of a Female Sprague Dawley Rat Exposed to 500 mg/L Sodium Tungstate Dihydrate for Two Years (H&E)

A) A low magnification photomicrograph of atypical hyperplasia of the uterus shows proliferation of the surface and glandular epithelium; the proliferative epithelium does not extend past the thick endometrial stroma into the underlying myometrial layers. This lack of invasion is consistent with atypical hyperplasia. B) A higher magnification of the atypical hyperplasia seen in panel A. There are vacuoles within the cytoplasm of numerous cells; this vacuolation of the epithelial cells is a common feature of atypical hyperplasia. C) A photomicrograph of atypical hyperplasia affecting an endometrial gland with large epithelial cells containing large nuclei, which are piling up on each other, resulting in protrusions into the glandular lumen. The large number of degenerate neutrophils within the lumen are incidental to the finding of atypical hyperplasia.

Other Tissues: In males, there was a significantly increased incidence of focal adrenal cortical hyperplasia in the 1,000 mg/L group, relative to the vehicle control group, when unilateral and bilateral lesions were combined (Appendix G). In the mandibular lymph node, there were significantly increased incidences of plasma cell hyperplasia in the 500 and 1,000 mg/L males, and of sinus dilation in the 250 mg/L males, relative to the vehicle control group (Appendix G). In the nose, goblet cell hyperplasia of the nasopharyngeal duct was significantly increased in incidence in the 250 mg/L males, and hyaline droplet accumulation in the respiratory epithelium was significantly increased in incidence in all exposed groups of females, relative to the vehicle control groups (Appendix G). These lesions were not considered related to ST exposure.

In females, there was a significantly decreased incidence of mammary gland fibroadenomas in the 1,000 mg/L group compared to the vehicle control group (Appendix G). Significant decreases in body weight have been shown to be associated with a decreased incidence of mammary gland tumors in rats.[92]

Mice

Three-month Study

There were no early deaths or exposure-related clinical observations in male or female B6C3F1/N mice exposed to ST for 3 months (Table 32, Table 33; Appendix G). Over the course of the study, group mean body weights were below 90% of the vehicle control group mean for the 250, 1,000, and 2,000 mg/L females and the 2,000 mg/L males (Table 32, Table 33; Figure 12). At study termination, the mean body weights of all exposed groups of males and females were within 10% of the vehicle control groups.

Weekly mean water consumption was reduced slightly in the 1,000 mg/L male group (11%), the 2,000 mg/L males (16%), and the 2,000 mg/L females (11%), relative to the respective vehicle control groups (Table 34; Appendix G). Drinking water concentrations of 125, 250, 500, 1,000, and 2,000 mg/L resulted in average daily ST doses of approximately 14, 27, 57, 108, and 212 mg/kg/day for males and 14, 29, 58, 113, and 202 mg/kg/day for females.

Table 32. Summary of Survival and Mean Body Weights of Male Mice in the Three-month Drinking Water Study of Sodium Tungstate Dihydrate

Study Day[a]	0 mg/L		125 mg/L			250 mg/L			500 mg/L			1,000 mg/L			2,000 mg/L		
	Av. Wt. (g)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N
1	22.2	10	22.4	100.9	10	22.6	101.7	10	22.7	102.3	10	22.1	99.8	10	22.4	100.9	10
8	24.4	10	24.8	101.5	10	24.6	100.7	10	24.3	99.6	10	23.9	98.0	10	23.9	97.7	10
15	25.9	10	26.3	101.7	10	26.0	100.5	10	25.7	99.5	10	25.3	97.6	10	24.8	95.7	10
22	27.9	10	28.7	102.7	10	28.3	101.5	10	27.9	99.9	10	27.1	97.2	10	26.5	95.0	10
29	28.9	10	29.6	102.4	10	29.4	101.7	10	29.0	100.3	10	28.4	98.1	10	27.3	94.4	10
36	30.4	10	31.4	103.3	10	31.0	102.0	10	30.3	99.8	10	29.7	97.7	10	28.4	93.3	10
43	32.7	10	33.5	102.7	10	33.0	101.0	10	32.4	99.2	10	31.6	96.8	10	30.0	91.9	10
50	34.6	10	35.6	103.0	10	34.7	100.4	10	34.0	98.3	10	33.9	98.1	10	31.8	92.1	10
57	35.8	10	37.3	104.2	10	36.5	101.9	10	35.3	98.7	10	35.3	98.7	10	32.8	91.5	10
64	36.2	10	38.0	105.0	10	37.0	102.3	10	35.9	99.1	10	35.9	99.4	10	32.9	91.0	10
71	37.6	10	39.3	104.5	10	38.1	101.3	10	37.2	98.8	10	36.9	98.2	10	34.0	90.4	10
78	39.0	10	40.7	104.6	10	39.2	100.5	10	38.3	98.4	10	38.2	98.0	10	35.0	89.7	10
85	39.4	10	41.2	104.5	10	40.0	101.5	10	39.1	99.3	10	39.1	99.3	10	35.2	89.4	10
EOS	**41.2**	**10**	**42.4**	**102.9**	**10**	**41.6**	**100.8**	**10**	**40.7**	**98.6**	**10**	**40.4**	**98.0**	**10**	**37.5**	**91.0**	**10**

EOS = end of study.
[a]Study day 1 is the day animals were placed on study.

Table 33. Summary of Survival and Body Weights of Female Mice in the Three-month Drinking Water Study of Sodium Tungstate Dihydrate

Study Day[a]	0 mg/L		125 mg/L			250 mg/L			500 mg/L			1,000 mg/L			2,000 mg/L		
	Av. Wt. (g)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N
1	17.1	10	16.7	97.6	10	17.2	100.1	10	17.3	101.0	10	16.9	98.8	10	16.8	98.1	10
8	19.4	10	18.6	95.9	10	18.8	96.9	10	19.1	98.3	10	18.8	96.5	10	18.5	95.1	10
15	20.3	10	19.8	97.6	10	19.6	96.5	10	20.2	99.5	10	19.5	95.8	10	19.8	97.3	10
22	21.9	10	21.3	97.4	10	21.2	96.8	10	22.0	100.5	10	20.8	95.1	10	20.8	95.2	10
29	23.0	10	22.1	96.2	10	21.2	92.2	10	22.4	97.5	10	21.0	91.6	10	21.6	94.0	10
36	24.8	10	22.8	91.9	10	22.6	91.0	10	23.6	94.9	10	22.1	89.0	10	22.2	89.3	10
43	26.0	10	24.3	93.6	10	23.5	90.5	10	24.3	93.4	10	23.1	89.0	10	23.4	90.2	10
50	27.8	10	26.0	93.6	10	24.6	88.4	10	26.2	94.3	10	24.9	89.5	10	24.5	88.1	10
57	28.9	10	27.9	96.5	10	25.0	86.6	10	27.0	93.4	10	26.1	90.4	10	26.1	90.3	10
64	30.3	10	28.5	93.9	10	26.0	85.7	10	28.2	92.9	10	26.4	86.9	10	27.8	91.6	10
71	31.3	10	29.4	93.7	10	26.7	85.3	10	30.3	96.6	10	27.9	89.0	10	28.1	89.8	10
78	32.1	10	31.1	96.9	10	27.6	86.2	10	30.3	94.4	10	29.2	91.0	10	28.7	89.6	10
85	31.7	10	32.7	103.1	10	29.0	91.5	10	31.1	98.0	10	29.5	93.1	10	28.7	90.6	10
EOS	**31.7**	**10**	**33.8**	**106.6**	**10**	**29.9**	**94.1**	**10**	**31.7**	**99.9**	**10**	**29.5**	**92.8**	**10**	**29.1**	**91.6**	**10**

EOS = end of study.
[a]Study day 1 is the day animals were placed on study.

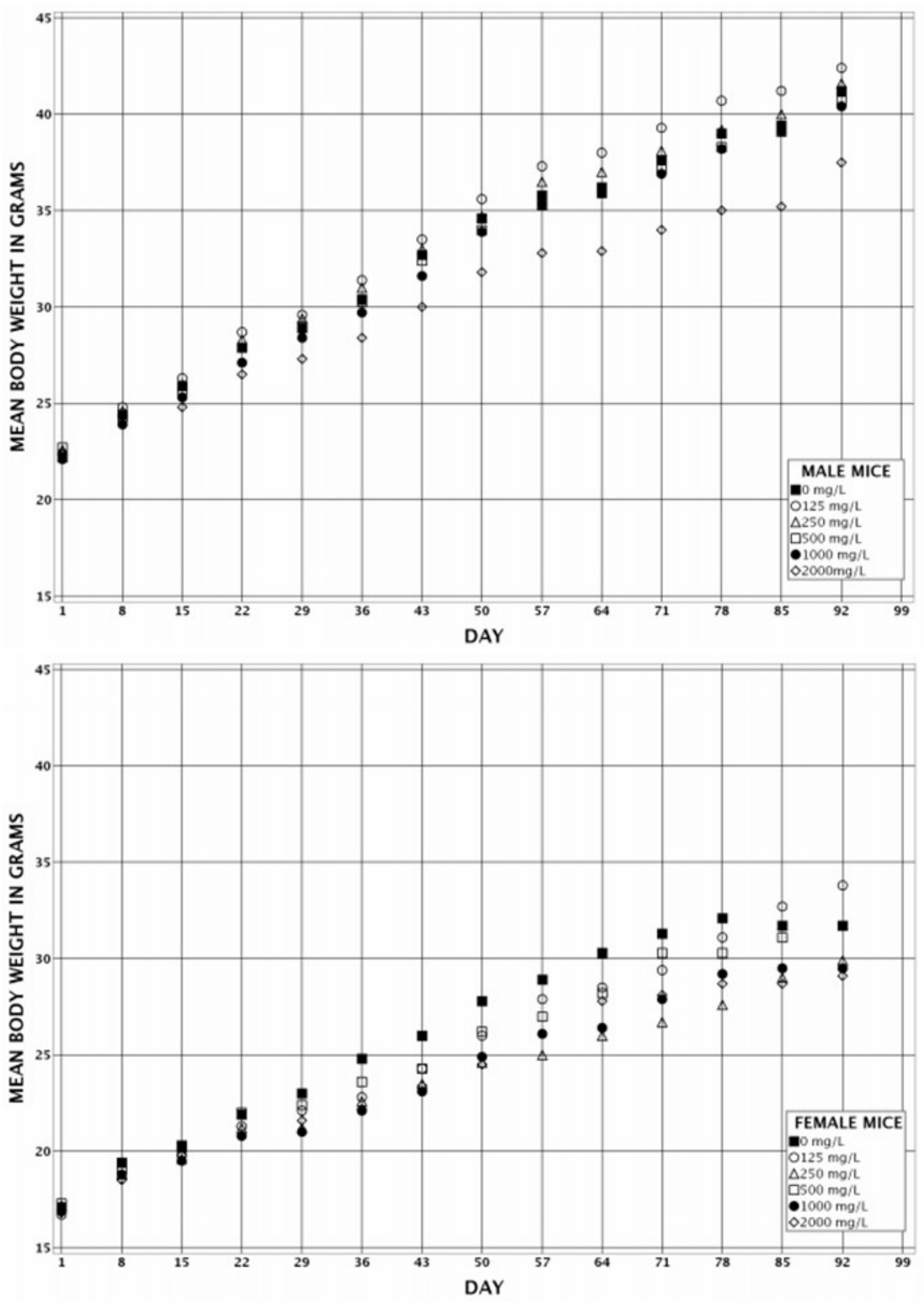


Figure 12. Growth Curves for Mice Exposed to Sodium Tungstate Dihydrate in Drinking Water for Three Months

Table 34. Summary of Water and Sodium Tungstate Dihydrate Consumption of Male and Female Mice in the Three-month Drinking Water Study

Week	0 mg/L	125 mg/L		250 mg/L		500 mg/L		1,000 mg/L		2,000 mg/L	
	Water (g/day)[a]	Water (g/day)	Dose (mg/kg/day)[b]	Water (g/day)	Dose (mg/kg/day)	Water (g/day)	Dose (mg/kg/day)	Water (g/day)	Dose (mg/kg/day)	Water (g/day)	Dose (mg/kg/day)
Male											
1	4.3[d]	3.8	21.2	3.7	41.0	3.5	77.2	3.6	162.6	3.4	303.7
4	3.7	3.5	15.3	3.4	30.0	3.4	61.0	3.3	121.7	3.1	234.0
13	3.5	3.5	10.6	3.6	22.5	3.4	43.4	3.1	79.2	3.0	170.4
Female											
1	2.5	2.4	17.9	2.6	37.9	2.5	72.2	2.2	129.9	2.0	237.8
4	3.0	2.6	15.2	2.6	30.7	2.7	61.4	2.7	129.8	2.3	220.9
13	2.2	2.6	9.9	2.6	22.4	2.6	41.8	2.4	81.4	2.3	160.1

[a]Water consumption data are presented as grams/animal/day.
[b]Milligrams of sodium tungstate dihydrate consumed/kilogram body weight/day.

Blood was collected from up to 10 animals per group on the morning of day 91. Total blood tungsten concentrations were determined using a validated analytical method (Appendix E); data are presented in Table 35. Tungsten was not detected in males in the vehicle control group above the LOD of the assay (0.0016 µg/g); however, low concentrations of tungsten were detected in females in the vehicle control group. In both males and females, the blood tungsten concentrations increased proportionally with the exposure concentration; there was no observed sex difference. In females, the tungsten concentrations in exposed groups were significantly higher than in the corresponding vehicle control group.

Table 35. Summary of Blood Tungsten Concentration Data for Male and Female Mice in the Three-month Drinking Water Study of Sodium Tungstate Dihydrate[a]

	0 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Male						
n	10	10	10	10	10	10
Blood (µg/g)	BD	0.17 ± 0.04	0.28 ± 0.04	0.47 ± 0.10	1.56 ± 0.27	2.63 ± 0.70
Female						
n	10	10	10	9	10	10
Blood (µg/g)[b,c]	0.03 ± 0.01**	0.13 ± 0.02**	0.27 ± 0.05**	0.44 ± 0.07**	1.06 ± 0.11**	2.87 ± 0.40**

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
**Statistically significant at $p \leq 0.01$.
BD = below detection; group did not have more than 20% of its values above the limit of detection (LOD).
[a]Data presented as mean ± standard error.
[b]Values below the LOD (0.0016 µg/g), were substituted with 1/2 the LOD value. If 80% or more of the values in the vehicle control group were below the LOD, no mean or standard error were calculated, and no statistical analysis was performed.
[c]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

In male mice, the white blood cell count was significantly decreased in the 1,000 and 2,000 mg/L groups relative to the vehicle control group (Table 36). These decreases were driven by a significant decrease in the lymphocyte count in the 1,000 and 2,000 mg/L groups and a significant decrease in the monocyte count in the 500 mg/L and higher groups (Table 36). Additionally, the eosinophil counts were significantly decreased in all ST-exposed male groups. These leukocyte changes are consistent with a stress leukogram (i.e., effects of chronic increase in endogenous corticosterone).[89]

Table 36. Summary of Select Hematology Data for Male Mice in the Three-month Drinking Water Study of Sodium Tungstate Dihydrate[a,b]

	0 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
n	10	10	10	10	10	10
White Blood Cells (10^3/μL)	5.91 ± 0.52**	4.87 ± 0.61	5.32 ± 0.42	4.94 ± 0.51	4.20 ± 0.37**	3.83 ± 0.56**
Lymphocytes (10^3/μL)	4.83 ± 0.45**	3.93 ± 0.49	4.38 ± 0.34	4.07 ± 0.43	3.41 ± 0.31**	3.12 ± 0.45**
Monocytes (10^3/μL)	0.20 ± 0.02**	0.13 ± 0.02	0.14 ± 0.02	0.10 ± 0.02**	0.09 ± 0.02**	0.09 ± 0.03**
Eosinophils (10^3/μL)	0.15 ± 0.03**	0.08 ± 0.01*	0.08 ± 0.01*	0.07 ± 0.02*	0.07 ± 0.01**	0.06 ± 0.02**

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data displayed as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

A higher group mean relative testis weight was observed in the 2,000 mg/L male group, relative to the vehicle control group, and was likely due to the lower mean body weights in that group (Appendix G). There were no histological lesions in the testes related to ST exposure. In female mice, there were several sporadic increases in group mean organ weights, but they lacked an exposure concentration response or other supporting evidence that they represented anything but biological variation (Appendix G). Relative kidney weights were significantly increased in the 1,000 and 2,000 mg/L females and 2000 mg/L males but were most likely due to reduced mean body weights in those groups compared to the vehicle control group (Table 37).

No exposure-related gross lesions were recorded. The only histological lesion associated with exposure was in the kidney. The incidences of renal tubule regeneration were higher in the 1,000 and 2,000 mg/L male and female groups compared to the respective vehicle control groups; the increases in the male groups were significant (Table 37). The lesion consisted of hyperplastic tubules, predominantly in the deep cortical to medullary region, lined by epithelial cells with increased cytoplasmic basophilia, nuclear crowding, prominent nucleoli, marginated chromatin, and karyomegaly. There were occasional mitotic figures.

Table 37. Summary of Renal Findings for Male and Female Mice in the Three-month Drinking Water Study of Sodium Tungstate Dihydrate

	0 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
n	10	10	10	10	10	10
Male						
Necropsy Body Wt. (g)[a]	41.2 ± 1.0**	42.4 ± 0.9	41.6 ± 1.5	40.7 ± 1.1	40.4 ± 0.8	37.5 ± 1.1*
R. Kidney Weight[a]						
Absolute (g)	0.30 ± 0.00	0.30 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.29 ± 0.00
Relative (mg/g)[b]	7.28 ± 0.15**	7.15 ± 0.16	7.47 ± 0.18	7.53 ± 0.22	7.75 ± 0.16	7.84 ± 0.18*
Histological Findings						
Kidney[c]	10	10	10	10	10	10
Renal tubule, regeneration[d]	0**	0	0	0	6** (1.0)[e]	10** (1.6)

	0 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Female						
Necropsy Body Wt. (g)	31.7 ± 1.7*	33.8 ± 1.3	29.9 ± 1.1	31.7 ± 1.3	29.5 ± 1.1	29.1 ± 1.2
R. Kidney Weight						
Absolute (g)	0.15 ± 0.00	0.17 ± 0.00	0.16 ± 0.00	0.16 ± 0.00	0.16 ± 0.00	0.16 ± 0.00
Relative (mg/g)	4.88 ± 0.19**	4.91 ± 0.11	5.28 ± 0.11	5.22 ± 0.13	5.48 ± 0.16*	5.42 ± 0.19*
Histological Findings						
Kidney	10	10	10	10	10	10
Renal tubule, regeneration	0*	0	0	0	1 (1.0)	2 (1.0)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
Statistical analysis for organ weight data performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
Statistical analysis for histological findings performed by the Poly-3 test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data presented as mean ± standard error.
[b]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.
[c]Number of animals examined microscopically.
[d]Number of animals with lesion.
[e]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

In male mice, no significant differences were observed between exposed groups and the vehicle control group in left testis weight, left epididymal weight, left cauda weight, or any of the sperm parameters, including number of sperm/mg cauda epididymis, total number sperm/cauda, sperm motility, number of homogenization-resistant spermatids/mg testis, and total number of spermatids (Appendix G). The testes and epididymides were evaluated to a no-effect level, and no histological findings associated with ST exposure were present at 3 months. Under the conditions of this 3-month study, ST administration via drinking water did not exhibit the potential to be a reproductive toxicant in B6C3F1/N mice. Testicular lesions observed in male mice after 2 years of exposure to ST, however, might possibly impair reproductive performance.

Exposure Concentration Selection Rationale for Two-year Studies in Mice

In the 3-month studies, there were no effects on survival and mean body weights in any exposure groups that were considered exposure concentration-limiting (Table 32, Table 33). There was a 16% reduction in water consumption in male mice at the top exposure group of 2,000 mg/L (Table 34), and minimal to mild renal tubule regeneration was noted in the 1,000 and 2,000 mg/L groups, but neither effect was considered exposure concentration-limiting (Table 37). Hence, exposure concentrations selected for the 2-year study were 0, 500, 1,000, and 2,000 mg/L.

Two-year Study (Interim Evaluations – 3, 6, 12, and 18 Months)

At 6 months in males, mean body weights were significantly decreased in the 500 mg/L, 1,000 mg/L, and 2,000 mg/L groups by approximately 7%, 8%, and 13%, respectively, relative to the vehicle control group. Mean body weights of the 2,000 mg/L females were significantly decreased by approximately 13% relative to the vehicle control group at 12 months (Table 38, Table 39). Mean body weights of all other groups, both males and females, were within 10% of the vehicle control groups at all time points.

At 3 months in males, mean absolute kidney weights were significantly increased in the 500 mg/L and 1,000 mg/L groups by approximately 10% and 13%, respectively, and mean relative kidney weight was significantly increased in the 500 mg/L group relative to the vehicle control group (Table 38). In females at 3 months, mean relative kidney weight was significantly decreased in the 1,000 mg/L group relative to the vehicle control group (Table 39). At 6 months in males, mean absolute kidney weights were significantly decreased in the 2,000 mg/L group by 9% and mean relative kidney weights were significantly increased in the 1,000 mg/L males relative to the vehicle control group; there were no significant differences in kidney weights among female groups. At 12 months, there were no significant differences in the kidney weights among male groups, but in females, mean relative kidney weights were significantly increased in the 1,000 and 2,000 mg/L groups relative to the vehicle control group. At 18 months, there were no significant pairwise differences in kidney weights in either male or female groups compared to the vehicle control groups.

At 3 months, mean relative liver weights were significantly increased in the 2,000 mg/L males and females relative to the vehicle control groups. At 6 months, mean absolute liver weights were significantly decreased by approximately 13% in the 500 mg/L males, 15% in the 1,000 mg/L males, and 21% in the 2,000 mg/L males relative to the vehicle control males (Table 38, Table 39). The biological importance of these changes in liver weights is unknown, but they are likely of little toxicological significance.

Other sporadic differences in organ weights were considered isolated changes of no toxicological significance (Appendix G).

Table 38. Summary of Select Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Mice Exposed to Sodium Tungstate Dihydrate in Drinking Water for 3, 6, 12, and 18 Months[a,b]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Three Months				
Necropsy Body Wt. (g)	34.7 ± 0.8 (10)	35.3 ± 1.2 (10)	37.0 ± 1.1 (10)	31.2 ± 1.0 (10)
Kidneys				
Absolute (g)	0.52 ± 0.01 (10)	0.57 ± 0.01* (10)	0.59 ± 0.01** (10)	0.50 ± 0.01 (10)
Relative (mg/g)[c]	15.03 ± 0.36 (10)	16.40 ± 0.48* (10)	15.91 ± 0.36 (10)	16.19 ± 0.27 (10)
Liver				
Absolute (g)	1.37 ± 0.03 (10)	1.43 ± 0.03 (10)	1.51 ± 0.06 (10)	1.30 ± 0.03 (10)
Relative (mg/g)	39.55 ± 0.30** (10)	40.72 ± 0.95 (10)	40.74 ± 0.62 (10)	41.83 ± 0.67* (10)
Six Months				
Necropsy Body Wt. (g)	45.7 ± 0.8** (10)	42.5 ± 0.8* (10)	42.1 ± 1.1** (10)	39.8 ± 0.8** (10)
Kidneys				
Absolute (g)	0.68 ± 0.02 (10)	0.67 ± 0.02 (10)	0.69 ± 0.02 (10)	0.62 ± 0.01* (10)
Relative (mg/g)	14.93 ± 0.30 (10)	15.87 ± 0.20 (10)	16.47 ± 0.30** (10)	15.69 ± 0.33 (10)
Liver				
Absolute (g)	2.03 ± 0.05** (10)	1.77 ± 0.10* (10)	1.72 ± 0.09** (10)	1.60 ± 0.05** (10)
Relative (mg/g)	44.45 ± 0.90* (10)	41.50 ± 1.63 (10)	40.67 ± 1.12 (10)	40.23 ± 0.66* (10)
Twelve Months				
Necropsy Body Wt. (g)	49.4 ± 1.0 (10)	48.1 ± 0.7 (10)	48.1 ± 0.8 (10)	46.9 ± 0.8 (10)
Kidneys				
Absolute (g)	0.81 ± 0.02 (10)	0.80 ± 0.02 (10)	0.82 ± 0.03 (10)	0.78 ± 0.02 (10)
Relative (mg/g)	16.43 ± 0.29 (10)	16.70 ± 0.25 (10)	17.12 ± 0.47 (10)	16.62 ± 0.29 (10)
Liver				
Absolute (g)	2.42 ± 0.15 (10)	2.18 ± 0.05 (10)	2.36 ± 0.14 (10)	2.13 ± 0.08 (10)
Relative (mg/g)	48.93 ± 3.00 (10)	45.37 ± 0.62 (10)	49.18 ± 3.15 (10)	45.56 ± 1.79 (10)
Eighteen Months				
Necropsy Body Wt. (g)	49.4 ± 1.7* (9)	48.4 ± 0.9 (10)	48.3 ± 1.2 (9)	46.4 ± 1.0 (6)
Kidneys				
Absolute (g)	0.79 ± 0.02 (9)	0.79 ± 0.02 (10)	0.85 ± 0.05 (9)	0.74 ± 0.02 (6)
Relative (mg/g)	16.07 ± 0.36 (9)	16.35 ± 0.24 (10)	17.69 ± 0.94 (9)	15.90 ± 0.35 (6)
Liver				
Absolute (g)	2.25 ± 0.19 (9)	2.45 ± 0.20 (10)	2.79 ± 0.30 (9)	1.86 ± 0.14 (6)
Relative (mg/g)	46.98 ± 6.36 (9)	50.98 ± 4.76 (10)	58.20 ± 6.42 (9)	39.95 ± 2.41 (6)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data presented as mean ± standard error (n).
[b]Statistical analysis performed by the Jonckheere (trend) and the Williams or Dunnett (pairwise) tests.
[c]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

Table 39. Summary of Select Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Mice Exposed to Sodium Tungstate Dihydrate in Drinking Water for 3, 6, 12, and 18 Months[a,b]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Three Months				
Necropsy Body Wt. (g)	27.3 ± 1.5 (10)	24.8 ± 0.7 (10)	29.2 ± 1.0 (10)	24.8 ± 0.9 (10)
Kidneys				
Absolute (g)	0.32 ± 0.01 (10)	0.31 ± 0.01 (10)	0.32 ± 0.01 (10)	0.31 ± 0.01 (10)
Relative (mg/g)[c]	11.88 ± 0.33 (10)	12.58 ± 0.28 (10)	10.89 ± 0.30* (10)	12.41 ± 0.21 (10)
Liver				
Absolute (g)	1.13 ± 0.04 (10)	1.09 ± 0.03 (10)	1.17 ± 0.02 (10)	1.13 ± 0.04 (10)
Relative (mg/g)	41.97 ± 1.23 (10)	43.76 ± 0.73 (10)	40.24 ± 0.92 (10)	45.60 ± 0.89* (10)
Six Months				
Necropsy Body Wt. (g)	39.8 ± 1.3 (10)	38.7 ± 1.0 (10)	39.3 ± 1.5 (10)	39.4 ± 1.4 (10)
Kidneys				
Absolute (g)	0.36 ± 0.01 (10)	0.36 ± 0.01 (10)	0.36 ± 0.01 (10)	0.36 ± 0.01 (10)
Relative (mg/g)	9.07 ± 0.26 (10)	9.36 ± 0.24 (10)	9.10 ± 0.24 (10)	9.15 ± 0.30 (10)
Liver				
Absolute (g)	1.37 ± 0.03 (10)	1.34 ± 0.02 (10)	1.36 ± 0.04 (10)	1.34 ± 0.03 (10)
Relative (mg/g)	34.63 ± 0.77 (10)	34.75 ± 0.59 (10)	34.83 ± 0.69 (10)	34.28 ± 0.86 (10)
Twelve Months				
Necropsy Body Wt. (g)	53.9 ± 0.8** (10)	50.3 ± 1.7 (10)	51.1 ± 1.6 (10)	46.7 ± 1.6** (10)
Kidneys				
Absolute (g)	0.41 ± 0.01 (10)	0.39 ± 0.02 (10)	0.41 ± 0.01 (10)	0.39 ± 0.01 (10)
Relative (mg/g)	7.51 ± 0.19** (10)	7.80 ± 0.14 (10)	8.10 ± 0.16* (10)	8.34 ± 0.27** (10)
Liver				
Absolute (g)	1.61 ± 0.08 (10)	1.48 ± 0.07 (10)	1.58 ± 0.05 (10)	1.43 ± 0.03 (10)
Relative (mg/g)	29.84 ± 1.34 (10)	29.38 ± 0.98 (10)	30.91 ± 0.71 (10)	30.97 ± 1.09 (10)
Eighteen Months				
Necropsy Body Wt. (g)	56.3 ± 1.3 (10)	55.0 ± 1.9 (9)	53.3 ± 2.6 (9)	56.3 ± 1.2 (10)
Kidneys				
Absolute (g)	0.44 ± 0.01 (10)	0.44 ± 0.02 (9)	0.45 ± 0.02 (9)	0.47 ± 0.01 (10)
Relative (mg/g)	7.72 ± 0.12* (10)	8.06 ± 0.23 (9)	8.55 ± 0.56 (9)	8.33 ± 0.17 (10)
Liver				
Absolute (g)	1.57 ± 0.07 (10)	1.51 ± 0.06 (9)	1.54 ± 0.06 (9)	1.61 ± 0.05 (10)
Relative (mg/g)	27.91 ± 0.78 (10)	27.61 ± 0.72 (9)	29.12 ± 1.08 (9)	28.63 ± 0.57 (10)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data presented as mean ± standard error (n).
[b]Statistical analysis performed by the Jonckheere (trend) and the Williams or Dunnett (pairwise) tests.
[c]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

Plasma, kidney, and urine from up to 10 animals per group were collected at 3, 6, 12, and 18 months from interim animals. Tungsten concentrations in all matrices were determined using validated analytical methods (Appendix E). In male mice, plasma tungsten concentrations increased proportionally with the exposure concentration at all time points (except at the 18 months in the 1,000 mg/L group) (Table 40; Figure 13A). In female mice, plasma tungsten concentrations increased proportionally with the exposure concentration up to 1,000 mg/L for all time points (except at 18 months in the 500 mg/L group); however, at 2,000 mg/L, the trend was toward a more-than-proportional increase in plasma tungsten concentration with increasing exposure concentration (Table 41; Figure 13A). There was also a trend toward decreasing plasma tungsten concentrations with increasing exposure duration in both male and female mice. Low tungsten concentrations were observed in the vehicle control groups at some interim evaluations; however, they were significantly lower than those in the lowest exposure groups (Table 40, Table 41). There was no observed sex difference in plasma tungsten concentration in mice (Table 40, Table 41; Figure 13A).

In male and female mice, tungsten concentrations in the kidney increased with increasing exposure concentration, but the trend was less than proportional (Figure 13B). There was also a trend toward decreasing tungsten concentrations with increasing exposure duration in both male and female mice, with the highest concentrations at 3 months. Low tungsten concentrations were observed in the kidneys of some animals in the vehicle control group; however, they were significantly lower than those in the lowest exposure group (Table 40, Table 41). The kidney-to-plasma ratios ranged from 1.43 to 4.36 suggesting retention of tungsten in the kidney. There were no consistent trends in the kidney-to-plasma ratios with increasing exposure concentration or duration. There was no observed sex difference in kidney tungsten concentrations in mice (Table 40, Table 41; Figure 13B).

The concentrations of tungsten in urine are presented as both µg/g of urine and µg/mg creatinine. Creatinine-corrected tungsten concentrations in urine increased proportionally with the exposure concentration for both males and females (Figure 13C). As with plasma and kidney, the trend was toward decreasing tungsten concentrations in urine with increasing exposure duration in both male and female mice. Low tungsten concentrations were observed in the urine of some vehicle control groups; however, tungsten concentrations in exposed groups were significantly higher than those in corresponding vehicle control groups (Table 40, Table 41). There was no observed sex difference in urinary tungsten concentrations in mice (Table 40, Table 41; Figure 13C).

Table 40. Summary of Plasma, Kidney, and Urine Tungsten Concentration Data for Male Mice Exposed to Sodium Tungstate Dihydrate in Drinking Water for 3, 6, 12, and 18 Months[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Three Months				
Kidney (µg/g)	BD	5.71 ± 0.88 (10)	6.50 ± 0.57 (10)	12.15 ± 2.16 (10)
Plasma (µg/mL)	BD	2.62 ± 0.47 (10)	4.11 ± 0.58 (10)	8.10 ± 1.14 (10)
Kidney/Plasma Ratio[b]	BD	2.45 ± 0.31 (10)	1.73 ± 0.16 (10)	1.51 ± 0.13 (10)
Urine[c,d]				
Urine (µg/mL urine)	0.02 ± 0.01** (7)	191.56 ± 41.89** (7)	592.00 ± 69.45** (10)	1,335.33 ± 148.26** (6)
Urine (µg/mg creatinine)	0.03 ± 0.01** (7)	353.16 ± 27.44** (7)	805.94 ± 70.58** (10)	1,672.99 ± 147.92** (6)

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Six Months				
Kidney (µg/g)[c,d]	0.06 ± 0.01** (10)	3.79 ± 0.29** (10)	5.89 ± 1.23** (10)	12.10 ± 1.39** (10)
Plasma (µg/mL)[c,d]	0.02 ± 0.00** (10)	1.68 ± 0.22** (10)	2.43 ± 0.57** (10)	5.82 ± 0.96** (10)
Kidney/Plasma Ratio[c]	3.46 ± 0.42* (10)	2.40 ± 0.18 (10)	2.58 ± 0.24 (10)	2.24 ± 0.19 (10)
Urine[c,d]				
Urine (µg/mL urine)	3.92 ± 2.77** (8)	204.50 ± 28.06** (8)	407.89 ± 92.69** (8)	816.50 ± 144.16** (8)
Urine (µg/mg creatinine)	6.43 ± 4.14** (8)	375.17 ± 45.85** (8)	709.28 ± 117.50** (7)	1,368.62 ± 149.28** (7)
Twelve Months				
Kidney (µg/g)	BD	3.67 ± 0.61 (10)	6.69 ± 0.59 (10)	10.45 ± 1.06 (10)
Plasma (µg/mL)[c,d]	0.02 ± 0.00** (10)	1.07 ± 0.16** (10)	2.37 ± 0.33** (10)	4.84 ± 0.73** (10)
Kidney/Plasma Ratio	BD	3.66 ± 0.48 (10)	3.10 ± 0.27 (10)	2.35 ± 0.18 (10)
Urine[c,d]				
Urine (µg/mL urine)	4.00 ± 1.69** (10)	152.57 ± 27.46** (10)	349.00 ± 38.70** (10)	661.09 ± 123.57** (10)
Urine (µg/mg creatinine)	15.57 ± 8.39** (10)	404.82 ± 34.78** (9)	905.34 ± 65.48** (10)	1,390.13 ± 183.53** (9)
Eighteen Months				
Kidney (µg/g)[c,d]	0.06 ± 0.01** (9)	2.67 ± 0.23** (9)[e]	5.50 ± 0.73** (8)[f]	8.71 ± 0.99** (6)
Plasma (µg/mL)[c,d]	0.01 ± 0.00** (9)	1.27 ± 0.19** (10)	4.95 ± 2.03** (9)	4.86 ± 0.65** (6)
Kidney/Plasma Ratio[c]	6.28 ± 1.23** (9)	2.41 ± 0.21** (9)	2.42 ± 0.34** (8)	1.85 ± 0.16** (6)
Urine[c,d]				
Urine (µg/mL urine)	0.02 ± 0.00** (9)	104.52 ± 11.81** (10)	194.00 ± 14.23** (8)	661.00 ± 161.34** (6)
Urine (µg/mg creatinine)	0.05 ± 0.01** (9)	278.92 ± 30.45** (10)	579.75 ± 52.95** (8)	1,279.52 ± 155.83** (6)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
BD = below detection; group did not have more than 20% of its values above the limit of detection (LOD).
[a]Data presented as mean ± standard error (n).
[b]For the kidney/plasma ratio calculation, a plasma density of 1 g/mL was assumed.
[c]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.
[d]Values below the LOD (0.013 µg/mL) were substituted with 1/2 the LOD value. If 80% or more of the values in the vehicle control group were below the LOD, no mean or standard error were calculated, and no statistical analysis was performed.
[e]The kidney concentration value for one male in the 500 mg/L group at 18 months was excluded from the analysis as an implausible value.
[f]The kidney concentration value for one male in the 1,000 mg/L group at 18 months was excluded from the analysis as an implausible value.

Table 41. Summary of Plasma, Kidney, and Urine Tungsten Concentration Data for Female Mice Exposed to Sodium Tungstate Dihydrate in Drinking Water for 3, 6, 12, and 18 Months[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Three Months				
Kidney (µg/g)	BD	3.54 ± 0.27 (10)	5.34 ± 0.49 (10)	11.44 ± 1.22 (10)
Plasma (µg/mL)	BD	1.84 ± 0.23 (10)	3.25 ± 0.49 (10)	8.72 ± 1.25 (10)
Kidney/Plasma Ratio[b]	BD	2.05 ± 0.12 (10)	1.86 ± 0.17 (10)	1.43 ± 0.11 (10)
Urine[c,d]				
Urine (µg/mL urine)	0.08 ± 0.02** (10)	442.78 ± 33.39** (9)	681.00 ± 118.08** (7)	1,470.00 ± 114.30** (10)

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Urine (µg/mg creatinine)	0.14 ± 0.04** (9)	596.49 ± 57.37** (9)	872.11 ± 105.33** (7)	2,149.65 ± 183.59** (10)
Six Months				
Kidney (µg/g)[c,d]	0.08 ± 0.02** (10)	2.90 ± 0.17** (10)	5.52 ± 0.40** (10)	10.00 ± 0.91** (10)
Plasma (µg/mL)[c,d]	0.02 ± 0.00** (10)	1.11 ± 1.13** (9)	1.95 ± 0.23** (10)	4.81 ± 0.61** (10)
Kidney/Plasma Ratio[c]	5.50 ± 2.51 (10)	2.79 ± 0.23 (9)	3.19 ± 0.40 (10)	2.19 ± 0.14 (10)
Urine[c,d]				
Urine (µg/mL urine)	0.11 ± 0.03** (9)	217.11 ± 20.48** (9)	419.60 ± 49.71** (10)	855.40 ± 106.56** (10)
Urine (µg/mg creatinine)	0.21 ± 0.05** (9)	432.30 ± 32.48** (9)	788.33 ± 89.19** (10)	1,594.25 ± 192.88** (10)
Twelve Months				
Kidney (µg/g)[c,d]	0.05 ± 0.01** (10)	2.30 ± 0.23** (10)	3.64 ± 0.22** (10)	6.59 ± 0.54** (10)
Plasma (µg/mL)[c,d]	0.04 ± 0.00** (10)	0.58 ± 0.09** (10)	1.35 ± 0.14** (10)	4.59 ± 1.25** (9)[e]
Kidney/Plasma Ratio[c]	1.13 ± 0.15 (10)	4.36 ± 0.42** (10)	2.84 ± 0.18** (10)	2.05 ± 0.32 (9)
Urine[c,d]				
Urine (µg/mL urine)	0.13 ± 0.03** (10)	167.01 ± 26.29** (10)	397.40 ± 63.15** (10)	1,070.20 ± 182.92** (10)
Urine (µg/mg creatinine)	0.26 ± 0.04** (10)	309.67 ± 42.88** (10)	645.14 ± 83.24** (10)	1,642.14 ± 306.17** (10)
Eighteen Months				
Kidney (µg/g)	BD	2.90 ± 0.26 (9)	3.85 ± 0.80 (9)	5.32 ± 0.44 (10)
Plasma (µg/mL)	BD	1.78 ± 0.32 (9)	1.66 ± 0.39 (9)	3.29 ± 1.15 (10)
Kidney/Plasma Ratio	BD	1.83 ± 0.16 (9)	2.45 ± 0.16 (9)	2.76 ± 0.60 (10)
Urine[c,d]				
Urine (µg/mL urine)	0.04 ± 0.01** (9)[f]	74.25 ± 15.40** (9)	106.61 ± 28.16** (9)	245.04 ± 37.25** (10)
Urine (µg/mg creatinine)[c,d,g]	0.19 ± 0.04** (4)	262.73 ± 28.01** (8)	397.77 ± 38.06** (6)	851.15 ± 59.51** (9)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
**Statistically significant at $p \leq 0.01$.
BD = below detection, group did not have more than 20% of its values above the limit of detection (LOD).
[a]Data presented as mean ± standard error (n).
[b]For the kidney/plasma ratio calculation, a plasma density of 1 g/mL was assumed.
[c]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests
[d]Values below the LOD (0.013 µg/mL) were substituted with 1/2 the LOD value. If 80% or more of the values in the vehicle control group were below the LOD, no mean or standard error were calculated, and no statistical analysis was performed.
[e]The plasma concentration value for one female in the 2,000 mg/L group at 12 months was excluded from the analysis as an implausible value.
[f]The urine concentration value for one female in the 0 mg/L group at 18 months was excluded from the analysis as an implausible value.
[g]The urine concentration values for five females in the 0 mg/L group, one female in the 500 mg/L group, three females in the 1,000 mg/L group, and one female in the 2,000 mg/L group were excluded because samples were determined to be dilute.

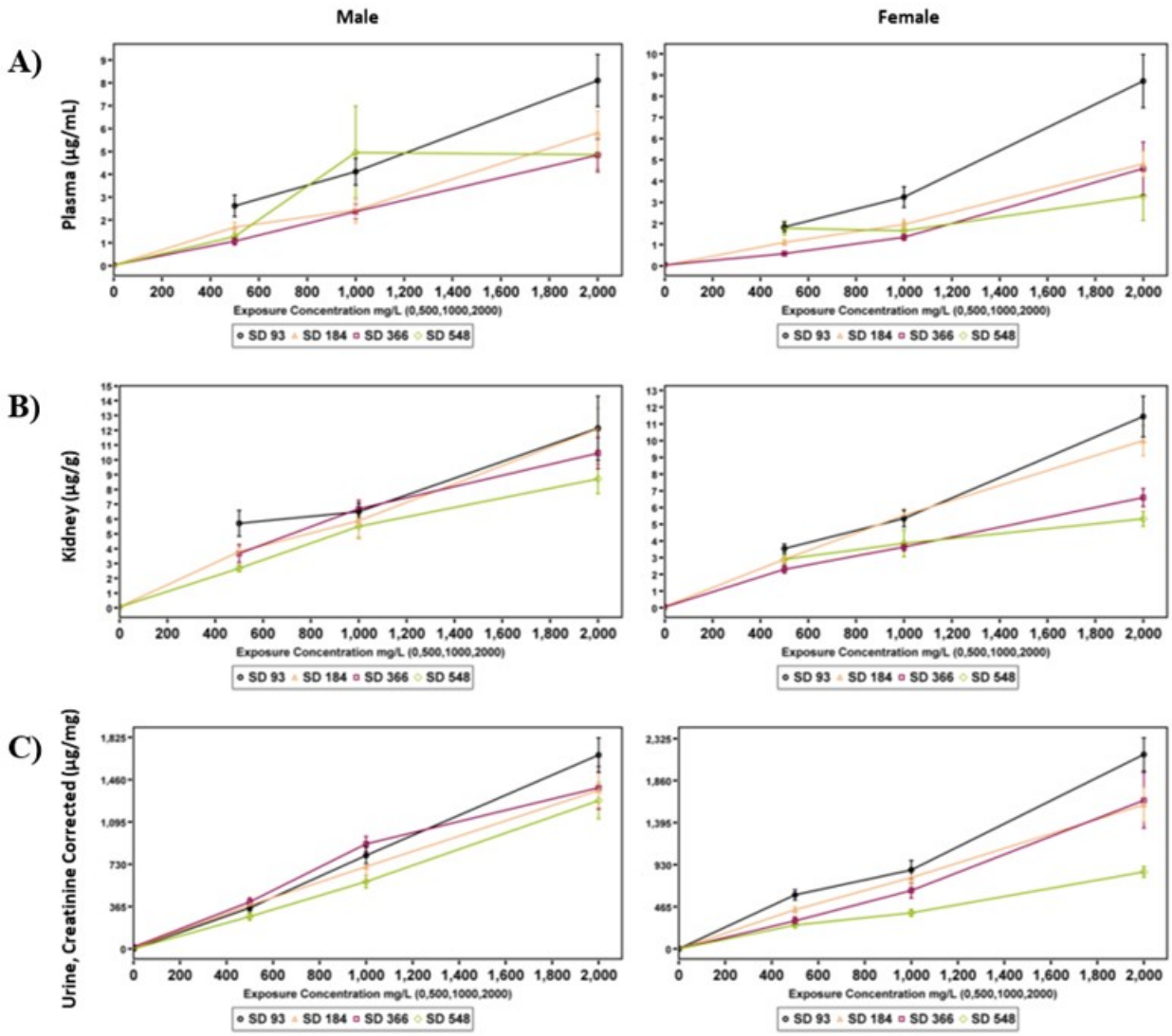


Figure 13. Tungsten Concentrations in Plasma, Kidney, and Urine in Mice Exposed to Sodium Tungstate Dihydrate in Drinking Water for 3, 6, 12, and 18 Months

Study day (SD) 93 = 3 months; SD 184 = 9 months; SD 366 = 12 months; SD 548 = 18 months.

Two-year Study

Survival

More mice in the exposed groups of males survived to study termination than in the vehicle control group of males; however, the differences were not significant (Table 42; Figure 14). Survival in females was similar across all groups.

Table 42. Summary of Survival of Male and Female Mice in the Two-year Drinking Water Study of Sodium Tungstate Dihydrate

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Male				
Animals Initially in Study	50	50	50	50
Moribund	5	3	4	6
Natural Deaths	18	16	11	9
Accidental Deaths	1	–	–	–
Animals Surviving to Study Termination	26	31	35	35
Percent Probability of Survival at End of Study[a]	53.1%[b]	62.0%	70.0%	70.0%
Mean Survival (Days)[c]	683.4 ± 9.7	684.4 ± 11.6	710.4 ± 6.4	686.4 ± 13.1
Survival Analysis[d]	p = 0.107N	p = 0.530N	p = 0.081N	p = 0.158N
Female				
Animals Initially in Study	50	50	50	50
Moribund	2	1	2	0
Natural Deaths	10	8	10	10
Animals Surviving to Study Termination	38	41[e]	38	40
Percent Probability of Survival at End of Study	76.0%	82.0%	76.0%	80.0%
Mean Survival (Days)	699.9 ± 10.2	713.5 ± 5.9	702.0 ± 10.9	704.0 ± 14.8
Survival Analysis	p = 0.809N	p = 0.582N	p = 1.000N	p = 0.743N

[a]Kaplan-Meier determinations.
[b]Calculation does not include the accidental death animal.
[c]Mean of litter means of all deaths (uncensored, censored, and study termination) ± standard error.
[d]The result of the Cox proportional hazards trend test with random litter effects is in the vehicle control group column, and the results of the proportional hazards pairwise comparisons to the vehicle control with random litter effects are in the exposed group columns. A negative trend or lower mortality in an exposure group is indicated by N.
[e]Includes one animal that died naturally during the last week of the study.

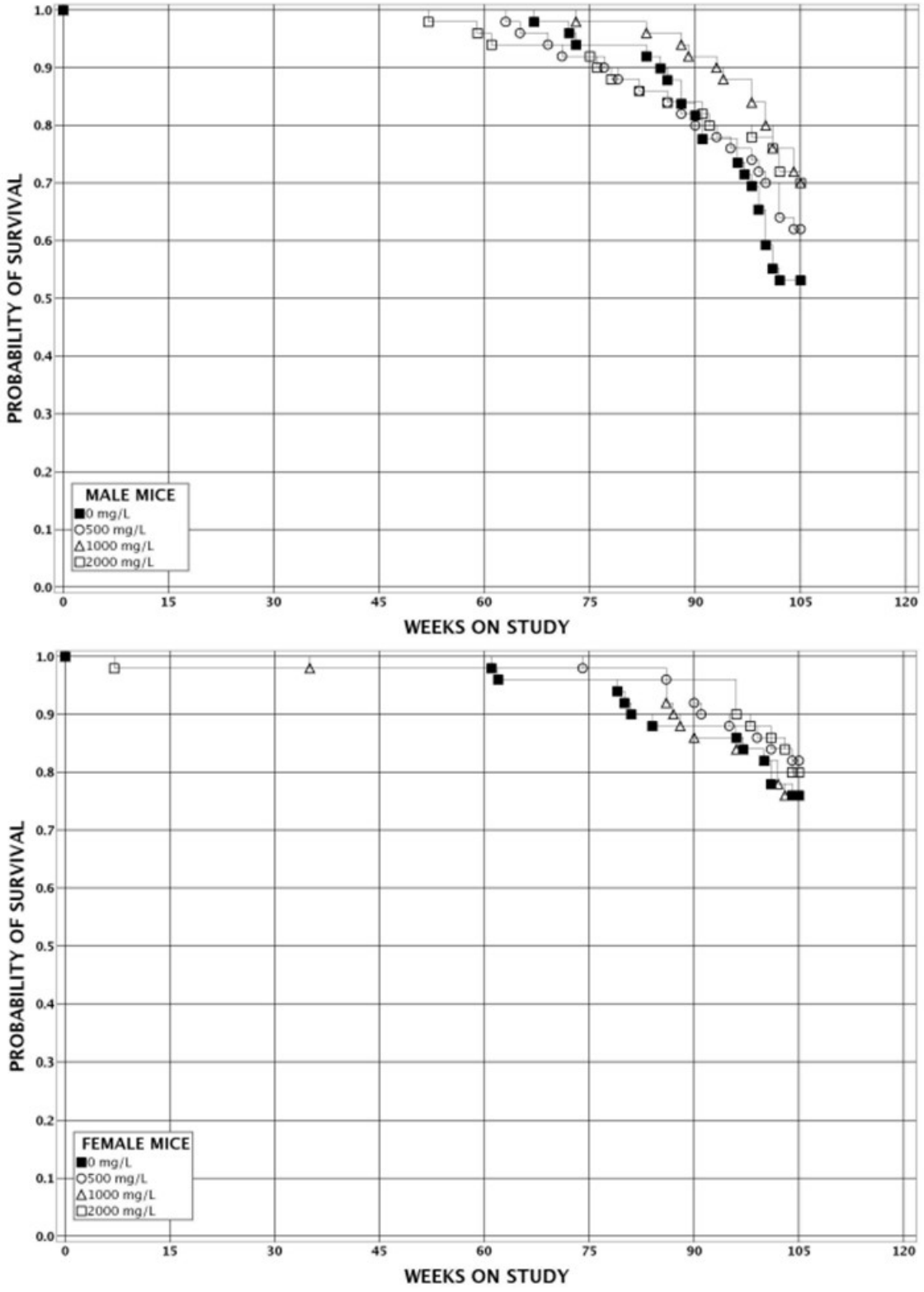


Figure 14. Kaplan-Meier Survival Curves for Mice Exposed to Sodium Tungstate Dihydrate in Drinking Water for Two Years

Body Weights, Water and Compound Consumption, and Clinical Observations

At study termination, the mean body weight of the 2,000 mg/L males was 88% of the vehicle control group; all other groups of exposed males and all groups of exposed females had mean body weights within 10% of their respective vehicle control groups (Table 43, Table 44; Figure 15).

Group mean water consumption over the course of the study for ST-exposed males for the 500, 1,000, and 2,000 mg/L groups averaged 96%, 90%, and 85% of the vehicle control group, respectively (Table 45). For the ST-exposed females, group water consumption values for the 500, 1,000, and 2,000 mg/L groups averaged 105%, 97%, and 93% of the vehicle control group, respectively (Table 46). Daily ST consumption for the 500, 1,000, and 2,000 mg/L groups averaged 42.5, 80.0 and 158.1 mg/kg/day, respectively, for the males and 29.0, 56.2, and 107.0 mg/kg/day, respectively, for the females (Table 45, Table 46). In general, ingested dose increased proportionally with the exposure concentration for both sexes. More occurrences of thinness and ruffled fur were recorded in exposed groups of male mice compared to vehicle control males; clinical observations were similar in all groups of females (Appendix G).

Table 43. Summary of Survival and Mean Body Weights of Male Mice in the Two-year Drinking Water Study of Sodium Tungstate Dihydrate

Study Day[a]	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	21.7	50	21.6	99.1	50	21.6	99.3	50	21.5	98.7	50
8	23.5	50	23.3	99.1	50	22.9	97.4	50	22.5	95.4	50
15	25.0	50	24.8	99.4	50	24.4	97.9	50	23.5	94.1	50
22	26.3	50	25.8	98.2	50	25.6	97.5	50	24.5	93.3	50
29	27.5	50	27.1	98.7	50	26.7	97.3	50	25.3	92.2	50
36	28.6	50	28.3	98.9	50	27.8	97.3	50	26.3	92.0	50
43	29.9	50	29.6	99.0	50	28.9	96.6	50	26.9	90.1	50
50	31.4	50	31.2	99.2	50	30.4	96.6	50	28.1	89.3	50
57	32.1	50	32.1	100.2	50	31.3	97.5	50	28.7	89.4	50
64	33.6	50	33.6	100.1	50	32.2	95.9	50	29.2	87.0	50
71	34.9	50	34.1	97.7	50	33.8	96.7	50	31.1	89.6	50
78	36.4	50	35.7	97.9	50	34.8	95.4	50	31.5	86.6	50
85	37.4	50	36.6	97.9	50	35.6	95.3	50	31.9	85.2	50
92	38.7	50	37.7	97.3	50	37.0	95.5	50	33.1	85.4	50
120	43.2	50	42.2	97.6	50	41.0	94.9	50	36.8	85.1	50
148	45.9	50	45.1	98.1	50	43.8	95.4	50	39.3	85.5	50
176	48.0	50	46.8	97.4	50	46.0	95.9	50	42.5	88.5	50
204	49.3	50	48.3	98.0	50	47.3	96.1	50	44.6	90.5	50
232	50.0	50	48.9	97.8	50	48.2	96.2	50	45.9	91.7	50
260	50.7	50	49.7	97.9	50	48.8	96.3	50	46.6	91.8	50
288	51.7	50	50.2	97.2	50	49.4	95.7	50	47.4	91.7	50
316	52.5	50	51.2	97.6	50	50.5	96.3	50	48.8	93.0	50
344	52.9	50	51.1	96.6	50	50.9	96.1	50	49.3	93.2	50
372	53.5	50	51.6	96.5	50	51.4	96.2	50	49.3	92.3	49
400	54.1	50	51.7	95.5	50	52.1	96.2	50	50.1	92.7	49
428	54.2	50	51.4	94.7	50	52.2	96.3	50	50.3	92.7	47
456	54.3	50	51.6	95.0	48	52.5	96.7	50	50.5	92.9	47
484	54.5	49	51.9	95.3	47	52.5	96.3	50	50.1	91.9	47
512	54.5	47	52.4	96.1	46	52.5	96.4	49	49.3	90.5	47
540	54.6	47	52.2	95.7	45	52.5	96.2	49	49.0	89.7	45
568	53.8	46	50.9	94.6	44	52.3	97.3	49	47.7	88.8	43
596	53.2	43	50.0	93.9	42	51.5	96.7	48	46.6	87.6	43
624	51.8	41	49.7	96.0	41	50.5	97.5	46	46.5	89.9	42
652	50.9	38	49.0	96.2	39	50.1	98.3	44	46.3	91.0	40
680	49.1	34	46.6	94.8	37	47.4	96.5	44	45.3	92.1	39
708	48.8	27	44.6	91.4	35	45.2	92.6	38	42.4	87.0	38
EOS	**49.8**	**26**	**47.1**	**94.4**	**31**	**45.9**	**92.0**	**35**	**43.9**	**88.0**	**35**

EOS = end of study.
[a]Study day 1 is the day animals were placed on study.

Table 44. Summary of Survival and Mean Body Weights of Female Mice in the Two-year Drinking Water Study of Sodium Tungstate Dihydrate

Study Day[a]	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	15.9	50	15.7	99.2	50	16.1	101.4	50	16.1	101.3	50
8	17.5	50	17.5	99.8	50	17.0	97.1	50	17.3	98.9	50
15	18.5	50	18.4	99.6	50	18.2	98.3	50	18.2	98.6	50
22	19.5	50	19.4	99.3	50	18.9	96.7	50	19.2	98.5	50
29	20.5	50	20.5	100.3	50	20.1	98.3	50	19.9	97.2	50
36	21.2	50	21.1	99.4	50	21.0	99.0	50	20.7	97.6	50
43	22.6	50	22.4	99.1	50	22.0	97.7	50	21.7	96.2	50
50	22.9	50	22.8	99.9	50	22.2	97.1	50	22.4	98.1	49
57	23.7	50	23.3	98.4	50	23.0	97.2	50	23.3	98.5	49
64	24.6	50	24.7	100.0	50	23.9	97.2	50	23.9	97.1	49
71	25.5	50	25.5	99.8	50	25.0	98.0	50	25.1	98.2	49
78	26.7	50	26.5	99.2	50	26.0	97.2	50	25.7	96.4	49
85	27.8	50	27.0	96.9	50	26.8	96.2	50	26.9	96.6	49
92	28.6	50	28.2	98.7	50	27.5	96.0	50	27.5	96.2	49
120	33.5	50	32.5	97.2	50	31.5	94.2	50	31.5	94.2	49
148	38.1	50	37.6	98.7	50	35.6	93.5	50	36.3	95.2	49
176	41.7	50	41.2	98.8	50	38.9	93.4	50	39.7	95.3	49
204	45.6	50	44.6	97.8	50	42.0	92.2	50	42.8	93.9	49
232	48.2	50	47.0	97.5	50	44.3	91.8	50	45.2	93.8	49
260	50.2	50	49.3	98.2	50	47.0	93.7	49	47.9	95.3	49
288	52.3	50	51.7	98.9	50	48.5	92.7	49	49.5	94.5	49
316	54.9	50	53.9	98.3	50	51.1	93.0	49	51.8	94.3	49
344	55.0	50	54.3	98.7	50	51.7	93.9	49	52.2	94.8	49
372	56.8	50	56.7	99.8	50	53.7	94.4	49	54.3	95.6	49
400	58.5	50	58.2	99.4	50	55.3	94.4	49	55.1	94.2	49
428	57.9	49	58.2	100.5	50	55.3	95.5	49	55.9	96.6	49
456	59.8	48	59.1	98.9	50	56.8	95.0	49	56.9	95.1	48
484	60.8	48	60.1	98.8	50	57.9	95.2	49	57.6	94.8	48
512	61.4	48	60.0	97.8	50	58.2	94.9	49	57.7	94.1	48
540	61.6	48	61.1	99.2	49	59.1	96.0	49	58.6	95.2	48
568	61.2	45	60.7	99.0	49	58.6	95.7	49	57.8	94.3	48
596	61.2	44	59.4	97.2	49	57.6	94.2	49	58.1	95.0	48
624	61.1	44	59.0	96.5	48	58.2	95.2	44	57.4	93.8	48
652	59.9	44	59.5	99.4	45	58.3	97.3	43	56.3	93.9	48
680	59.0	42	57.6	97.6	44	57.8	97.9	42	56.0	95.0	45
708	56.6	39	53.6	94.7	42	54.3	96.0	41	52.7	93.1	43
EOS	**57.1**	**38**	**54.9**	**96.1**	**40**	**54.9**	**96.2**	**38**	**53.8**	**94.2**	**40**

EOS = end of study.
[a]Study day 1 is the day animals were placed on study.

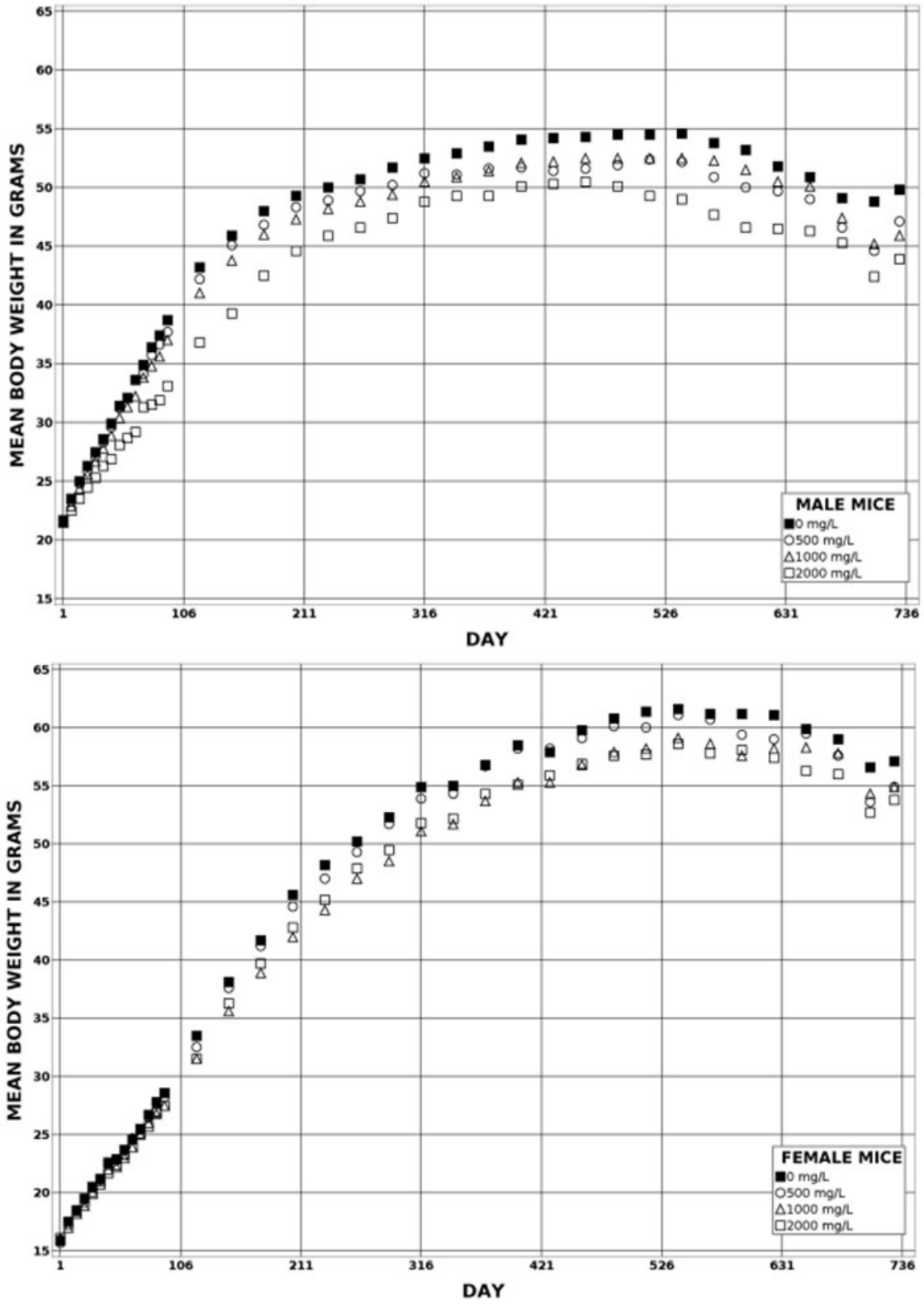


Figure 15. Growth Curves for Mice Exposed to Sodium Tungstate Dihydrate in Drinking Water for Two Years

Table 45. Summary of Water and Sodium Tungstate Dihydrate Consumption of Male Mice in the Two-year Drinking Water Study

Week	0 mg/L	500 mg/L		1,000 mg/L		2,000 mg/L	
	Water (g/day)[a]	Water (g/day)	Dose (mg/kg/day)[b]	Water (g/day)	Dose (mg/kg/day)	Water (g/day)	Dose (mg/kg/day)
1	–	–	–	–	–	–	–
13	3.2	3.3	45.1	3.0	84.2	2.8	175.7
54	4.1	4.1	39.7	3.7	72.0	3.3	133.8
102	4.9	4.1	46.0	4.3	95.1	3.8	179.1

[a]Grams of water consumed/animal/day.
[b]Milligrams of sodium tungstate dihydrate consumed/kilogram body weight/day.

Table 46. Summary of Water and Sodium Tungstate Dihydrate Consumption of Female Mice in the Two-year Drinking Water Study

Week	0 mg/L	500 mg/L		1,000 mg/L		2,000 mg/L	
	Water (g/day)[a]	Water (g/day)	Dose (mg/kg/day)[b]	Water (g/day)	Dose (mg/kg/day)	Water (g/day)	Dose (mg/kg/day)
1	1.9	1.8	57.2	1.8	111.9	1.7	211.5
13	2.5	2.6	48.2	2.3	85.9	2.2	163.7
54	2.4	2.5	22.0	2.3	42.9	2.4	88.4
102	2.9	3.4	31.7	2.8	51.5	2.6	98.6

[a]Grams of water consumed/animal/day.
[b]Milligrams of sodium tungstate dihydrate consumed/kilogram body weight/day.

Histopathology

This section describes the significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the kidney, liver, large intestine (cecum), and testes. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms are presented as supplemental data in Appendix G.

Kidney: Renal tubule neoplasms were only recorded in exposed males; there was one renal tubule adenoma in the 1,000 mg/L males and two renal tubule carcinomas in the 2,000 mg/L males (Table 47). Additional step sections of the kidneys were examined from all male and female mice, but additional renal tubule neoplasms were not observed. Proliferative lesions identified by the kidney step section pathologist were considered part of the spectrum of lesions associated with CPN by the pathology working group (PWG). Renal tubule regeneration was significantly increased in all exposed groups of males and in the 1,000 and 2,000 mg/L females, relative to vehicle control groups (Table 47). The incidences of pigment in the kidney were significantly increased compared to the vehicle control groups in the 2,000 mg/L males and 1,000 mg/L females, and lower in the 500 and 1,000 mg/L males compared to the vehicle control group (Table 47). Given the high incidence of pigment in the male vehicle control group, and the lack of an exposure concentration response in either the males or females, the finding of pigment in the kidney of exposed animals is of questionable toxicological importance. Also, in males, there was a positive trend for renal tubule necrosis with increased exposure (Table 47).

The renal tubule adenoma was a discrete nodule that protruded slightly above the cortical surface (Figure 16). It was a well-circumscribed cellular mass composed of well-differentiated renal tubule epithelial cells. The renal cell carcinomas, in contrast to the adenoma, were large and invasive, effacing and replacing more than 60% of the renal parenchyma (Figure 17). The carcinomas had solid and cystic areas and displayed marked cellular pleomorphism and a high mitotic rate.

A normal kidney from a B6C3F1/N mouse is shown in Figure 18. Renal tubule regeneration was characterized by basophilia, karyomegaly, hypertrophy, and hyperplasia of the renal tubule epithelium (Figure 19). Occasional mitotic figures were also present. Severity grading was based on the amount of renal cortex involved, with minimal regeneration involving <10% of the cortex; mild regeneration involving approximately 10%–25% of the cortex; moderate regeneration involving approximately 26%–75% of the cortex, and marked regeneration involving over 75% of the cortex. Renal tubule regeneration often occurred in kidneys that also had CPN, and the lesions had to be separated diagnostically. Basophilic and hyperplastic tubules could also be seen in CPN, but with CPN, the tubules would also have thickened basement membranes. Affected renal tubules of CPN tended to lack the amount of karyomegaly, hypertrophy, and hyperplasia that was characteristic of the epithelium seen in regeneration. Also, the pattern of the regeneration was somewhat different from that of CPN—with low magnification, moderate to severe regeneration could be seen to affect the cortex in a thick band sparing the medulla (generally affecting the proximal convoluted tubule); the lesions of CPN typically were not so evenly distributed.

Pigment in the kidney was light golden brown and was usually located in or around tubules with CPN or regeneration, either in the renal tubule epithelium or in macrophages. All of the occurrences of kidney pigment in females, and almost all in males, were of minimal severity. This pigment most likely represents a background change, with the high incidences due to the lack of any threshold used for diagnosis.

Renal tubule necrosis was recorded in three 2,000 mg/L males and was characterized by renal tubule epithelial cells with brightly eosinophilic cytoplasm and pyknotic or missing nuclei; affected cells were often shrunken, irregular in shape, or sloughed off of their basement membrane (Table 47).

Table 47. Incidences of Neoplastic and Nonneoplastic Lesions of the Kidney in Male and Female Mice in the Two-year Drinking Water Study of Sodium Tungstate Dihydrate

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
n[a]	50	50	50	50
Male				
Renal Tubule, Regeneration[b]	2** (1.0)[c]	21** (1.4)	32** (1.4)	38** (1.6)
Renal Tubule, Necrosis	0*	0	0	3 (1.3)
Pigment	30** (1.0)	10 (1.0)	18 (1.2)	44** (1.0)

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Renal Tubule, Adenoma[d]	0	0	1	0
Renal Tubule, Carcinoma[e]				
Overall rate[f]	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
Adjusted rate[g]	0%	0%	0%	4.6%
Terminal rate[h]	0/26 (0%)	0/31 (0%)	0/35 (0%)	2/35 (6%)
First incidence (days)	_[i]	_	_	730 (T)
Poly-3 test[j]	p = 0.047	(e)	(e)	p = 0.244
Renal Tubule, Adenoma or Carcinoma (Combined)[k]				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	2/50 (4%)
Adjusted rate	0%	0%	2.2%	4.6%
Terminal rate	0/26 (0%)	0/31 (0%)	1/35 (3%)	2/35 (6%)
First incidence (days)	_	_	730 (T)	730 (T)
Poly-3 test	p = 0.073	(e)	p = 0.520	p = 0.244
Female				
Renal Tubule, Regeneration	0**	1 (3.0)	7** (1.1)	7** (1.3)
Renal Tubule, Necrosis	1 (2.0)	5 (1.0)	1 (1.0)	3 (2.7)
Pigment	0	0	6* (1.0)	1 (1.0)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Poly-3 test; **$p \leq 0.01$.
(T) = study termination; (e) = statistic could not be computed.
[a]Number of animals examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.
[d]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 1/687 (0.15% ± 0.55%); range: 0% to 2%.
[e]Historical control incidence: 2/687 (0.31% ± 1.11%); range: 0% to 4%.
[f]Number of animals with neoplasm/number of animals necropsied.
[g]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[h]Observed incidence at terminal euthanasia.
[i]Not applicable; no neoplasms in group.
[j]Beneath the control incidence is the p value associated with the trend test. Beneath the exposed group incidences are the p values corresponding to pairwise comparisons between the vehicle control group and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal euthanasia. A negative trend or a lower incidence in an exposure group is indicated by N.
[k]Historical control incidence: 3/687 (0.46% ± 1.66%); range: 0% to 6%.

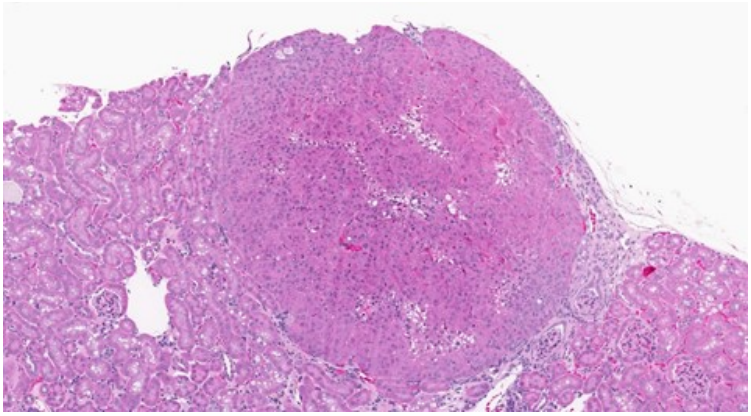


Figure 16. Renal Tubule Adenoma in a Male B6C3F1/N Mouse Exposed to 1,000 mg/L Sodium Tungstate Dihydrate for Two Years (H&E)

The adenoma is a well-circumscribed mass, which is protruding above the surface of the kidney. It is composed of a solid sheet of well-differentiated epithelial cells.

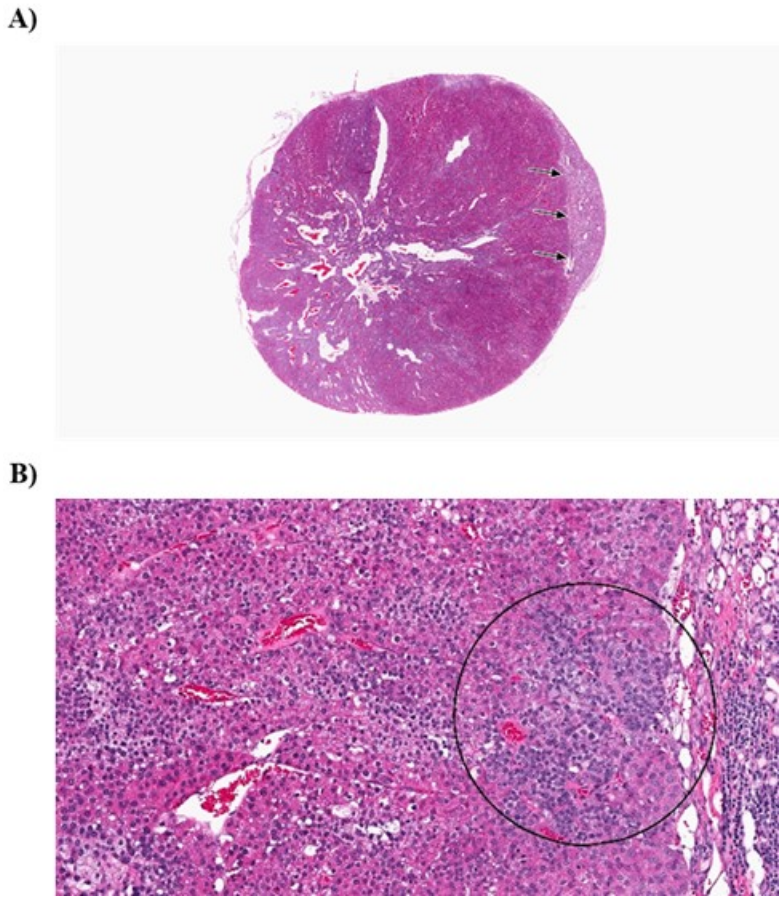
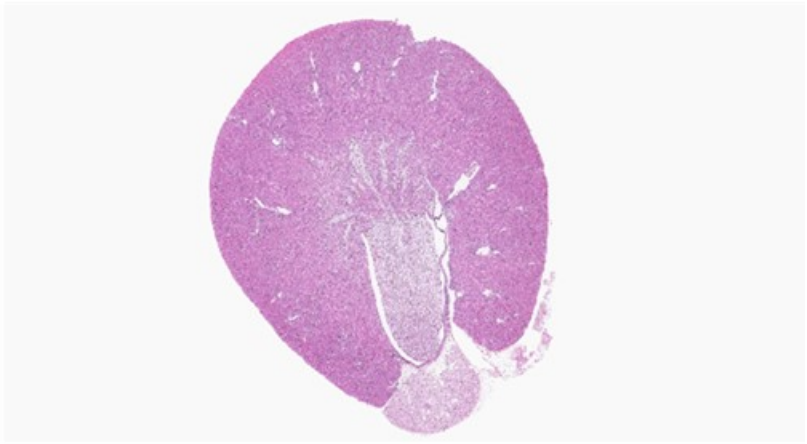


Figure 17. Renal Tubule Carcinoma in a Male B6C3F1/N Mouse Exposed to 2,000 mg/L Sodium Tungstate Dihydrate for Two Years (H&E)

A) The neoplasm is very large and has almost completely obliterated the kidney; only a small amount of uninvolved kidney remains (arrows). B) Higher magnification of the renal tubule carcinoma in panel A; the neoplasm is densely cellular and one area in particular (circle) displays cells with a very high nuclear-to-cytoplasmic ratio.

A)



B)

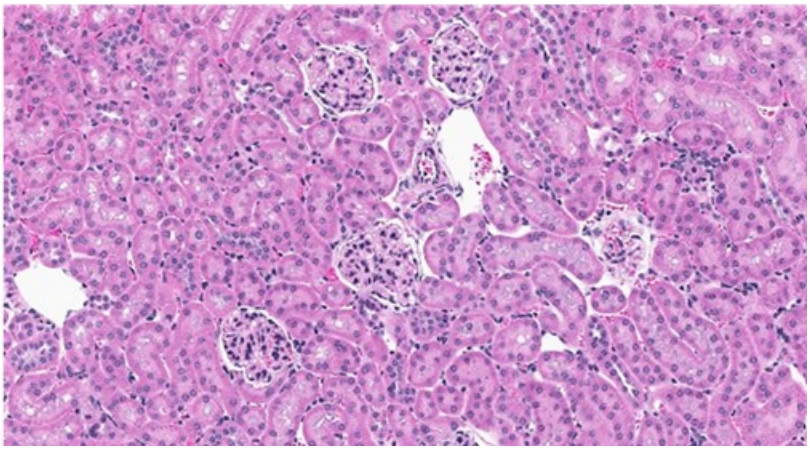
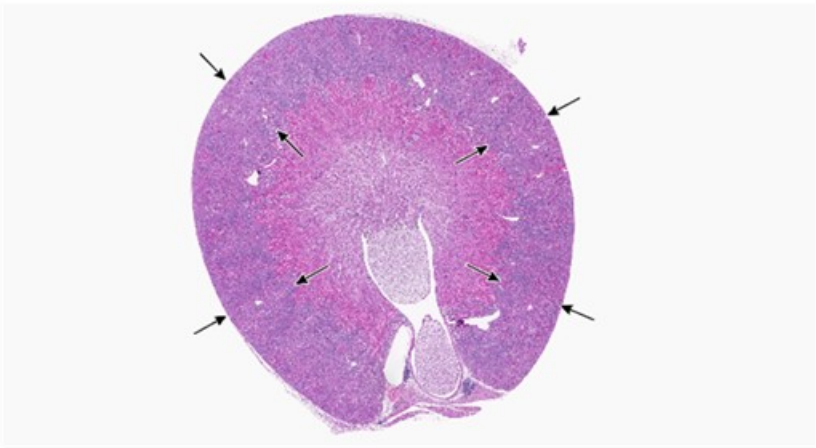


Figure 18. Normal Kidney from a Vehicle Control Female B6C3F1/N Mouse in the Two-year Study of Sodium Tungstate Dihydrate (H&E)

A) This normal kidney is from a vehicle control female B6C3F1/N mouse. B) This shows a higher magnification of the normal kidney in panel A.

A)



B)

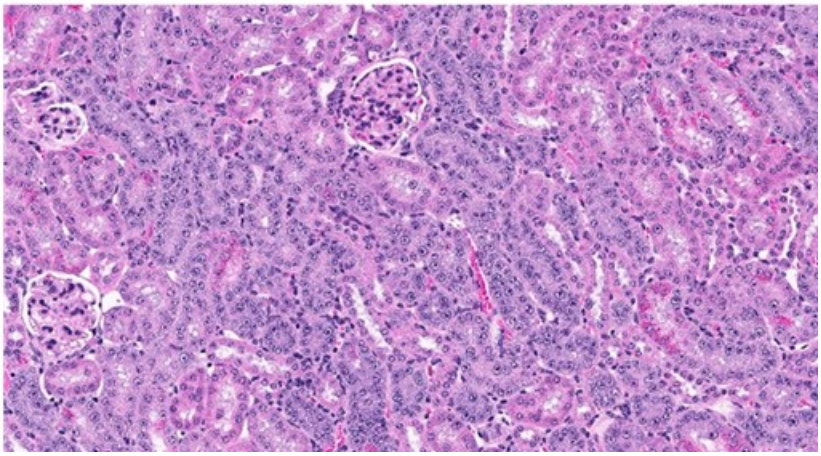


Figure 19. Moderate Renal Tubule Regeneration in a Female B6C3F1/N Mouse Exposed to 2,000 mg/L Sodium Tungstate Dihydrate for Two Years (H&E)

A) This low magnification photomicrograph shows a thick band of basophilia due to regeneration of the renal tubules involving the cortex (arrows). Compare with the kidney in Figure 18A. B) This higher magnification of the renal tubule regeneration in panel A shows that the tubules are basophilic and the cells are crowded, consistent with hyperplasia and regeneration. There are no thickened basement membranes or other evidence of chronic progressive nephropathy, such as associated interstitial inflammation. Compare with the section of kidney in Figure 18B.

Large Intestine, Cecum: The incidences of pigment in the cecum were significantly increased in the 1,000 and 2,000 mg/L males and females (Table 48). The pigment was found in macrophages, and possibly fibroblasts, within the lamina propria of the cecum, and was similar in character to that observed in the kidney; it was light golden brown (Figure 20). Cells containing pigment were usually observed in small clusters, but were not associated with other lesions, such as mucosal inflammation or ulceration. Special staining with Perl's stain for iron did not reveal iron in the pigment, therefore, the pigment was not hemosiderin. Other possibilities include pigments associated with cell breakdown, such as lipofuscin or ceroid.

Testes: There were increased incidences of germinal epithelium degeneration recorded in the testes of all exposed groups of males compared to the vehicle control group, and the increase was

significant in the 500 mg/L group (Table 48). Germinal epithelial degeneration consisted of several changes, including vacuolation of the germinal epithelium, disorganization of the germinal epithelium, or depletion of germs cells (Figure 21). Typically, especially with minimal and mild lesions, only one or two seminiferous tubules were affected; however, the involvement of only one seminiferous tubule might present as several cross sections of tubules within a section of testis. Marked lesions were characterized by seminiferous tubules with only Sertoli cells remaining (atrophy); this was usually a widespread to diffuse change throughout the testis.

Bone Marrow and Spleen: Hypercellularity of the bone marrow was significantly increased in incidence in the 500 and 1,000 mg/L males; the incidence of extramedullary hematopoiesis in the spleen was significantly increased in the 500 and 1,000 mg/L females (Table 48). Hypercellularity of the bone marrow was characterized by cells filling the marrow cavity, throughout the length of the femur. Little adipose tissue was observed. Both the erythroid and myeloid cell lines appeared to have increased in most cases. Megakaryocytes were also abundant in affected animals. Most of the cases were minimal to mild in severity, although an occasional animal had moderate to marked hypercellularity recorded; severity was graded subjectively and was based on the amount of blood cell precursors and the lack of adipocytes compared to the vehicle control animals and what would be expected in a chronic study. Extramedullary hematopoiesis in the spleen is common in mice, even at 2 years. As such, extramedullary hematopoiesis was recorded when it appeared increased over that which would be expected in the spleen. It usually involved an expansion of the red pulp, which was filled with cells containing little cytoplasm and dense basophilic nuclei. Evidence of both erythroid and myeloid lineages were typically present, as were abundant megakaryocytes. The toxicological significance of the differences in the incidences of bone marrow hyperplasia and extramedullary hematopoiesis in the spleen is unknown.

Other Tissues: There were significantly increased incidences of hepatocellular adenomas and carcinomas, combined, in the 500 mg/L females. This group also had a significant increase in the incidences of eosinophilic foci and focal inflammation. Hepatocellular neoplasms are common in B6C3F1 female mice, with a historical control range of up to 28% for adenomas, and up to 40% for adenomas and carcinomas combined. There was no exposure concentration relationship in the incidences of either the neoplastic or nonneoplastic lesions observed in the liver, and the incidences of hepatocellular adenomas and hepatocellular carcinomas were within the historical control range for each group (Appendix G). Therefore, the increased incidence in the 500 mg/L females was considered biological variation, and not related to ST exposure.

Table 48. Incidences of Select Nonneoplastic Lesions in Male and Female Mice in the Two-year Drinking Water Study of Sodium Tungstate Dihydrate

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
n[a]	50	50	50	50
Male				
Large Intestine, Cecum				
Pigment[b]	3** (1.0)[c]	7 (1.0)	17** (1.0)	32** (1.0)
Testis				
Germinal epithelium, degeneration	11 (1.5)	20* (1.3)	20 (1.5)	20 (2.2)

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Female				
Large Intestine, Cecum				
Pigment	0**	3 (1.0)	7** (1.0)	14** (1.0)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Poly-3 test; **$p \leq 0.01$.
[a]Number of animals examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

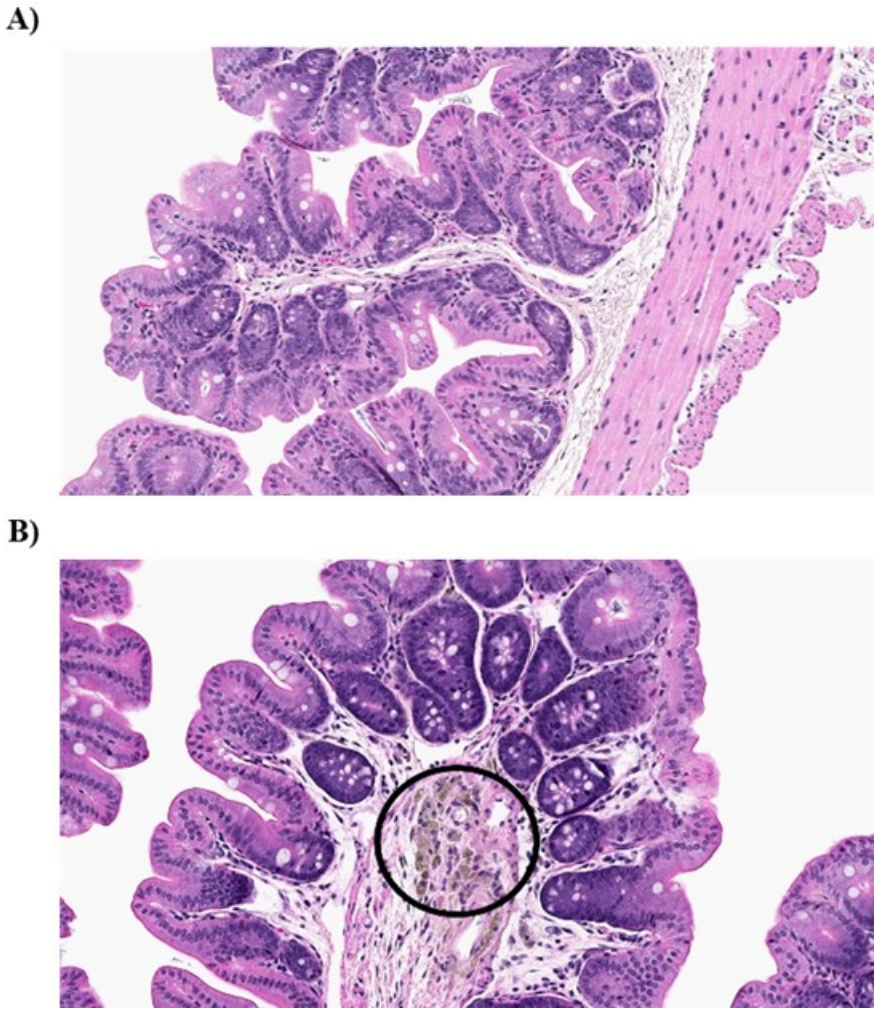


Figure 20. Pigment in the Cecum of B6C3F1/N Mice Exposed to 0 or 2,000 mg/L Sodium Tungstate Dihydrate for Two Years (H&E)

A) This normal cecum is from a B6C3F1/N vehicle control female mouse. B) In this cecum in a female B6C3F1/N mouse exposed to 2,000 mg/L sodium tungstate dihydrate, the golden-brown pigment is contained primarily in macrophages within the lamina propria (circle). Compare with the section of cecum in panel A.

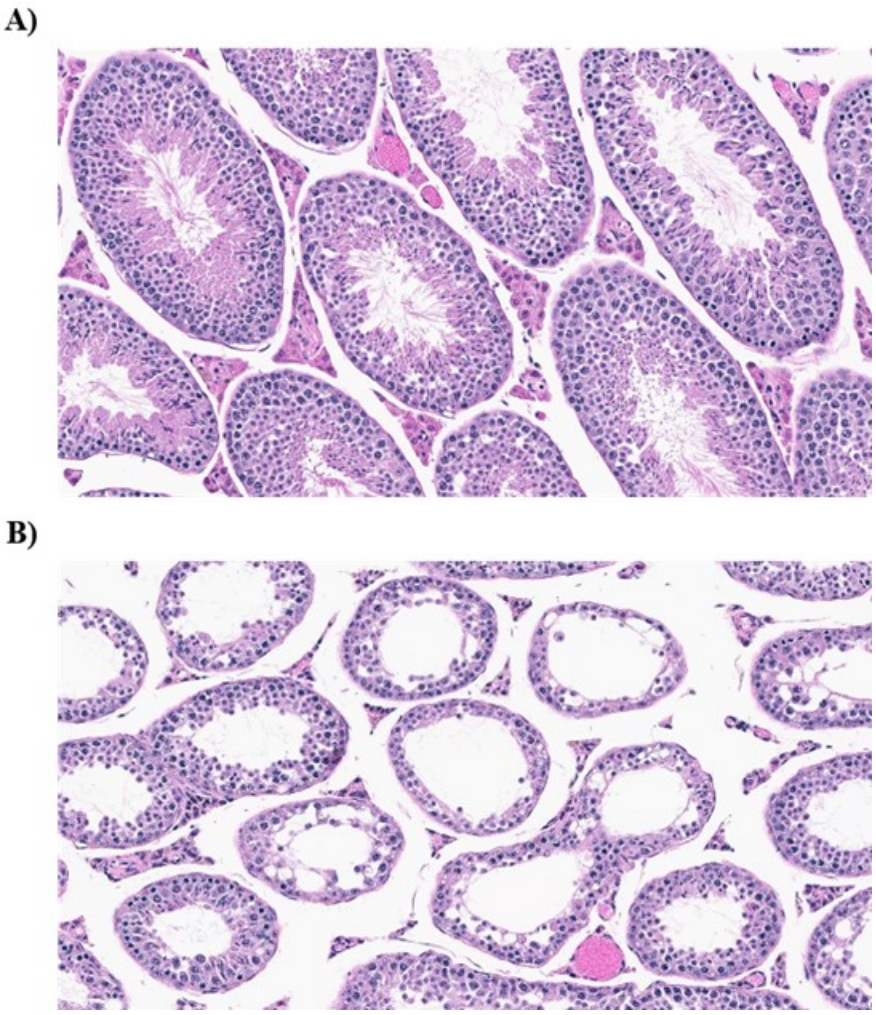


Figure 21. Germinal Epithelium Degeneration in the Testis from B6C3F1/N Mice Exposed to 2,000 mg/L Sodium Tungstate Dihydrate for Two Years (H&E)

A) Testis from a B6C3F1/N mouse exposed to 2,000 mg/L sodium tungstate dihydrate (ST) is within normal limits. B) Testis from a B6C3F1/N mouse exposed to 2,000 mg/L ST shows widespread degeneration of the germinal epithelium. A thinning of the germinal epithelium of the seminiferous tubules is observed due to a decrease in the number of cells; compare with the testis in panel A.

Genetic Toxicology

The genetic toxicity of ST was evaluated in bacterial reverse mutation assays and in both the peripheral blood micronucleus test and the comet assay in rats and mice. ST significantly increased DNA damage in the comet assay in liver cells from male and female rats, and in cells from liver and ileum tissues in male mice (Appendix D).

ST (12.5 to 6,000 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA98 or TA100, or in *Escherichia coli* WP2 *uvrA* (pKM101), when tested with or without exogenous metabolic activation provided by phenobarbital/benzoflavone-induced rat S9 and cofactors (Table D-1).

At the end of the 3-month studies, peripheral blood samples were obtained from male and female rats and mice and analyzed for the frequency of micronucleated reticulocytes and erythrocytes (Table D-2, Table D-3). In male and female rats, the reticulocyte population (polychromatic erythrocytes, or PCEs)—which is the only red blood cell population that can be accurately assessed for micronucleus frequency in peripheral blood of rats due to efficient splenic scavenging of damaged erythrocytes—did not show an increase in micronucleated cells after 3 months of exposure to ST via drinking water (0, 125, 250, 500, 1,000, and 2,000 mg/L) (Table D-2). Significant increases in the percent reticulocytes were seen in both male and female rats suggesting that ST could have stimulated erythropoiesis in the bone marrow; however, the absolute increases in the percentages were small compared to the vehicle control animals.

In male and female mice, there were no significant increases in micronucleated reticulocytes or in micronucleated erythrocytes in either sex following 3 months of exposure to ST via drinking water (0, 125, 250, 500, 1,000, and 2,000 mg/L) (Table D-3). A significant increase in the percent reticulocytes was seen in male mice suggesting that ST could have stimulated erythropoiesis in the bone marrow; however, the absolute increase was small compared to the vehicle control group.

In addition to evaluating the potential for chromosomal damage, the potential for DNA damage was assessed using the comet assay in the same animals in which micronucleus induction was evaluated. DNA damage from exposure to ST was assessed in liver, ileum, and kidney cell samples and in blood leukocytes (Table D-4, Table D-5). Significant increases in DNA damage, measured as percent tail DNA, were observed in liver cells from male and female rats. Significant increases in DNA damage were also observed in liver and ileum cells from male mice. No increases in percent tail DNA were observed in female mice. Although cells from kidney tissue were evaluated for male and female rats, and ileum tissue was evaluated from male rats, results from these tissues were considered invalid due to unusually high percent tail DNA in the vehicle control group.

Discussion

Tungsten was nominated to the National Toxicology Program (NTP) by the Centers for Disease Control and Prevention for toxicity and carcinogenicity studies due to inadequate data on effects in humans and concern about potential human exposure via drinking water.[93; 94] Tungstate (WO_4^{2-}) is the most common of the naturally occurring forms of soluble tungsten; sodium tungstate dihydrate (ST) was selected for these studies because it is the most water-soluble form of tungstate.

In the current perinatal and 3-month rat study, no exposure-related effects were noted on pregnancy status, maternal survival, or the number of dams that littered at any of the exposure concentrations tested. There were significant decreases in the mean body weights of dams in the 1,000 and 2,000 mg/L groups (approximately 10% and 18%, respectively) at the end of lactation. The mean body weights of pups (male and female combined) on postnatal day 21 in the 2,000 mg/L group were also significantly decreased by approximately 14%. There were no ST-related findings in the testes or epididymides, or changes in sperm parameters in either rat or mouse after 3 months of exposure. A previous study of Sprague Dawley rats exposed daily to ST at 5 or 125 mg/kg/day by gavage for 70 days (including through mating, gestation, and lactation) similarly found no reproductive effects.[95]

In the 3-month drinking water studies, the kidney was the major target organ of toxicity in both rats and mice. In rats, renal tubule regeneration was increased in the male and female 1,000 and 2,000 mg/L groups; the increases in the 2,000 mg/L groups were significant. Renal tubule regeneration was distinct from chronic progressive nephropathy (CPN), which had similar incidences and severities between exposed and vehicle control groups. Renal tubule regeneration was also significantly increased in 1,000 and 2,000 mg/L male mice. In female mice, although there was a significant positive trend, there were no pairwise significant differences between vehicle control animals and exposed animals, indicating sex-related differences in ST sensitivity in mice.

The urine xanthine/creatinine ratios were significantly increased in all the male and female rat groups at 13 weeks. This finding was not unexpected as ST has been shown to antagonize the normal metabolic action of molybdate in its role as a cofactor for several enzymes, including xanthine oxidase.[1; 96; 97] Xanthine oxidase catalyzes the oxidation of xanthine to uric acid; thus, inhibition of this enzyme can lead to increases of xanthine in the urine.[1]

Serum insulin concentrations were also measured in the perinatal and 3-month rat study. Serum insulin concentrations were significantly decreased in the high-exposure male group whereas serum glucose was unchanged. Studies have shown that ST has antidiabetic effects in rats in that when administered to streptozotocin-induced (STZ) diabetic rats, serum glucose normalizes without changes to the serum insulin concentrations.[98; 99] In these same studies, both glucose and insulin concentrations were unchanged when ST was administered to healthy rats. These studies suggest that in STZ diabetic rats, serum glucose is lowered in part due to ST's ability to restore hepatic glucose metabolism by increasing the capacity of the liver to use glucose through glycolysis and glycogenesis. The effects have previously been shown to occur through an insulin receptor-independent pathway.[8] Given the known effects of tungstate on glucose metabolism, the

finding of decreased insulin was unexpected; the reason for this finding in the current study is not known.

Significant decreases noted in body weight gain in rat dams during the lactation phase, and reductions in final mean body weight in weaned pups, informed the decision to lower the top exposure concentration in rats to 1,000 mg/L in the chronic studies and to expose mice up to 2,000 mg/L.

In the perinatal and 2-year rat study, no exposure-related effects were noted on pregnancy status, maternal survival, or the number of dams that littered at any of the exposure concentrations tested (up to approximately 143 mg/kg/day). These findings are consistent with previous findings in the literature, which showed no reproductive effects in Sprague Dawley rats exposed to ST at 5 or 125 mg/kg/day.[95]

At study termination, an approximately 22% reduction in mean body weight was observed in the 1,000 mg/L female rats compared to the vehicle control group. Mean body weights of all groups of exposed males were within 10% of the vehicle control group. Mean water consumption was reduced by approximately 16% in the 1,000 mg/L males; in all other groups it was within 10% of the vehicle control group.

In the chronic studies, female rats in the 500 mg/L group had a significant increase in the incidence of thyroid C-cell adenomas compared to the vehicle control group, and there was a doubling of the incidence of C-cell adenomas in the 250 and 500 mg/L groups compared to that seen in the vehicle control group. Although there was no significant difference between the combined incidences of C-cell adenoma or carcinoma in the exposed groups when compared to the control group, the incidences in the 250 and 500 mg/L groups were above the historical control range. The highest incidence of C-cell carcinomas was found in the 1,000 mg/L group, and there was an earlier onset of C-cell neoplasms in exposed groups compared to the vehicle control group. However, C-cell neoplasms are a fairly common background tumor in Sprague Dawley rats, with a historical control range of up to 24% for adenomas and 28% for adenomas and carcinomas combined. There were no significant differences of C-cell hyperplasia in female rats between exposed groups and the vehicle control group, nor were there any significant differences between exposed and vehicle control male rats in the incidences of C-cell lesions. Therefore, NTP concluded that there was equivocal evidence of carcinogenic activity based on the incidences of C-cell adenoma or carcinoma (combined) in female rats; this was driven primarily by the adenomas.

Similar to the 3-month studies, the kidney was confirmed as a target organ of toxicity in both male and female rats following chronic exposure to ST. Suppurative inflammation of the renal tubules was significantly increased in the 1,000 mg/L males and females. Renal tubule regeneration was significantly increased in the 1,000 mg/L females but was not observed in male rats. By the end of the chronic study, the CPN of moderate to marked severity present in male rats made it challenging to distinguish changes of renal tubule regeneration from those seen with CPN. Given the findings in the 3-month study and the findings in the female rats, the renal tubular epithelium is likely a target of ST in both male and female rats. Despite the observation of renal tubule regeneration at 3 months, there was no progression to renal tubule neoplasms at 2 years. Consistent with the nephrotoxicity, kidney tungsten concentration increased with the

exposure concentration and the kidney/plasma ratios were higher than 1.0 at all exposure concentrations and time points demonstrating retention of tungsten in the kidney.

Exposure to ST for 2 years also resulted in a significantly increased incidence of atypical hyperplasia in the uterus of female rats in the 500 mg/L group. The incidences of atypical hyperplasia were greater than that of the vehicle control group, but not significantly so, in the 250 and 1,000 mg/L groups. Atypical hyperplasia in the uterus is considered a preneoplastic finding, but neither the incidences of adenoma nor adenocarcinoma of the uterus were significantly increased in exposed groups compared to the vehicle control group.

In mice, toxicological effects included moderate reductions in final mean body weight and water consumption for males in the 2,000 mg/L group. There were no exposure-related reductions in mean body weights or water consumption for females in any of the ST-exposed groups. Daily estimated ST consumption indicated that the consumed doses of females were approximately 70% of males at a given exposure concentration. Absolute kidney weights were significantly increased in the 500 and 1,000 mg/L males compared to the vehicle control group at 3 months, however, this effect was not observed at the later interim time points.

Histologically, the kidney was a target of ST administration in mice after chronic exposure. Similar to what was seen at 3 months, renal tubule regeneration was recorded in significantly increased incidences with increasing exposure. Three renal tubule neoplasms were observed in exposed male mice; no renal tubule neoplasms occurred in vehicle control mice, and no renal tubule neoplasms were found in female mice. Although there was no statistical difference between the incidences of renal tubule neoplasms in exposed groups versus the vehicle control group, they are uncommon in mice, with only three males out of a total of 687 control male mice in the historical database having renal tubule neoplasms. Of the 13 studies that are in the current NTP historical control database for mice, all three of the renal tubular neoplasms that occurred in control animals occurred in the same study. There were no renal tubule neoplasms recorded in control animals in the other 12 studies. In this study, the findings of renal tubule neoplasms in the mid (adenoma) and high (carcinoma) exposed groups, the lack of any such neoplasms in the concurrent control group, and the fact that the kidney was a target for nonneoplastic lesions—balanced by the lack of statistical significance or a neoplastic response in the kidneys of rats or female mice—led to the conclusion that the renal tubular neoplasms in male mice were considered equivocal evidence of carcinogenic activity. Consistent with this, the kidney/plasma tungsten ratios of higher than 1 suggest retention of tungsten in the kidney.

Overall, the kidney was considered a major target organ of toxicity in rats and mice in the subchronic and chronic studies. As indicated by the tissue burden data, tungsten accumulated in the kidney in an exposure concentration-dependent manner. Some species differences in tungsten accumulation in the kidney was observed in these studies. In mice, kidney/plasma ratios (1.4–4.4) remained similar regardless of the exposure duration with no observed sex difference; however, in rats, the ratios increased with exposure duration, with male rats showing a higher ratio (1.83–2.51 at 3 months to 12.63–24.26 at 18 months) than female rats (1.17–2.06 at 3 months to 3.99–7.11 at 18 months). The general trend was toward a decreasing kidney/plasma ratio with increasing exposure concentration in both rats and mice.

Other nonneoplastic lesions observed in the chronic mouse study associated with exposure to ST included pigment in the cecum and germinal epithelium degeneration in the testes. Hence, there

was evidence of systemic exposure to ST following oral administration in the drinking water based on the tissue distribution data and on toxicity in multiple tissues.

Although human biomonitoring data on tungsten concentrations in serum are relatively limited in the literature, the plasma tungsten concentration in the current studies following exposure of male rats (2.64 µg/mL) and male mice (1.27 µg/mL) to 250 ppm ST are approximately 18,000 and 8,500 times higher, respectively, compared to humans (arithmetic mean 0.15 µg/L or 0.00015 µg/mL, n = 290).[100] Several studies have evaluated urinary tungsten concentrations in humans (summarized in Lemus et al.[34]). Urinary tungsten concentrations in male rat (104.37 µg/mg creatinine) and male mice (278.92 µg/mg creatinine) exposed to 250 ppm ST in these studies are >1,000,000 times the urinary concentrations reported by a National Health and Nutrition Examination Survey program (2015–2016) (geometric mean 0.076 µg/g creatinine or 0.000076 µg/mg creatinine) based on a representative sample of 3,057 individuals.[101]

Conclusions

Under the conditions of these 2-year drinking water studies, there was *no evidence of carcinogenic activity* of sodium tungstate dihydrate (ST) in male Hsd:Sprague Dawley® SD® rats at exposure concentrations of 250, 500, or 1,000 mg/L. There was *equivocal evidence of carcinogenic activity* of ST in female Hsd:Sprague Dawley® SD® rats based on increased incidences of C-cell adenoma or carcinoma (combined) of the thyroid gland.

There was *equivocal evidence of carcinogenic activity* of ST in male B6C3F1/N mice based on the occurrences of renal tubule adenoma or carcinoma (combined) in exposed animals. There was *no evidence of carcinogenic activity* of ST in female B6C3F1/N mice at exposure concentrations of 500, 1,000, or 2,000 mg/L.

Exposure to ST in drinking water caused increased incidences of nonneoplastic lesions in the kidney of male and female rats and mice, in the uterus of female rats, in the large intestine of male and female mice, and in the testes of male mice.

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Appendix A. Chemical Characterization and Dose Formulation Studies

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A.1. Procurement and Characterization of Sodium Tungstate Dihydrate

Sodium tungstate dihydrate (ST) was procured from Sigma-Aldrich (St. Louis, MO) in two lots (lot 12330JO and lot MKBG9975V). Lot 12330JO was obtained directly from Sigma-Aldrich (St. Louis, MO), whereas lot MKBG9975V was produced by Sigma-Aldrich and obtained from Government Scientific Source, Inc. (Reston, VA). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory and study laboratory at Battelle (Columbus, OH). Reports on analyses performed in support of the ST studies are on file at the National Institute of Environmental Health Sciences.

ST is a white solid composed of fine crystals. The 3-month studies used lot 12330JO, which was homogenized by mixing for 15 minutes and transferred to 1 L amber storage bottles. For the 2-year studies, the remainder of lot 12330JO was combined with lot MKBG9975V to create lot 07072011 by mixing in a blender for 15 minutes.

The identities of the lots were confirmed using infrared spectroscopy (Figure A-1). The x-ray diffraction patterns of both lots were in good agreement with the reference pattern. Purity assessment with proton-induced x-ray emission identified magnesium (0.7%–0.9%) and aluminum (approximately 0.3%) impurities in both lots. The average concentrations of tungsten and sodium in lot 12330JO were 54.09% and 18.24%, respectively. Lot 07072011 had average tungsten and sodium concentrations of 47.6% and 15.8%, respectively. Elemental analysis using inductively coupled plasma atomic emission spectrometry yielded purities of approximately 99%, based on weight percentages of tungsten (55.2%–56.4%) and sodium (13.4%–13.8%). Karl Fisher titration yielded a water content of 9.5% for lot 12330JO and 10.0%–10.3% for lot 07072011, slightly lower than the anticipated 10.9%. Titration with lead nitrate indicated a purity of 97.6% for lot 12330JO and 98.2% for lot 07072011. Ion chromatography (IC) with a suppressed conductivity detector and liquid chromatography with an inductively coupled plasma (ICP) mass spectrometer indicated a purity of 100% for both lots. Additional information on chromatography systems used can be found in Table A-1.

Accelerated stability studies were conducted on samples of ST stored at 60°C, 25°C, 5°C, and −20°C using IC with a suppressed conductivity detector. Stability was confirmed for at least 2 weeks when stored in sealed amber glass bottles at 25°C, 5°C, and −20°C. Given these findings, bulk ST was stored in sealed amber glass bottles at 25°C. Periodic analyses of the bulk chemical using IC with a suppressed conductivity detector were conducted during the 3-month and 2-year studies by the study laboratory and confirmed that no degradation occurred.

A.2. Preparation and Analysis of Dose Formulations

The presence of tungsten and molybdenum in animal feed (NIH-07 and NTP-2000) and deionized water used in the 3-month and 2-year studies were evaluated with ICP optical emission spectrometry at Galbraith Laboratories, Inc. (Knoxville, TN). NIH-07 feed contained approximately 2 ppm tungsten, and the concentration in NTP-2000 feed was at the detection limit of the assay (0.80 ppm). Concentrations of tungsten in deionized water, and the concentration of molybdenum in all feed and water samples, were below the limits of detection of the assay (0.20 to 0.80 ppm).

Stability analysis conducted on the 20 μg/mL (20 mg/mL) formulation found that the formulations were stable when sealed and stored in Nalgene bottles for 42 days at 5°C and room temperature (approximately 25°C). An animal room simulation was conducted using the 20 mg/mL formulation stored in a drinking water bottle filled near capacity with aliquots periodically removed to simulate animal drinking. There was no significant loss in tungsten over 7 days at room temperatures.

Dose formulations of ST were prepared monthly (Table A-2). Formulations were prepared with deionized water. The 3-month studies used formulations of 0, 125, 250, 500, 1,000, and 2,000 mg/L for both mice and rats. These formulations were prepared four times in the mouse study (May–July 2009) and five times in the rat study (May–August 2009). The 2-year mouse study used 0, 500, 1,000, and 2,000 mg/L formulations prepared 27 times from January 2012 to January 2014. The 2-year rat study used 0, 250, 500, and 1,000 mg/L formulations prepared 28 times from December 2011 to January 2014. Formulations were determined to be homogeneous and of appropriate concentration using IC with a suppressed conductivity detector. Stability was confirmed for 42 days at room temperature.

Preadministration and postadministration (animal room) analysis of formulations was conducted monthly throughout the 3-month studies (Table A-3, Table A-4). During the 2-year studies, preadministration formulations were analyzed every 1–3 months, whereas postadministration (animal room) formulation were analyzed every 6–8 months (Table A-5, Table A-6). All preadministration formulations in the 3-month rat and mouse studies were within 10% of the target concentration. In the 3-month mouse study, four postadministration samples were more than 10% below the target concentration in postadministration samples collected from bottles used to expose females and carboys, with the largest difference being 12.8% below the target (Table A-4). Postadministration samples collected from bottles or carboys in the 3-month rat study prepared in May 2009 (125 mg/L), July 2009 (500 mg/L), and August 2009 (2,000 mg/L) were 12.3%, 11.5%, and 10.8% below the target concentrations, respectively. All preadministration and postadministration samples in the 2-year studies were within 10% of the target concentration.

Table A-1. Chromatography Systems Used in the Three-month and Two-year Studies of Sodium Tungstate Dihydrate

Chromatography	Detection System	Column	Mobile Phase
System A			
Ion chromatography	Suppressed conductivity (50 mA)	Dionex IonPac AS11-HC, 250 mm × 2 mm ID	24 mM sodium hydroxide, flow rate 0.4 mL/min
System B			
Ion chromatography	Suppressed conductivity (40°C)	Dionex IonPac AS11-HC, 250 mm × 4 mm ID	27 mM sodium hydroxide, flow rate 0.9 mL/min
System C			
Liquid chromatography	Inductively coupled plasma-mass spectrometer	IonPac AS11-HC, 250 × 2 mm ID	Approximately 14.4 mM sodium hydroxide, flow rate 0.4 mL/min

ID = internal diameter.

Table A-2. Preparation and Storage of Dose Formulations in the Three-month and Two-year Studies of Sodium Tungstate Dihydrate

Preparation
Stock solutions of sodium tungstate dihydrate were created by weighing an appropriate amount of lot 12330JO (3-month studies) or lot 07072011 (2-year studies) in a weighing container. The contents were transferred to a volumetric flask and rinsed with water to ensure complete transfer. Flasks were brought to volume with deionized water. Dose formulations were prepared monthly throughout the 3-month and 2-year studies.
Chemical Lot Number
3-month: 12330JO 2-year: 07072011 (Sigma lot 12330JO and MKBG9975V)
Maximum Storage Time
42 days
Storage Conditions
Formulations were stored in sealed Nalgene bottles at 5°C or 25°C
Study Laboratory
Battelle (Columbus, OH)

Table A-3. Results of Analyses of Dose Formulations Administered to Rats in the Perinatal and Three-month Study of Sodium Tungstate Dihydrate

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)[a]	Difference from Target (%)
May 11, 2009	May 12, 2009	125	118 ± 9	−5.6
		250	257 ± 2	2.7
		500	511 ± 12	2.2
		1,000	1,070 ± 20	6.7
		2,000	2,200 ± 10	10.0
	June 25, 2009 (bottle)[b]	125	123 ± 6	−1.9
		250	259 ± 2	3.7
		500	515 ± 9	3.0
		1,000	945 ± 53	−5.5
		2,000	1,890 ± 130	−5.5
	June 25, 2009 (carboy)[c]	125	110 ± 8	−12.3
		250	245 ± 8	−2.1
		500	485 ± 27	−2.9
		1,000	973 ± 19	−2.7
		2,000	2,120 ± 70	6.0

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)[a]	Difference from Target (%)
June 5, 2009	June 16, 2009	125	123 ± 3	−1.6
		250	243 ± 4	−2.7
		500	494 ± 4	−1.2
		1,000	1,000 ± NA[d]	0.0
		2,000	1,940 ± 40	−3.2
	July 24, 2009 (bottle)[b]	125	121 ± 4	−3.5
		250	245 ± 8	−1.9
		500	508 ± 11	1.6
		1,000	1,040 ± 10	3.7
		2,000	2,070 ± 20	3.7
	July 24, 2009 (carboy)[c]	125	127 ± 7	1.3
		250	248 ± NA[e]	−0.8
		500	523 ± NA[e]	4.5
		1,000	1,050 ± 10	4.7
		2,000	2,080 ± 30	4.2
July 31, 2009	August 3, 2009	125	118 ± 7	−5.6
		250	254 ± 4	1.6
		500	510 ± 13	2.0
		1,000	1,010 ± 80	1.3
		2,000	1,950 ± 170	−2.5
	September 22, 2009 (bottles)[b]	125	117 ± 4	−6.7
		250	243 ± 22	−2.7
		500	442 ± 32	−11.5
		1,000	1,050 ± 30	4.7
		2,000	1,870 ± 130	−6.7
	September 22, 2009 (carboy)[c]	125	129 ± 8	3.2
		250	271 ± 8	8.5
		500	479 ± 24	−4.2
		1,000	1,030 ± 80	2.9
		2,000	1,920 ± 20	−3.8
August 27, 2009	August 31, 2009	125	114 ± 4	−8.5
		250	244 ± 3	−2.4
		500	467 ± 19	−6.5
		1,000	964 ± 4	−3.6
		2,000	1,890 ± 40	−5.7

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)[a]	Difference from Target (%)
	September 30, 2009 (bottle)[b]	125	114 ± 12	−8.5
		250	240 ± 7	−4.0
		500	486 ± 4	−2.9
		1,000	1,010 ± 10	1.3
		2,000	2,050 ± 20	2.3
	September 30, 2009 (carboy)[c]	125	126 ± 3	1.1
		250	263 ± 1	5.1
		500	522 ± 10	4.3
		1,000	1,020 ± 50	2.2
		2,000	1,780 ± 240	−10.8

NA = not applicable.
[a]Average of triplicate analysis.
[b]Animal room sample from the formulation remaining in the drinking water bottle.
[c]Animal room sample from the formulation collected from the carboy.
[d]Duplicate sample analyzed with a precision of duplicates value of 0.96.
[e]Duplicate sample analyzed with a precision of duplicates value of 1.0.

Table A-4. Results of Analyses of Dose Formulations Administered to Mice in the Three-month Study of Sodium Tungstate Dihydrate

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)[a]	Difference from Target (%)
May 11, 2009	May 12, 2009	125	118 ± 9	−5.6
		250	257 ± 2	2.7
		500	511 ± 12	2.2
		1,000	1,070 ± 20	6.7
		2,000	2,200 ± 10	10.0
	June 25, 2009 (bottle)[b]	125	123 ± 2	−1.9
		250	263 ± 3	5.1
		500	508 ± 3	1.7
		1,000	1,050 ± 10	4.7
		2,000	2,140 ± 30	7.0
	June 25, 2009 (carboy)[c]	125	128 ± 9	2.7
		250	269 ± 5	7.7
		500	530 ± 5	6.0
		1,000	1,090 ± 20	9.3
		2,000	2,190 ± 50	9.3
June 5, 2009	June 16, 2009	125	123 ± 3	−1.6
		250	243 ± 4	−2.7
		500	494 ± 4	−1.2

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)[a]	Difference from Target (%)
		1,000	1,000 ± NA[d]	0.0
		2,000	1,940 ± 40	−3.2
	July 24, 2009 (bottle)[b]	125	127 ± 4	1.9
		250	257 ± 1	2.9
		500	513 ± 18	2.6
		1,000	1,060 ± 0	6.0
		2,000	2,100 ± 10	5.0
	July 24, 2009 (carboy)[c]	125	128 ± 4	2.1
		250	265 ± 7	5.9
		500	537 ± 12	7.3
		1,000	1,100 ± 20	9.7
		2,000	2,110 ± 30	5.5
July 31, 2009	August 3, 2009	125	118 ± 7	−5.6
		250	254 ± 4	1.6
		500	510 ± 13	2.0
		1,000	1,010 ± 80	1.3
		2,000	1,950 ± 170	−2.5
	September 1, 2009 (bottles, males)[b]	125	115 ± 6	−8.0
		250	239 ± 5	−4.3
		500	486 ± 8	−2.7
		1,000	981 ± 17	−1.9
		2,000	1,850 ± 80	−7.7
	September 1, 2009 (bottles, females)[b]	125	109 ± 7	−12.8
		250	220 ± 5	−12.0
		500	456 ± 17	−8.8
		1,000	901 ± 6	−9.9
		2,000	1,770 ± 30	−11.3
	September 1, 2009 (carboy)[c]	125	112 ± 3	−10.1
		250	241 ± 2	−3.7
		500	494 ± 5	−1.3
		1,000	1,000 ± 10	0.4
		2,000	1,920 ± 60	−4.0

[a]Average of triplicate analysis.
[b]Animal room sample from the formulation remaining in the drinking water bottle.
[c]Animal room sample from the formulation collected from the carboy.
[d]Duplicate sample analyzed with a precision of duplicates value of 0.96.

Table A-5. Results of Analyses of Dose Formulations Administered to Rats in the Perinatal and Two-year Study of Sodium Tungstate Dihydrate

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)[a]	Difference from Target (%)
December 12, 2011	December 15, 2011	250	246 ± 6 / 247 ± 2	−1.7 / −1.1
		500	495 ± 3 / 483 ± 13	−0.9 / −3.3
		1,000	963 ± 10 / 945 ± 17	−3.7 / −5.5
	January 19, 2012[b] (bottle)	250	236 ± 6	−5.7
		500	486 ± 2	−2.8
		1,000	998 ± 13	−0.2
	January 19, 2012[c] (carboy)	250	239 ± 7	−4.3
		500	485 ± 3	−3.1
		1,000	967 ± 6	−3.3
January 5, 2012	January 6, 2012	250	238 ± 10 / 243 ± 5	−4.8 / −2.7
		500	473 ± 28 / 471 ± 24	−5.3 / −5.7
		1,000	918 ± 57 / 960 ± 32	−8.2 / −4.0
March 29, 2012	March 29, 2012	250	245 ± 1 / 238 ± 3	−2.0 / −4.7
		500	480 ± 3 / 479 ± 1	−4.1 / −4.1
		1,000	957 ± 3 / 962 ± 3	−4.3 / −3.8
May 22, 2012	May 22, 2012	250	242 ± 4 / 242 ± 1	−3.1 / −3.2
		500	490 ± 9 / 477 ± 7	−2.0 / −4.6
		1,000	958 ± 3 / 957 ± 16	−4.2 / −4.3
August 15, 2012	August 20, 2012	250	236 ± 11 / 249 ± 1	−5.6 / −0.4
		500	508 ± 0 / 504 ± 8	1.6 / 0.9
		1,000	997 ± 13 / 1,000 ± 10	−0.3 / 0.0
	September 25, 2012[b] (bottle)	250	233 ± 7	−6.7
		500	480 ± 8	−4.1
		1,000	1,000 ± 20	0.0
	September 25, 2012[c] (carboy)	250	240 ± 8	−4.1
		500	498 ± 0	−0.4
		1,000	1,010 ± 10	0.7
October 11, 2012	October 14, 2012	250	237 ± 15 / 256 ± 6	−5.1 / 2.4
		500	490 ± 5 / 499 ± 4	−1.9 / −0.3
		1,000	998 ± 3 / 1,050 ± 20	−0.2 / 5.3
December 4, 2012	December 6, 2012	250	240 ± 10 / 253 ± 3	−4.0 / 1.1
		500	515 ± 8 / 538 ± 6	3.1 / 7.5
		1,000	1,050 ± 20 / 1,060 ± 10	5.0 / 5.7

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)[a]	Difference from Target (%)
February 26, 2013	February 27, 2013	250	231 ± 8 / 238 ± 3	−7.6 / −4.9
		500	478 ± 3 / 476 ± 7	−4.5 / 4.8
		1,000	970 ± 22 / 950 ± 17	−3.0 / −5.0
	April 9, 2013[b] (bottle)	250	233 ± 1	−6.7
		500	482 ± 3	−3.7
		1,000	953 ± 18	−4.7
	April 9, 2013[c] (carboy)	250	241 ± 1	−3.6
		500	468 ± 3	−6.5
		1,000	945 ± 18	−5.5
April 25, 2013	April 26, 2013	250	240 ± 5 / 250 ± 7	−4.1 / −0.1
		500	527 ± 23 / 494 ± 17	5.4 / −1.3
		1,000	995 ± 9 / 988 ± 6	−0.5 / −1.2
July 16, 2013	July 18. 2013	250	258 ± 10 / 240 ± 14	3.1 / −4.0
		500	479 ± 17 / 509 ± 21	−4.1 / 1.7
		1,000	955 ± 71 / 970 ± 10	−4.5 / −3.0
September 10, 2013	September 14, 2013	250	228 ± 5 / 227 ± 6	−8.9 / −9.1
		500	454 ± 8 / 501 ± 23	−9.1 / 0.3
		1,000	1,090 ± 20 / 1,080 ± 0	8.7 / 8.0
	October 23, 2013[b] (bottle)	250	228 ± 12	−8.8
		500	491 ± 5	−1.8
		1,000	1,070 ± 10	6.7
	October 23, 2013[c] (carboy)	250	246 ± 4	−1.7
		500	485 ± 16	−2.9
		1,000	1,060 ± 30	6.0
December 4, 2013	December 6, 2013	250	232 ± 9 / 256 ± 12	−7.3 / 2.5
		500	495 ± 8 / 480 ± 21	−1.1 / −4.1
		1,000	973 ± 13 / 900 ± 69	−2.7 / −10.0

Values on either side of the / represent multiple formulations of the same dose prepared on that date.
[a]Average of triplicate analysis.
[b]Animal room sample from the formulation remaining in the drinking water bottle.
[c]Animal room sample from the formulation collected from the carboy.

Table A-6. Results of Analyses of Dose Formulations Administered to Mice in the Two-year Study of Sodium Tungstate Dihydrate

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)[a]	Difference from Target (%)
January 5, 2012	January 6, 2012	500	473 ± 28 / 471 ± 24	−5.3 / −5.7
		1,000	918 ± 57 / 960 ± 32	−8.2 / −4.0
		2,000	1,840 ± 60	−8.2
	February 16, 2012[b] (bottle)	500	476 ± 26	−4.7
		1,000	938 ± 57	−6.2
		2,000	2,020 ± 30	1.0
	February 16, 2012[c] (carboy)	500	456 ± 30	−8.8
		1,000	987 ± 32	−1.3
		2,000	2,030 ± 90	1.7
March 29, 2012	March 29, 2012	500	480 ± 3 / 479 ± 1	−4.1 / −4.1
		1,000	957 ± 3 / 962 ± 3	−4.3 / −3.8
		2,000	1,830 ± 40	−8.7
May 22, 2012	May 22, 2012	500	490 ± 9 / 477 ± 7	−2.0 / −4.6
		1,000	958 ± 3 / 957 ± 16	−4.2 / −4.3
		2,000	1,940 ± 10	−3.0
August 15, 2012	August 20, 2012	500	508 ± 0 / 504 ± 8	1.6 / 0.9
		1,000	997 ± 13 / 1,000 ± 10	−0.3 / 0.0
		2,000	1,980 ± 10	−0.8
	September 25, 2012[b] (bottle)	500	494 ± 22	−1.3
		1,000	1,020 ± 10	2.0
		2,000	2,070 ± 20	3.7
	September 25, 2012[c] (carboy)	500	529 ± 4	5.9
		1,000	NA[d]	NA[d]
		2,000	2,090 ± 30	4.7
October 11, 2012	October 14, 2012	500	490 ± 5 / 499 ± 4	−1.9 / −0.3
		1,000	998 ± 3 / 1,050 ± 20	−0.2 / 5.3
		2,000	2,110 ± 30	5.7
December 4, 2012	December 6, 2012	500	515 ± 8 / 538 ± 6	3.1 / 7.5
		1,000	1,050 ± 20 / 1,060 ± 10	5.0 / 5.7
		2,000	2,060 ± 30	3.2

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)[a]	Difference from Target (%)
February 26, 2013	February 27, 2013	500	478 ± 3 / 476 ± 7	−4.5 / −4.8
		1,000	970 ± 22 / 950 ± 17	−3.0 / −5.0
		2,000	1,890 ± 10	−5.3
	April 9, 2013[b] (bottle)	500	453 ± 16	−9.5
		1,000	915 ± 13	−8.5
		2,000	1,900 ± 20	−5.0
	April 9, 2013[c] (carboy)	500	468 ± 3	−6.5
		1,000	937 ± 10	−6.3
		2,000	1,880 ± 20	−5.8
April 25, 2013	April 26, 2013	500	527 ± 23 / 494 ± 17	5.4 / −1.3
		1,000	995 ± 9 / 988 ± 6	−0.5 / −1.2
		2,000	1,900 ± 10	−5.2
July 16, 2013	July 18, 2013	500	479 ± 17 / 509 ± 21	−4.1 / 1.7
		1,000	955 ± 71 / 970 ± 10	−4.5 / −3.0
		2,000	2,000 ± 70	0.2
September 10, 2013	September 14, 2013	500	454 ± 8 / 501 ± 23	−9.1 / 0.3
		1,000	1,090 ± 20 / 1,080 ± 0	8.7 / 8.0
		2,000	2,150 ± 100	7.5
	October 23, 2013[b] (bottle)	500	503 ± 7	0.5
		1,000	1,040 ± 10	3.7
		2,000	2,090 ± 10	4.5
	October 23, 2013[c] (carboy)	500	485 ± 16	−2.9
		1,000	1,060 ± 30	6.0
		2,000	1,980 ± 30	−0.8
December 4, 2013	December 6, 2013	500	495 ± 8 / 480 ± 21	−1.1 / −4.1
		1,000	973 ± 13 / 900 ± 69	−2.7 / −10.0
		2,000	1,910 ± 30	−4.5

Values on either side of the / represent multiple formulations of the same dose prepared on that date.
NA = not analyzed.
[a]Average of triplicate analysis.
[b]Animal room sample from the formulation remaining in the drinking water bottle.
[c]Animal room sample from the formulation collected from the carboy.
[d]Sample not collected from carboy.

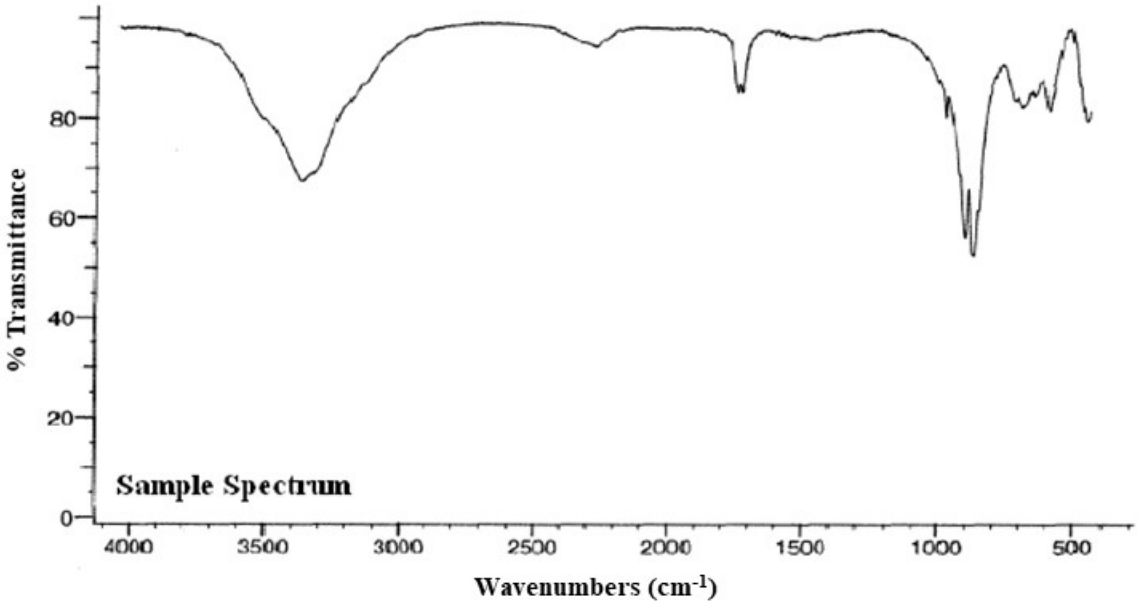

Figure A-1. Infrared Absorption Spectrum of Sodium Tungstate Dihydrate

Appendix B. Ingredients, Nutrient Composition, and Contaminant Levels in NIH-07 Rat Ration and NTP-2000 Rat and Mouse Ration

Tables

Table B-1. Ingredients of NIH-07 Rat Ration B-2
Table B-2. Vitamins and Minerals in NIH-07 Rat Ration B-2
Table B-3. Nutrient Composition of NIH-07 Rat Ration B-3
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B.1. NIH-07 Feed

Table B-1. Ingredients of NIH-07 Rat Ration

Ingredients	Percent by Weight
Ground Hard Winter Wheat	23.00
Ground #2 Yellow Shelled Corn	24.25
Wheat Middlings	10.0
Alfalfa Meal (Dehydrated, 17% Protein)	4.0
Soybean Meal (47% Protein)	12.0
Fish Meal (62% Protein)	10.0
Soy Oil (Without Preservatives)	2.5
Dried Brewer's Yeast	2.0
Calcium Carbonate (USP)	0.5
Vitamin Premix[a]	0.25
Mineral Premix[b]	0.15
Calcium Phosphate, Dibasic (USP)	1.25
Sodium Chloride	0.5
Choline Chloride (70% Choline)	0.10
Dried Skim Milk	5.00
Dried Molasses	1.50
Corn Gluten Meal (60% Protein)	3.00
Methionine	0.0

USP = United States Pharmacopeia.
[a]Wheat middlings as carrier.
[b]Calcium carbonate as carrier.

Table B-2. Vitamins and Minerals in NIH-07 Rat Ration

	Amount[a]	Source
Vitamins		
Vitamin A	6,062 IU	Stabilized vitamin A palmitate or acetate
Vitamin D	5,070 IU	D-activated animal sterol
Vitamin K	3.1 mg	Menadione sodium bisulfite complex
Vitamin E	22 IU	α-Tocopheryl acetate
Niacin	33 mg	–
Folic Acid	2.4 mg	–
d-Pantothenic Acid	19.8 mg	d-Calcium pantothenate
Riboflavin	3.8 mg	–
Thiamine	11 mg	Thiamine mononitrate

	Amount[a]	Source
B_{12}	50 µg	–
Pyridoxine	6.5 mg	Pyridoxine hydrochloride
Biotin	0.15 mg	d-Biotin
Minerals		
Iron	132 mg	Iron sulfate
Zinc	18 mg	Zinc oxide
Manganese	66 mg	Manganese oxide
Copper	4.4 mg	Copper sulfate
Iodine	2.0 mg	Calcium iodate
Cobalt	0.44 mg	Cobalt carbonate

[a]Per kg of finished product.

Table B-3. Nutrient Composition of NIH-07 Rat Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	24.65 ± 1.344	23.7–25.6	2
Crude Fat (% by Weight)	5.3 ± 0.141	5.2–5.4	2
Crude Fiber (% by Weight)	3.57 ± 0.184	3.44–3.70	2
Ash (% by Weight)	6.565 ± 0.049	6.53–6.60	2
Amino Acids (% of Total Diet)			
Arginine	1.380 ± 0.06	1.3–1.49	10
Cystine	0.322 ± 0.031	0.274–0.372	10
Glycine	1.150 ± 0.070	1.06–1.31	10
Histidine	0.518 ± 0.024	0.497–0.553	10
Isoleucine	0.984 ± 0.024	0.952–1.03	10
Leucine	2.018 ± 0.067	1.93–2.13	10
Lysine	1.243 ± 0.051	1.13–1.32	10
Methionine	0.488 ± 0.016	0.468–0.515	10
Phenylalanine	1.097 ± 0.022	1.07–1.12	10
Threonine	0.918 ± 0.031	0.883–0.961	10
Tryptophan	0.277 ± 0.020	0.265–0.326	10
Tyrosine	0.860 ± 0.037	0.785–0.894	10
Valine	1.134 ± 0.025	1.11–1.17	10
Essential Fatty Acids (% of Total Diet)			
Linoleic	2.30 ± 0.219	1.99–2.59	10
Linolenic	0.25 ± 0.275	0.217–0.296	10

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Vitamins			
Vitamin A (IU/kg)	4,545 ± 67.2	4,070–5,020	2
α-Tocopherol (ppm)	6,704 ± 21,045	40.3–66,600	10
Thiamine (ppm)[a]	14.95 ± 0.778	14.4–15.5	2
Riboflavin (ppm)	14.47 ± 3.352	10.0–19.8	10
Niacin (ppm)	99.33 ± 8.235	87.0–112.0	10
Pantothenic Acid (ppm)	44.38 ± 3.806	38.2–51.1	10
Pyridoxine (ppm)[a]	12.876 ± 3.171	9.63–19.7	10
Folic Acid (ppm)	2.482 ± 0.487	1.68–3.09	10
Biotin (ppm)	0.3283 ± 0.172	0.0–0.638	10
B_{12} (ppb)	49.4 ± 6.83	41.8–61.6	10
Choline (as chloride) (ppm)	1,821.0 ± 197.5	1,570–2,200	10
Minerals			
Calcium (%)	1.205 ± 0.078	1.15–1.26	2
Phosphorus (%)	0.938 ± 0.036	0.912–0.963	2
Potassium (%)	0.830 ± 0.036	0.769–0.88	10
Chloride (%)	0.652 ± 0.106	0.441–0.8	10
Sodium (%)	0.378 ± 0.46	0.318–0.469	10
Magnesium (%)	0.187 ± 0.014	0.17–0.218	10
Iron (ppm)	385.1 ± 54.9	276.0–469.0	10
Manganese (ppm)	90.81 ± 7.566	80.7–104.0	10
Zinc (ppm)	64.15 ± 10.07	52.4–89.2	10
Copper (ppm)	14.13 ± 2.57	11.9–21.1	10
Iodine (ppm)	1.811 ± 0.992	0.54–3.45	10
Chromium (ppm)	3.946 ± 0.036	3.89–4.0	8
Cobalt (ppm)	0.5155 ± 0.267	0.01–0.963	10

[a]As hydrochloride.

Table B-4. Contaminant Levels in NIH-07 Rat Ration

	Mean ± Standard Deviation	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.2965 ± 0.029	0.26–0.317	2
Cadmium (ppm)	0.082 ± 0.004	0.079–0.085	2
Lead (ppm)	0.0785 ± 0.001	0.078–0.079	2
Mercury (ppm)[a]	0.0135 ± 0.002	0.012–0.015	2

	Mean ± Standard Deviation	Range	Number of Samples
Selenium (ppm)	0.4065 ± 0.110	0.329–0.484	2
Aflatoxins (ppb)[a]	5	–	2
Nitrate Nitrogen (ppm)[b]	19.55 ± 1.344	18.6–20.5	2
Nitrite Nitrogen (ppm)[a,b]	<0.61	–	2
BHA (ppm)[a,c]	<1.0	–	2
BHT (ppm)[a,c]	<1.0	–	2
Aerobic Plate Count (CFU/gm)[a]	<10	–	2
Coliform (MPN/gm)[a]	<3	–	2
Escherichia coli (MPN/gm)[a]	<10	–	2
Salmonella (MPN/gm)	0	–	2
Total Nitrosamines (ppb)[d]	6.6 ± 1.70	5.4–7.8	2
N-Ndimethylamine (ppb)[d]	0	–	2
N-Npyrrolidine (ppb)[d]	6.6 ± 1.70	5.4–7.8	2
Pesticides (ppm)			
α-BHC[a]	<0.01	–	2
β-BHC[a]	<0.02	–	2
γ-BHC[a]	<0.01	–	2
δ-BHC[a]	<0.01	–	2
Heptachlor[a]	<0.01	–	2
Aldrin[a]	<0.01	–	2
Heptachlor Epoxide[a]	<0.01	–	2
DDE[a]	<0.01	–	2
DDD[a]	<0.01	–	2
DDT[a]	<0.01	–	2
HCB[a]	<0.01	–	2
Mirex[a]	<0.01	–	2
Methoxychlor[a]	<0.05	–	2
Dieldrin[a]	<0.01	–	2
Endrin[a]	<0.01	–	2
Telodrin[a]	<0.01	–	2
Chlordane[a]	<0.05	–	2
Toxaphene[a]	<0.10	–	2
Estimated PCBs[a]	<0.20	–	2
Ronnel[a]	<0.01	–	2
Ethion[a]	<0.02	–	2

	Mean ± Standard Deviation	Range	Number of Samples
Trithion[a]	<0.05	–	2
Diazinon[a]	<0.10	–	2
Methyl Chlorpyrifos	0.0391 ± 0.027	0.0200–0.0582	2
Methyl Parathion[a]	<0.02	–	2
Ethyl Parathion[a]	<0.02	–	2
Malathion[a]	<0.02	–	2
Endosulfan I[a]	<0.01	–	2
Endosulfan II[a]	<0.01	–	2
Endosulfane Sulfate[a]	<0.03	–	2

All samples were irradiated.
BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane; HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.
[a]All values were below the detection limit. The detection limit is given as the mean.
[b]Sources of contamination include alfalfa, grains, and fish meal.
[c]Sources of contamination include soy oil and fish meal.
[d]All values were corrected for percent recovery.

B.2. NTP-2000 Feed

Table B-5. Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground Hard Winter Wheat	22.26
Ground #2 Yellow Shelled Corn	22.18
Wheat Middlings	15.0
Oat Hulls	8.5
Alfalfa Meal (Dehydrated, 17% Protein)	7.5
Purified Cellulose	5.5
Soybean Meal (49% Protein)	5.0
Fish Meal (60% Protein)	4.0
Corn Oil (without Preservatives)	3.0
Soy Oil (without Preservatives)	3.0
Dried Brewer's Yeast	1.0
Calcium Carbonate (USP)	0.9
Vitamin Premix[a]	0.5
Mineral Premix[b]	0.5
Calcium Phosphate, Dibasic (USP)	0.4
Sodium Chloride	0.3

Ingredients	Percent by Weight
Choline Chloride (70% Choline)	0.26
Methionine	0.2

USP = United States Pharmacopeia.
[a]Wheat middlings as carrier.
[b]Calcium carbonate as carrier.

Table B-6. Vitamins and Minerals in NTP-2000 Rat and Mouse Ration

	Amount[a]	Source
Vitamins		
Vitamin A	4,000 IU	Stabilized vitamin A palmitate or acetate
Vitamin D	1,000 IU	D-activated animal sterol
Vitamin K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl Acetate	100 IU	–
Niacin	23 mg	–
Folic Acid	1.1 mg	–
d-Pantothenic Acid	10 mg	d-Calcium pantothenate
Riboflavin	3.3 mg	–
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	–
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	d-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a]Per kg of finished product.

Table B-7. Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	14.61 ± 0.537	13.9–16.5	32
Crude Fat (% by Weight)	8.39 ± 0.376	7.7–9.2	32
Crude Fiber (% by Weight)	9.26 ± 0.603	7.1–10.1	32
Ash (% by Weight)	4.93 ± 0.138	4.66–5.2	32

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Amino Acids (% of Total Diet)			
Arginine	0.805 ± 0.075	0.67–0.97	29
Cystine	0.220 ± 0.021	0.15–0.25	29
Glycine	0.702 ± 0.038	0.62–0.8	29
Histidine	0.342 ± 0.070	0.27–0.68	29
Isoleucine	0.549 ± 0.040	0.43–0.66	29
Leucine	1.100 ± 0.063	0.96–1.24	29
Lysine	0.700 ± 0.104	0.31–0.86	29
Methionine	0.409 ± 0.042	0.26–0.49	29
Phenylalanine	0.623 ± 0.047	0.471–0.72	29
Threonine	0.513 ± 0.041	0.43–0.61	29
Tryptophan	0.155 ± 0.027	0.11–0.2	29
Tyrosine	0.422 ± 0.066	0.28–0.54	29
Valine	0.666 ± 0.040	0.55–0.73	29
Essential Fatty Acids (% of Total Diet)			
Linoleic	3.94 ± 0.235	3.49–4.55	29
Linolenic	0.30 ± 0.064	0.005–0.368	29
Vitamins			
Vitamin A (IU/kg)	3,757 ± 70.50	2,520–5,450	32
Vitamin D (IU/kg)[a]	1,000	–	–
α-Tocopherol (ppm)	2,456 ± 128.17	13.6–69,100	29
Thiamine (ppm)[b]	7.5 ± 0.614	6.1–9.0	32
Riboflavin (ppm)	8.17 ± 2.841	42–17.5	29
Niacin (ppm)	78.66 ± 8.11	66.4–98.2	29
Pantothenic Acid (ppm)	26.42 ± 11.05	17.4–81.0	29
Pyridoxine (ppm)[b]	9.75 ± 2.045	6.44–14.3	29
Folic Acid (ppm)	1.58 ± 0.43	1.15–3.27	29
Biotin (ppm)	0.323 ± 0.093	0.2–0.704	29
B_{12} (ppb)	50.41 ± 34.89	18.3–174	29
Choline (as Chloride) (ppm)	2,593 ± 633.8	1,160–3,790	29
Minerals			
Calcium (%)	0.902 ± 0.056	0.697–1.02	32
Phosphorus (%)	0.551 ± 0.023	0.504–0.615	32
Potassium (%)	0.668 ± 0.029	0.626–0.733	29
Chloride (%)	0.392 ± 0.044	0.3–0.517	29

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Sodium (%)	0.195 ± 0.027	0.16–0.283	29
Magnesium (%)	0.217 ± 0.054	0.185–0.49	29
Sulfur (%)	0.170 ± 0.029	0.116–0.209	14
Iron (ppm)	191.6 ± 36.18	135–311	29
Manganese (ppm)	50.11 ± 9.42	21–73.1	29
Zinc (ppm)	57.3 ± 25.54	43.3–184	29
Copper (ppm)	7.57 ± 2.49	3.21–16.3	29
Iodine (ppm)	0.513 ± 0.221	0–0.972	29
Chromium (ppm)	1.02 ± 1.04	0.33–3.97	28
Cobalt (ppm)	0.222 ± 0.152	0.0857–0.864	27

[a]From formulation.
[b]As hydrochloride.

Table B-8. Contaminant Levels in NTP-2000 Rat and Mouse Ration

	Mean ± Standard Deviation	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.199 ± 0.045	0.143–0.307	32
Cadmium (ppm)	0.065 ± 0.080	0.015–0.5	32
Lead (ppm)	0.16 ± 0.202	0.059–1.19	32
Mercury (ppm)[a]	0.012 ± 0.004	0.01–0.026	32
Selenium (ppm)	0.169 ± 0.039	0.029–0.266	32
Aflatoxins (ppb)[a]	<5.0	–	32
Nitrate Nitrogen (ppm)[b]	18.88 ± 9.5	10.0–45.9	32
Nitrite Nitrogen (ppm)[a,b]	0.61	–	32
BHA (ppm)[a,c]	<1.00	–	32
BHT (ppm)[a,c]	1.03 ± 0.156	1.0–1.88	32
Aerobic Plate Count (CFU/gm)	14.84 ± 18.56	10.0–110.0	32
Coliform (MPN/gm)	3.0	–	32
Escherichia coli (MPN/gm)[a]	<10.0	–	32
Salmonella (MPN/gm)	Negative	–	32
Total Nitrosamines (ppb)[d]	9.8 ± 5.12	0–19.9	32
N-Ndimethylamine (ppb)[d]	2.3 ± 2.6	0–11.1	32
N-Npyrrolidine (ppb)[d]	7.8 ± 4.24	0–18.6	32
Pesticides (ppm)			
α-BHC[a]	<0.01	–	32
β-BHC[a]	<0.02	–	32

	Mean ± Standard Deviation	Range	Number of Samples
γ-BHC[a]	<0.01	–	32
δ-BHC[a]	<0.01	–	32
Heptachlor[a]	<0.01	–	32
Aldrin[a]	<0.01	–	32
Heptachlor Epoxide[a]	<0.01	–	32
DDE[a]	<0.01	–	32
DDD[a]	<0.01	–	32
DDT[a]	<0.01	–	32
HCB[a]	<0.01	–	32
Mirex[a]	<0.01	–	32
Methoxychlor[a]	<0.05	–	32
Dieldrin[a]	<0.01	–	32
Endrin[a]	<0.01	–	32
Telodrin[a]	<0.01	–	32
Chlordane[a]	<0.05	–	32
Toxaphene[a]	<0.10	–	32
Estimated PCBs[a]	<0.20	–	32
Ronnel[a]	<0.01	–	32
Ethion[a]	<0.02	–	32
Trithion[a]	<0.05	–	32
Diazinon[a]	<0.10	–	32
Methyl Chlorpyrifos	0.112 ± 0.141	0.02–0.686	32
Methyl Parathion[a]	<0.02	–	32
Ethyl Parathion[a]	<0.02	–	32
Malathion	0.07 ± 0.07	0.02–0.234	32
Endosulfan I[a]	<0.01	–	32
Endosulfan II[a]	<0.01	–	32
Endosulfane Sulfate[a]	<0.03	–	32

All samples were irradiated.
BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane; HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.
[a]All values were below the detection limit. The detection limit is given as the mean.
[b]Sources of contamination include alfalfa, grains, and fish meal.
[c]Sources of contamination include soy oil and fish meal.
[d]All values were corrected for percent recovery.

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C.1. Methods

Rodents used in the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that might affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicological evaluation of test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) or exposed animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals come from the same production source and weanling groups as the animals used for the studies of test compounds.

In these toxicology and carcinogenesis studies, blood samples were collected from each sentinel animal, allowed to clot, and the serum was separated. Additionally, fecal samples were collected and tested for endoparasites and *Helicobacter* species. All samples were processed appropriately, with serology and *Helicobacter* testing sent to IDEXX BioResearch (formerly Rodent Animal Diagnostic Laboratory [RADIL], University of Missouri), Columbia, MO, for determination of the presence of pathogens. Evaluation for endo- and ectoparasites was performed in-house by the testing laboratory.

The laboratory methods and agents for which testing was performed are tabulated in Table C-1 and Table C-2 below; the times at which samples were collected during the studies are also listed.

C.2. Results

Rats: Positive for endoparasites – pinworms (*Syphacia* spp.) for the 2-year study. All other test results were negative.

Mice: All test results were negative.

Table C-1. Methods and Results for Sentinel Animal Testing in Male and Female Rats

Collection Time Points	Three-month Study			Two-year Study							
	Quarantine[a]	3.5 Weeks[b]	End of Study	Quarantine[a]	1 Month	6 Months	12 Months	16 Months[c]	17 Months[c]	18 Months	End of Study
Number Examined (Males/Females)	0/10	0/8	5/5	0/10	5/5	5/5	5/5	1/0	1/0	5/6	5/5
Method/Test											
Multiplex Fluorescent Immunoassay (MFI)											
Kilham rat virus (KRV)	−	−	−	−	−	−	−	−	−	−	−
Mycoplasma pulmonis	−	−	−	−	−	−	−	−	−	−	−
Parvo NS-1	−	−	−	NT	NT	NT	NT	NT	NT	NT	NT
Pneumonia virus of mice (PVM)	−	−	−	−	−	−	−	−	−	−	−
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	−	−	−	−	−	−	−	−	−	−	−
Rat minute virus (RMV)	−	−	−	−	−	−	−	−	−	−	−
Rat parvo virus (RPV)	−	−	−	−	−	−	−	−	−	−	−
Rat theilovirus (RTV)	−	−	−	−	−	−	−	−	−	−	−
Sendai	−	−	−	−	−	−	−	−	−	−	−
Theiler's murine encephalomyelitis virus (TMEV)	−	−	−	NT	NT	NT	NT	NT	NT	NT	NT
Toolan's H1	−	−	−	−	−	−	−	−	−	−	−
Immunofluorescence Assay (IFA)											
Pneumocystis carinii	NT	NT	NT	NT	NT	−	NT	NT	NT	NT	NT
In-house Evaluation											
Endoparasite evaluation (evaluation of cecal content)	NT	NT	NT	−	−	NT	+	−	+	+[d]	NT
Ectoparasite evaluation (evaluation of perianal surface)	NT	NT	NT	−	−	NT	+	+	+	+[d]	NT

− = negative; + = positive; NT = not tested.
[a]Age-matched nonpregnant females.
[b]Time-mated females that did not have a litter.
[c]Single sentinel rat tested at this time point.
[d]Number Examined (Males/Females) = 8/10

Table C-2. Methods and Results for Sentinel Animal Testing in Male and Female Mice

Collection Time Points	Three-month Study			Two-year Study					
	1 Month	End of Study	Quarantine	1 Month	6 Months	12 Months	15 Months	18 Months	End of Study
Number Examined (Males/Females)	5/5	5/5	5/5	5/5	5/5	5/5	1/0	5/5	5/5
Method/Test									
Multiplex Fluorescent Immunoassay (MFI)									
Ectromelia virus	–	–	–	–	–	–	NT	–	–
Epizootic diarrhea of infant mice	–	–	–	–	–	–	NT	–	–
Lymphocytic choriomeningitis virus (LCMV)	–	–	–	–	–	–	NT	–	–
Mycoplasma pulmonis	–	–	–	–	–	–	NT	–	–
Mouse hepatitis virus (MHV)	–	–	–	–	–	–	NT	–	–
Mouse norovirus (MNV)	–	–	–	–	–	–	NT	–	–
Parvo NS-1	–	–	–	–	–	–	NT	–	–
Mouse parvovirus (MPV)	–	–	–	–	–	–	NT	–	–
Minute virus of mice (MVM)	–	–	–	–	–	–	NT	–	–
Pneumonia virus of mice (PVM)	–	–	–	–	–	–	NT	–	–
Reovirus (REO3)	–	–	–	–	–	–	NT	–	–
Sendai	–	–	–	–	–	–	NT	–	–
Theiler's murine encephalomyelitis virus (TMEV) GDVII	–	–	–	–	–	–	NT	–	–
Immunofluorescence Assay (IFA)									
Epizootic diarrhea of infant mice	NT	NT	NT	NT	NT	NT	NT	–	NT
Mouse norovirus (MNV)	NT	NT	NT	NT	NT	NT	NT	–	NT
Polymerase Chain Reaction (PCR)									
Helicobacter species	NT	NT	NT	NT	NT	NT	NT	–	NT
In-house Evaluation									
Endoparasite evaluation (evaluation of cecal content)	NT	NT	–	–	–[a]	–[b]	–	–[c]	NT
Ectoparasite evaluation (evaluation of perianal surface)	NT	NT	–	–	–[a]	–[b]	–	–[c]	NT

– = negative; + = positive; NT = not tested.
[a]Number Examined (Males/Females) = 0/1
[b]Number Examined (Males/Females) = 1/0
[c]Number Examined (Males/Females) = 8/9

Appendix D. Genetic Toxicology

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D.1. Bacterial Mutagenicity

D.1.1. Bacterial Mutagenicity Test Protocol

Testing procedures were modified from those originally reported by Zeiger et al. (1992). Coded samples of sodium tungstate dihydrate (ST; the same chemical lot that was used in the 2-year bioassays) were incubated with the *Salmonella typhimurium* (TA98, TA100) or *Escherichia coli* (WP2 *uvrA* (pKM101)) tester strains either in buffer or S9 mix (metabolic activation enzymes and cofactors from phenobarbital/benzoflavone-induced male Sprague Dawley rat liver) for 20 minutes at 37°C. Top agar supplemented with *L*-histidine (or tryptophan for the *E. coli* strain) and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine- or tryptophan-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37°C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of ST. The highest concentration tested was limited by toxicity in strain TA100; the other two strains were tested up to the assay limit dose of 6,000 µg/plate. All trials were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose-related, not reproducible, or not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. No minimum percentage or fold increase is required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background.

D.1.2. Results

ST (12.5 to 6,000 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA98 or TA100, or in *Escherichia coli* WP2 *uvrA* (pKM101), when tested with or without exogenous metabolic activation provided by phenobarbital/benzoflavone-induced rat S9 and cofactors (Table D-1).

Table D-1. Mutagenicity of Sodium Tungstate Dihydrate in Bacterial Tester Strains[a]

Strain	Concentration (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
TA98					
	0	28 ± 2	21 ± 2	46 ± 2	28 ± 1
	12.5	25 ± 1	25 ± 5	33 ± 3	28 ± 3
	50	27 ± 2	30 ± 4	29 ± 2	30 ± 4
	125	23 ± 2	19 ± 3	32 ± 1	31 ± 4
	500	22 ± 6	23 ± 5	34 ± 5	24 ± 1
	1,500	19 ± 2	17 ± 3	34 ± 6	38 ± 4
	6,000	30 ± 2	23 ± 6	33 ± 4	29 ± 6

Strain	Concentration (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
Trial Summary		Negative	Negative	Negative	Negative
Positive Control[b]		518 ± 31	465 ± 25	1,216 ± 77	1,017 ± 26
TA100					
	0	99 ± 3	123 ± 8	113 ± 2	117 ± 6
	12.5	111 ± 8	129 ± 8	112 ± 6	113 ± 5
	50	106 ± 7	100 ± 5	106 ± 5	108 ± 10
	125	118 ± 8	107 ± 9	116 ± 3	111 ± 1
	500	113 ± 3	97 ± 3	120 ± 2	105 ± 4
	1,500	100 ± 5	113 ± 7	117 ± 6	138 ± 10
	6,000	100 ± 6	110 ± 6	111 ± 11	117 ± 12
Trial Summary		Negative	Negative	Negative	Negative
Positive Control		850 ± 9	800 ± 43	2,640 ± 135	1,724 ± 18
***Escherichia coli* WP2 *uvrA* (pKM101)**					
	0	186 ± 8	191 ± 12	194 ± 11	219 ± 7
	12.5	159 ± 3	183 ± 7	219 ± 6	183 ± 11
	50	183 ± 7	161 ± 6	221 ± 21	179 ± 8
	125	166 ± 18	156 ± 6	194 ± 4	181 ± 12
	500	162 ± 6	171 ± 14	200 ± 14	206 ± 15
	1,500	171 ± 8	185 ± 10	213 ± 17	211 ± 16
	6,000	185 ± 12	165 ± 18	193 ± 14	197 ± 16
Trial Summary		Negative	Negative	Negative	Negative
Positive Control		1,105 ± 49	959 ± 21	1,234 ± 70	1,009 ± 36

[a]Studies performed at Integrated Laboratory Systems, LLC. Data are presented as revertants/plate (mean ± standard error) from three plates; 0 µg/plate served as the solvent control.
[b]The positive controls in the absence of metabolic activation were sodium azide (TA100), 2-nitrofluorene (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

D.2. Micronucleus Assay

D.2.1. Peripheral Blood Micronucleus Test Protocol

At termination of the 3-month toxicity studies of ST, blood samples (approximately 200 µL) were collected from male and female rats and mice, placed in ethylenediaminetetraacetic acid (EDTA)-coated tubes, and shipped overnight to the testing laboratory. Upon arrival, blood samples were fixed in ultracold methanol using a MicroFlowPLUS Kit (Litron Laboratories, Rochester, NY) according to the manufacturer's instructions. Fixed samples were stored in a −80°C freezer until analysis. Thawed blood samples were analyzed for frequency of micronucleated immature erythrocytes (polychromatic erythrocytes [PCEs], reticulocytes) and mature erythrocytes (normochromatic erythrocytes, NCEs) using a flow cytometer;[102] both the mature and the immature erythrocyte populations can be analyzed separately by employing

special cell surface markers to differentiate the two cell types. Because the very young reticulocyte subpopulation (CD71-positive cells) can be targeted using this technique, rat blood samples can be analyzed for damage that occurred in the bone marrow within the past 24–48 hours, before the rat spleen appreciably alters the percentage of micronucleated reticulocytes in circulation.[103] In mice, both the immature and mature erythrocyte populations can be evaluated for micronucleus frequency because the mouse spleen does not sequester and eliminate damaged erythrocytes. Damaged erythrocytes achieve steady state in the peripheral blood of mice following 4 weeks of continuous exposure. Approximately 20,000 reticulocytes and 1×10^6 erythrocytes were analyzed per animal for frequency of micronucleated cells, and the percentage of immature erythrocytes (% PCE) was calculated as a measure of bone marrow toxicity resulting from ST exposure.

Prior experience with the large number of cells scored using flow cytometric scoring techniques[104] suggests it is reasonable to assume that the proportion of micronucleated reticulocytes is approximately normally distributed. The statistical tests selected for trend and for pairwise comparisons with the control group depend on whether the variances among the groups are equal. The Levene test at $\alpha = 0.05$ is used to test for equal variances. In the case of equal variances, linear regression is used to test for a linear trend with exposure concentration and the Williams test is used to test for pairwise differences between each exposed group and the control group. In the case of unequal variances, the Jonckheere test is used to test for linear trend and the Dunn test is used for pairwise comparisons of each exposed group with the control group. To correct for multiple pairwise comparisons, the p value for each comparison with the control group is multiplied by the number of comparisons made. In the event that this product is >1.00, it is replaced with 1.00. Trend tests and pairwise comparisons with the controls are considered significant at $p \leq 0.025$.

In the micronucleus test, it is preferable to base a positive result on the presence of both a significant trend as well as at least one significantly elevated exposure group compared with the corresponding control group. In addition, historical control data are used to evaluate the biological significance of any observed response. Both statistical significance and biological significance are considered when arriving at a call. The presence of either a significant trend or a single significant exposure group generally results in an equivocal call. The absence of both a trend and a significant exposure group results in a negative call. Ultimately, the scientific staff determines the final call after considering the results of statistical analyses, reproducibility of any effects observed (in acute studies), and the magnitudes of those effects.

D.2.2. Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple samples of a chemical were tested in the same assay, and different results were obtained among these samples and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly those concerning pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the in vitro assays are conducted with and without exogenous metabolic activation. Results obtained in the absence

of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that is a scientific judgment of the overall evidence for activity of ST in an assay.

D.2.3. Results

At the end of the 3-month studies, peripheral blood samples were obtained from male and female rats and mice and analyzed for the frequency of micronucleated reticulocytes and erythrocytes (Table D-2, Table D-3). In male and female rats, the reticulocyte population (PCEs), which is the only red blood cell population that can be accurately assessed for micronucleus frequency in peripheral blood of rats due to efficient splenic scavenging of damaged erythrocytes, did not show an increase in micronucleated cells following 3 months of exposure to ST via drinking water (0, 125, 250, 500, 1,000, and 2,000 mg/L) (Table D-2). Significant increases in the percent reticulocytes were seen in both male and female rats, suggesting that ST could have stimulated erythropoiesis in the bone marrow; however, the absolute increases in the percentages were small compared to the vehicle control animals.

In male and female mice, there were no significant increases in micronucleated reticulocytes or in micronucleated erythrocytes in either sex following 3 months of exposure to ST via drinking water (0, 125, 250, 500, 1,000, and 2,000 mg/L) (Table D-3). A significant increase in the percent reticulocytes was seen in male mice suggesting that ST could have stimulated erythropoiesis in the bone marrow; however, the absolute increase was small compared to the vehicle control group.

Table D-2. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Rats in the Three-month Drinking Water Study of Sodium Tungstate Dihydrate[a]

	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
Male							
Exposure Concentration (mg/L)							
0	5	0.379 ± 0.04		0.055 ± 0.01		1.048 ± 0.08	
125	5	0.447 ± 0.06	0.483	0.037 ± 0.01	0.934	1.138 ± 0.08	1.000
250	5	0.320 ± 0.10	0.563	0.034 ± 0.01	0.969	0.926 ± 0.04	1.000
500	5	0.450 ± 0.03	0.386	0.051 ± 0.01	0.978	1.036 ± 0.04	1.000
1,000	5	0.450 ± 0.06	0.400	0.033 ± 0.00	0.983	1.182 ± 0.08	0.222
2,000	5	0.390 ± 0.09	0.412	0.025 ± 0.00	0.985	1.318 ± 0.08	0.016
Trend[d]		p = 0.439		p = 0.988		p = 0.003	

	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
Female							
Exposure Concentration (mg/L)							
0	5	0.520 ± 0.03		0.074 ± 0.02		0.891 ± 0.11	
125	5	0.560 ± 0.14	0.649	0.038 ± 0.00	0.861	0.799 ± 0.08	1.000
250	5	0.488 ± 0.05	0.735	0.064 ± 0.02	0.921	1.050 ± 0.10	0.278
500	5	0.520 ± 0.04	0.770	0.056 ± 0.01	0.939	1.085 ± 0.14	0.275
1,000	5	0.506 ± 0.09	0.787	0.050 ± 0.01	0.948	1.240 ± 0.07	0.042
2,000	5	0.280 ± 0.04	0.800	0.045 ± 0.01	0.954	1.191 ± 0.09	0.043
Trend		p = 0.995		p = 0.853		p = 0.012	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data presented as mean ± standard error.
[c]Pairwise comparisons with the vehicle control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Exposure concentration-related trends evaluated by linear regression or the Jonckheere test ($p \leq 0.025$).

Table D-3. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Mice in the Three-month Drinking Water Study of Sodium Tungstate Dihydrate[a]

	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
Male							
Exposure Concentration (mg/L)							
0	5	2.530 ± 0.22		1.433 ± 0.05		1.558 ± 0.04	
125	5	2.690 ± 0.11	0.299	1.467 ± 0.04	1.000	1.444 ± 0.06	1.000
250	5	2.830 ± 0.19	0.357	1.491 ± 0.04	1.000	1.552 ± 0.08	1.000
500	5	2.840 ± 0.30	0.381	1.559 ± 0.04	0.181	1.608 ± 0.04	0.939
1,000	5	2.380 ± 0.23	0.395	1.504 ± 0.07	1.000	1.547 ± 0.04	0.955
2,000	5	2.700 ± 0.12	0.383	1.470 ± 0.01	1.000	1.745 ± 0.03	0.021
Trend[d]		p = 0.580		p = 0.240		p = 0.002	
Female							
Exposure Concentration (mg/L)							
0	5	1.810 ± 0.18		0.909 ± 0.01		1.302 ± 0.13	
125	5	2.103 ± 0.15	0.339	0.952 ± 0.04	0.293	1.938 ± 0.20	0.311
250	5	1.940 ± 0.09	0.875	0.992 ± 0.02	0.350	1.568 ± 0.20	0.373
500	5	1.730 ± 0.13	1.000	0.890 ± 0.01	0.373	1.613 ± 0.22	0.400
1,000	5	1.960 ± 0.27	1.000	0.934 ± 0.04	0.387	1.187 ± 0.08	0.411

	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
2,000	5	1.930 ± 0.08	0.922	0.874 ± 0.02	0.398	1.509 ± 0.13	0.418
Trend		p = 0.522		p = 0.968		p = 0.567	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data presented as mean ± standard error.
[c]Pairwise comparisons with the vehicle control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Exposure concentration-related trends evaluated by linear regression or the Jonckheere test ($p \leq 0.025$).

D.3. Comet Assay

D.3.1. Comet Assay Protocol

For preparation of samples for the comet assay, a 50 µL sample of blood was transferred to a tube containing 1 mL of freshly prepared cold mincing buffer [Mg^{+2}, Ca^{+2}, and phenol-free Hank's Balanced Salt Solution (Life Technologies, Carlsbad, CA) with 20 mM EDTA, pH 7.3 to 7.5, and 10% v/v fresh dimethyl sulfoxide (DMSO)]. The ileum, liver, and kidney were rinsed with cold mincing buffer to remove residual blood and were held on ice briefly (≤5 minutes) until processed. Small portions (3 to 4 mm) of the ileum, liver, and kidney were placed in tubes containing cold mincing solution and rapidly minced until finely dispersed. All samples prepared for the comet assay were immediately flash frozen in liquid nitrogen[105] and subsequently transferred to a −80°C freezer for storage until shipment by overnight courier on dry ice to the analytical laboratory. Upon receipt, all samples were immediately placed in a −80°C freezer for storage until further processing.

Blood and tissue samples were thawed on ice and maintained on ice during slide preparation. Just before use, each cell suspension was shaken gently to mix the cells and placed back on ice for 15 to 30 seconds to allow clumps to settle. A portion of the supernatant was empirically diluted with 0.5% low melting point agarose (Lonza, Walkersville, MD) dissolved in Dulbecco's phosphate buffer (Ca^{+2}, Mg^{+2}, and phenol-free) at 37°C and layered onto each well of a 2-well CometSlide™ (Trevigen, Gaithersburg, MD). Slides were immersed in cold lysing solution [2.5 M NaCl, 100 mM Na_2EDTA, 10 mM tris(hydroxymethyl)aminomethane (Tris), pH 10, containing freshly added 10% DMSO (Fisher Scientific, Pittsburgh, PA), and 1% Triton X-100] overnight in a refrigerator, protected from light. The following day, the slides were rinsed in 0.4 M Trizma base (pH 7.5), randomly placed onto the platform of a horizontal electrophoresis unit, and treated with cold alkali solution (300 mM NaOH, 1 mM Na_2EDTA, pH > 13) for 20 minutes to allow DNA unwinding, then electrophoresed at 4°C to 9°C for 20 minutes at 25 V (0.7 V/cm), with a current of approximately 300 mA. After electrophoresis, slides were neutralized with 0.4 M Trizma base (pH 7.5) for 5 minutes and then dehydrated by immersion in absolute ethanol (Pharmco-AAPER, Shelbyville, KY) for at least 5 minutes and allowed to air dry. Slides were prepared in a laboratory with a relative humidity no more than 60% and stored at room temperature in a desiccator with a relative humidity of no more than 60% until stained and scored; stained slides were stored in a desiccator. NaCl, Na_2EDTA, Triton X-100, and Trizma base were purchased from Sigma-Aldrich (St. Louis, MO); NaOH was purchased from Fisher Scientific (Pittsburgh, PA).

After staining with SYBR® Gold (Molecular Probes, Life Technologies, Grand Island, NY), the slides, independently coded to mask treatment, were scored using Comet Assay IV Imaging Software, Version 4.3.1 (Perceptive Instruments, Ltd., Suffolk, UK), validated for Good Laboratory Practice Part 11 compliance. In the alkaline (pH > 13) comet assay, damaged nuclear DNA fragments undergo unidirectional migration through the agarose gel within an electrical field, forming an image that resembles a comet, and the greater the amount of fragmentation, the greater the amount of DNA migration that will occur. The image analysis software partitions the intensity of the fluorescent signal of the DNA in the entire comet image into the percent that is attributable to the comet head and the percent attributable to the tail. Manual adjustment of the automated detection of head and tail features is sometimes required. To evaluate DNA damage levels, the extent of DNA migration was characterized for 100 scorable comet figures per animal/tissue as percent tail DNA (intensity of all tail pixels divided by the total intensity of all pixels in the comet, expressed as a percentage).

D.3.2. Results

In addition to evaluating the potential for chromosomal damage, the potential for DNA damage was assessed using the comet assay in the same animals in which micronucleus induction was evaluated. DNA damage from exposure to ST was assessed in liver, ileum, and kidney cell samples, and in blood leukocytes (Table D-4, Table D-5). Significant increases in DNA damage, measured as percent tail DNA, were observed in liver cells from male and female rats. Increases in DNA damage were not observed for peripheral blood leukocytes in male or female rats, or for ileum cells in female rats. Although cells from kidney tissue were evaluated from male and female rats, and ileum tissue was evaluated from male rats, results from these tissues were considered invalid due to unusually high levels of percent tail DNA in the control group. In male mice, significant increases in DNA damage were observed in liver and ileum cells, but not in kidney cells or peripheral blood leukocytes. No increases in percent tail DNA were observed in female mice for liver, kidney, ileum, or peripheral blood leukocyte cells.

Table D-4. DNA Damage in Male and Female Rats Exposed to Sodium Tungstate Dihydrate in Drinking Water for Three Months[a]

	Exposure Concentration (mg/L)	Blood		Ileum		Kidney		Liver	
		Percent Tail DNA[b]	P Value[c]	Percent Tail DNA[b]	P Value	Percent Tail DNA[b]	P Value	Percent Tail DNA[b]	P Value
Male									
	0	1.32 ± 0.22		IT		IT		3.22 ± 0.12	
	125	1.15 ± 0.15	0.615	IT		IT		11.00 ± 1.44	0.078
	250	1.21 ± 0.14	0.702	IT		IT		4.87 ± 0.70	1.000
	500	1.59 ± 0.11	0.313	IT		IT		16.06 ± 1.43	0.003
	1,000	1.49 ± 0.21	0.323	IT		IT		14.74 ± 1.43	0.010
	2,000	1.77 ± 0.39	0.109	IT		IT		20.02 ± 2.08	<0.001
	Trend[d]	p = 0.021		–		–		p < 0.001	
Female									
	0	1.48 ± 0.19		17.38 ± 4.29		IT		9.84 ± 0.61	
	125	1.58 ± 0.17	0.679	24.02 ± 2.57	0.445	IT		13.53 ± 1.63	0.460
	250	1.28 ± 0.36	0.764	16.00 ± 1.73	0.522	IT		17.02 ± 2.57	0.049
	500	1.35 ± 0.13	0.798	15.58 ± 3.15	0.555	IT		14.51 ± 1.07	0.131
	1,000	1.10 ± 0.20	0.814	17.07 ± 2.73[e]	0.588	IT		14.32 ± 1.87	0.196
	2,000	1.22 ± 0.18	0.827	16.95 ± 2.69	0.587	IT		22.47 ± 1.91	<0.001
	Trend	p = 0.892		p = 0.741		–		p < 0.001	

IT = invalid test due to unusually high control percent tail DNA.
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data presented as mean ± standard error; n = 5.
[c]Pairwise comparisons with the vehicle control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Exposure concentration-related trends evaluated by linear regression of the Jonckheere test ($p \leq 0.025$).
[e]n = 4.

Table D-5. DNA Damage in Male and Female Mice Exposed to Sodium Tungstate Dihydrate in Drinking Water for Three Months[a]

Exposure Concentration (mg/L)	Blood		Ileum		Kidney		Liver	
	Percent Tail DNA[b]	P Value[c]	Percent Tail DNA[b]	P Value	Percent Tail DNA[b]	P Value	Percent Tail DNA[b]	P Value
Male								
0	2.49 ± 0.43		11.69 ± 2.34		6.20 ± 0.57		4.14 ± 0.32[e]	
125	3.90 ± 0.48	0.049	20.26 ± 1.91	0.019	5.48 ± 0.73	0.838	17.55 ± 0.50	<0.001
250	3.86 ± 0.54	0.058	13.27 ± 0.68	0.023	5.07 ± 0.70	0.904	17.51 ± 1.25	<0.001
500	3.29 ± 0.37	0.061	22.91 ± 1.17	0.023	4.84 ± 0.34	0.924	17.01 ± 1.03	<0.001
1,000	3.34 ± 0.54	0.062	9.35 ± 1.56	0.023	5.67 ± 0.54	0.867	19.86 ± 1.02	<0.001
2,000	3.61 ± 0.33	0.060	19.96 ± 0.91	<0.001	6.63 ± 0.38	0.402	19.97 ± 1.25	<0.001
Trend[d]	p = 0.199		p = 0.212		p = 0.308		p < 0.001	
Female								
0	0.93 ± 0.19		20.50 ± 1.80		6.81 ± 1.87		6.40 ± 0.52	
125	1.24 ± 0.24	0.830	21.85 ± 1.80	0.340	4.84 ± 0.47	1.000	5.92 ± 0.89	0.573
250	1.03 ± 0.11	1.000	21.07 ± 1.85	0.404	8.54 ± 3.13	1.000	10.63 ± 1.22	0.053
500	1.04 ± 0.23	1.000	24.65 ± 1.01	0.131	5.36 ± 0.59	1.000	7.99 ± 1.14	0.056
1,000	1.28 ± 0.29	1.000	23.91 ± 1.94	0.134	12.16 ± 3.75	0.403	8.90 ± 1.00	0.056
2,000	1.42 ± 0.09	0.167	21.88 ± 1.05	0.137	7.01 ± 1.02	1.000	7.50 ± 0.64	0.056
Trend	p = 0.081		p = 0.112		p = 0.057		p = 0.105	

[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data presented as mean ± standard error; n = 5.
[c]Pairwise comparisons with the vehicle control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Exposure concentration-related trends evaluated by linear regression of the Jonckheere test ($p \leq 0.025$).
[e]n = 4.

Appendix E. Tungsten Concentration Determination

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E.1. Sample Collection

E.1.1. Three-month Studies

E.1.1.1. Urine

At week 12, all F_1 rats were removed from exposure and placed in individual metabolism cages to allow for collection of urine. Rats were fasted during the collection period and had access to untreated water ad libitum. Urine samples were collected on wet ice, overnight, for approximately 16 hours. Urine samples (1 mL from each rat) were frozen at −20°C and shipped to the analytical laboratory (Battelle Toxicology Northwest, Richland, WA).

E.1.1.2. Blood

At study termination, rats and mice were anesthetized with a CO_2/O_2 mixture, and blood was collected from the retroorbital plexus (rats) or sinus (mice) into heparinized centrifuge tubes containing ethylenediaminetetraacetic acid (EDTA). Blood samples were frozen at −80°C and shipped to Battelle Toxicology Northwest (Richland, WA).

E.1.2. Two-year Studies (Interim Evaluations)

At the beginning of the 2-year study, groups of 40 interim study male and female rats and mice were randomly assigned to the tissue distribution study and exposed identically to the core study groups.

At the 3-, 6-, 12-, and 18-month interim evaluations, urine, feces, blood, and tissues (liver, kidneys, stomach, small intestine, and bone) were collected from up to 10 predesignated F_1 rats/sex/exposure group and up to 10 predesignated mice/sex/exposure group. Early death animals were not replaced. On the morning of the day before scheduled blood collection, animals were moved to metabolism cages (one animal/cage); while in the metabolism cages, the animals had ad libitum access to feed and their assigned concentration of dosed drinking water. Urine and feces were collected over a 24-hour period. Blood was collected via cardiac puncture into tubes containing K_3 EDTA, centrifuged, and the plasma harvested. Immediately after blood collection, the animals were euthanized and the entire liver, both kidneys, stomach (separated into glandular and non-glandular), small intestine, and both femurs were collected, weighed, and maintained on dry ice until moved into storage. All samples were stored at −85°C to −60°C until shipped to Battelle Toxicology Northwest (Richland, WA).

E.2. Sample Analysis

E.2.1. Three-month Studies

Tungsten concentrations in samples were quantified using validated analytical methods, and method validation data are given in Table E-1. Blood and urine samples from the study were stored at −70°C and −20°C, respectively, following collection. Samples were allowed to thaw at room temperature and mixed well. A 0.1 mL aliquot of blood was transferred into a Teflon digestion tube (CEM Corporation, Matthews, NC) and 0.15 mL of concentrated HNO_3, 1.5 mL of deionized water, and 0.1 mL of 10 µg/mL bismuth (internal standard) in approximately 1% HNO_3 (using 1 mg/mL procured from SPEX CertiPrep, Metuchen, NJ) was added. Urine samples were prepared similarly to blood except that to 0.1 mL of urine, 0.375 mL of

concentrated HNO_3, 1 mL of deionized water, and 0.25 mL of 0.2 µg/mL bismuth were added. The final acid strength of samples was approximately 1% HNO_3. Samples were capped, allowed to stand for approximately 15 minutes, and digested at 1,600 W and 200°C for 40 minutes for blood or 20 minutes for urine using a MARS5 Microwave Digestion System (CEM Corporation, Mathews, NC). Samples were cooled to room temperature and diluted to 10 mL with water.

Corresponding matrix calibration standards, blanks, and quality control (QC) standards were prepared and analyzed similarly to study samples. Calibration curves were run on blood with six calibration standards (blood, 0.1 to 100 µg tungsten/L; urine, 0.5 to 150 µg tungsten/L). Blood QC standards were prepared at 0.5 and 50 µg tungsten/L, and urine QC standards were prepared at 2 and 100 µg/L. Study samples with responses greater than the highest calibration standard were diluted with 1% HNO_3 such that the final concentration in the sample was within the validated range. All samples were analyzed for tungsten concentration using an inductively coupled plasma-mass spectrometry (ICP-MS) method as described below.

E.2.2. Two-year Studies (Interim Evaluations)

Tungsten concentrations in samples were quantified using validated analytical methods and corresponding validation data are given in Table E-2, Table E-3, and Table E-4 for urine, plasma, and kidney, respectively. All study samples were stored at −70°C following collection. Plasma and urine samples were prepared using a method similar to the 3-month studies. Kidney samples were weighed and homogenized with approximately 5 volumes of water for 5 minutes using a polytron homogenizer. The homogenate was sonicated for approximately 30 minutes and then vortexed to mix. A 0.1-mL aliquot of the homogenate was digested similar to other matrices as before.

Corresponding solvent calibration standards, blanks, and matrix QC standards were prepared and analyzed similarly to study samples. Calibration curves were run with six calibration standards (0.1 to 100 µg tungsten/mL to quantitate plasma; 0.5 to 150 µg tungsten/mL to quantitate urine; 60 to 2,500 ng/mL to quantitate kidney homogenate). QC samples were prepared in all three matrices at 75 and 1,875 µg tungsten/mL. Study samples with responses greater than the highest calibration standard were diluted with 1% HNO_3 such that final concentration in the sample was within the validated range. All samples were analyzed for tungsten concentration using an ICP-MS method as described below.

E.2.3. Instrumentation and Quantitation

Samples from the 3-month studies were analyzed using an Agilent 7500ce (Agilent, Palo Alto, CA) ICP-MS. The detector mode was set to auto with a total acquisition time of 3.3 seconds. The ions monitored were m/z 182 and 209 for tungsten and bismuth, respectively. Samples from the 2-year studies were analyzed using a Perkin Elmer NexION 300 (Waltham, MA) ICP-MS. The detector mode was set to dual and total acquisition time of 4.008 seconds. The ions monitored were m/z 181.948 and 208.980 for tungsten and bismuth, respectively.

The performance of the calibration curve was evaluated before the analysis of each sample set. A successful calibration was indicated by the following: correlation coefficient (r) ≥ 0.98; relative standard deviation (RSD) ≤ ± 15% (except at the limit of quantitation (LOQ) where RSD was ≤ ± 20%); relative error (RE) ≤ ± 15% (except at LOQ where RE was ≤ ± 20%).

Calibration curves relating response ratio of analyte to internal standard (following correction for the background concentrations for the 3-month studies only) and concentration of tungsten in matrix were constructed using a 1/X weighted linear regression. The concentrations of tungsten in samples were calculated using response ratio, the regression equation, initial sample weight or volume, digestion volume, and dilution when applicable. The concentrations were reported as µg tungsten/mL (µg tungsten/g in 3-month studies) for blood, plasma, and urine and µg tungsten/g for kidney in the 2-year studies.

Data from study samples were considered valid if they were bracketed by valid QC sets. In general, each sample set, method blanks, and controls were bracketed by two QC sets, which consisted of a calibration blank and two concentrations of calibration standards (QC low and QC high). A QC set passed when the measured concentration for QC standards were within 20% of its nominal value. If the QC standard failed, it was necessary to reanalyze the bracketed samples. All QC standards were within 20% of nominal concentrations.

E.3. Analysis of Xanthine and Methionine

Xanthine and methionine concentrations in rat urine were quantified using validated analytical methods using a standard addition approach. Validation data are listed in Table E-5. Study samples were stored at −20°C until used. Samples were allowed to thaw at room temperature and mixed well. Five 50 µL aliquot samples were transferred to individual wells in a 96-well plate, and 50 µL aliquots of the spiking standards of either methionine or xanthine (target concentrations 0, 0.075, 0.15, 0.3, 0.6 µg/mL) were added. After the addition of internal standard (400 µL of 1,500 ng/mL $^{15}N_2$ xanthine or 2 µg/mL $^{2}H_3$ methionine), the plate was covered with a pierceable sealing mat and the samples were mixed for approximately 10 seconds.

All samples were analyzed by liquid chromatography with tandem mass spectrometry using either an Agilent (Santa Clara, CA) or Shimadzu liquid chromatograph coupled to a Sciex (Toronto, Ontario, Canada) 4000 or 5000 mass spectrometer. For analysis of xanthine, chromatography was performed using a Phenomenex (Torrance, CA) Synergi Hydro RP (2 × 50 mm) column. Mobile phases A (water with 1 mM ammonium acetate, 0.1% formic acid) and B (acetonitrile with 0.1% formic acid) were run at a flow rate of 0.275 mL/minute with a linear gradient from 3% B to 95% B over 3 minutes followed by a 2.5-minute hold. The turbospray ion source was operated in negative mode. Transitions monitored for xanthine and $^{15}N_2$ xanthine were 151: >108 and 153: >109, respectively. For analysis of methionine, chromatography was performed using a Phenomenex (Torrance, CA) Develosil C30 (50 × 2 mm) column. Mobile phases were the same as for analysis of xanthine and were run at a flow rate of 0.275 mL/minute with a linear gradient from 3% B (3 minutes) to 95% B over 1 minute followed by a 0.5-minute hold. The turbospray ion source was operated in negative mode. Transitions monitored for methionine and $^{2}H_3$ methionine were 150: >104 and 153: >107, respectively.

Calibration curves relating response ratio of analyte to internal standard and concentration of xanthene or methionine in matrix were constructed using linear regression. The xanthine concentration in each was determined as the negative x-intercept of the standard addition curve. The concentration was reported as µg/mL of urine. Data from study samples were considered valid if the QCs were within 30% of the nominal values.

Table E-1. Analytical Method Validation and Stability Data for Tungsten in Blood and Urine for the Three-month Studies[a]

Validation Parameter	Rat Blood	Rat Urine	Mouse Blood
Matrix Concentration Range (µg/g)	0.01–10	0.002–3	–
LOQ (µg/g)	0.0068	0.0018	–
LOD (µg/g)	0.002	0.00054	–
Correlation Coefficient (r)	≥0.999	≥0.999	–
Recovery (%)[b]	101.3–117.2	57.1–127.5	–
Precision and Accuracy[c,d]			
Intra-day % RSD	≤7.9	≤4.6	≤6.8
Intra-day % RE	≤ ± 7.1	≤ ± 4.4	≤ ± 3.1
Inter-day % RSD	≤5.9	≤4.0	–
Inter-day % RE	≤ ± 5.0	≤ ± 1.9	–
Dilution Verification	up to 50 µg/g	up to 30 µg/g	up to 100 µg/g
% RSD	4.4	1.3	2.8
% RE	−10.5	−14.0	−10.8
Extract Stability (Ambient Storage) (Average % RE)[d]	≤ ± 2.5 up to 7 days	≤ ± 4.3 up to 3 days	≤ ± 1.5 up to 8 days
Matrix Stability (Average % RE)[d]			
Freeze-thaw (three cycles)	≤ ± 9.4	≤ ± 6.4	≤ ± 6.3
Frozen matrix (−70°C for blood and −20°C for urine, up to approximately 60 days)	≤ ± 11.8	≤ ± 10.0	≤ ± 7.6

LOQ = limit of quantitation; LOD = limit of detection; RSD = relative standard deviation; RE = relative error.
[a]Method was fully validated in Sprague Dawley rat blood and urine and cross-validated in B6C3F1/N mouse blood using quality control (QC) samples prepared in mouse blood at three concentrations (0.05, 1, 5 µg tungsten/g) and analyzed using a rat blood calibration curve.
[b]Estimated by comparing response of matrix standards to solvent standards.
[c]Precision was estimated as % RSD. Accuracy was estimated as average % RE.
[d]Determined for six replicate QCs at three levels: 0.05, 1, and 5 µg tungsten/g for blood and 0.01, 0.2, and 2 µg tungsten/g for urine.

Table E-2. Analytical Method Validation and Stability Data for Tungsten in Urine for the Two-year Studies[a]

Validation Parameter	Rat	Mouse
Solvent Standard Concentration Range (ng/mL)	60–2,500	60–2,500
LOQ (ng/mL)	60	60
LOD (ng/mL)	13.0	13.0
Correlation Coefficient (r)	≥0.998	≥0.999
Recovery (%)[b]	102–105	104–117
Precision and Accuracy[c,d,e]		
Intra-day % RSD	≤7.1	≤2.9
Intra-day % RE	≤ ± 11.9	≤ ± 29.3

Validation Parameter	Rat	Mouse
Inter-day % RSD	≤5.8	–
Inter-day % RE	≤ ± 10.6	–
Dilution Verification – Water Predigestion	up to 100,000 ng/mL	up to 100,000 ng/mL
% RSD	1.4	3.3
% RE	7.9	−5.1
Dilution Verification – Acid Postdigestion	up to 100,000 ng/mL	up to 100,000 ng/mL
% RSD	2.9	3.4
% RE	4.0	4.0
Matrix Evaluation[d,e,f]		
Sprague Dawley rat % RSD	≤11.4	–
Sprague Dawley rat % RE	≤ ± 27.0	–
B6C3F1/N mouse % RSD	–	≤3.6
B6C3F1/N mouse % RE	–	≤ ± 17.1
Postpreparative Stability (Ambient Storage) (% RE)[e,g]	≤ ± 15.8 up to 16 days	≤ ± 9.3 up to 16 days
Matrix Stability (% RE)[d,e]		
Freeze-thaw (four cycles)	≤ ± 11.7	≤ ± 2.7
Frozen matrix (−70°C up to approximately 61 days)	≤ ± 15.0	≤ ± 12.8

LOQ = limit of quantitation; LOD = limit of detection; RSD = relative standard deviation; RE = relative error.
[a]Method was fully validated in Sprague Dawley rat urine using a solvent standard curve and cross-validated in B6C3F1 mouse urine using quality control (QC) samples prepared in mouse urine at three concentrations (75, 375, 1,875 ng tungsten/mL) and analyzed using solvent curve.
[b]Estimated by comparing response of matrix QC samples to solvent QC samples.
[c]Precision was estimated as % relative standard deviation (RSD). Accuracy was estimated as average % relative error (RE).
[d]Determined for six replicate QCs at three concentrations: 75, 375, and 1,875 ng tungsten/mL for rat and mouse urine.
[e]Corrected for endogenous tungsten in urine.
[f]Study samples matrices were assessed using six replicate QCs at three concentrations: 75,375,1875 ng tungsten/mL.
[g]Determined for six replicate QCs at three concentrations: 75, 375, and 1,875 ng tungsten/mL for rat and mouse urine evaluated using freshly extracted standards and stored standard extracts.

Table E-3. Analytical Method Validation and Stability Data for Tungsten in Plasma for the Two-year Studies[a]

Validation Parameter	Rat	Mouse
Solvent Concentration Range (ng/mL)	60–2,500	60–2,500
LOQ (ng/mL)	60	60
LOD (ng/mL)	13.0	13.0
Correlation Coefficient (r)	≥0.99	≥0.99
Recovery (%)[b]	103–117	90.5–101
Precision and Accuracy[c,d,e]		
Intra-day % RSD	≤5.1	≤2.9
Intra-day % RE[e]	≤ ± 12.9	≤ ± 14.6
Inter-day % RSD	–	–

Validation Parameter	Rat	Mouse
Inter-day % RE	–	–
Dilution Verification – Water Predigestion	up to 25,000 ng/mL	up to 25,000 ng/mL
% RSD	2.5	2.2
% RE	5.2	5.2
Dilution Verification – Acid Postdigestion	up to 25,000 ng/mL	up to 25,000 ng/mL
% RSD	2.2	1.2
% RE	−1.6	10.4
Matrix Evaluation[d,e,f]		
Sprague Dawley rat % RSD	≤4.7	–
Sprague Dawley rat % RE	≤ ± 17.4	–
B6C3F1/N mouse % RSD	–	≤3.5
B6C3F1/N mouse % RE	–	≤ ± 19.1
Postpreparative Stability (Ambient Storage) (% RE)[e,g]	≤ ± 18.4 up to 14 days	≤ ± 18.2 up to 14 days
Matrix Stability (Average % RE)[d,e]		
Freeze-thaw (four cycles)	≤ ± 9.3	≤ ± 11.8
Frozen matrix (−70°C up to approximately 60 days)	≤ ± 16.2	≤ ± 18.3

LOQ = limit of quantitation; LOD = limit of detection; RSD = relative standard deviation; RE = relative error.
[a]Method was fully validated in Sprague Dawley rat urine using a solvent curve and crossed-validated in rat plasma and B6C3F1 mouse plasma using three concentrations (75, 375, 1,875 ng tungsten/mL) of quality control (QC) samples prepared in rat and mouse plasma and analyzed using solvent curve.
[b]Estimated by comparing response of matrix sample to solvent sample.
[c]Precision was estimated as % relative standard deviation (RSD). Accuracy was estimated as average % relative error (RE).
[d]Determined for six replicate QCs at three concentrations: 75, 375, and 1,875 ng tungsten/mL for rat and mouse plasma.
[e]Corrected for endogenous tungsten in plasma.
[f]Study samples matrices were assessed using six replicate QCs at three concentrations: 75, 375, 1,875 ng tungsten/mL.
[g]Determined for six replicate QCs at three concentrations: 75, 375, and 1,875 ng tungsten/mL for rat and mouse plasma evaluated using freshly extracted standards and stored standard extracts.

Table E-4. Analytical Method Validation and Stability Data for Tungsten in Kidney for the Two-year Studies[a]

Validation Parameter	Rat	Mouse
Matrix Concentration Range (ng/mL)	60–2,500	60–2,500
LOQ (ng/mL)	60	60
LOD (ng/mL)	13.0	13.0
Correlation Coefficient (r)	≥0.99	≥0.99
Recovery (%)[b]	118–120	111–124
Precision and Accuracy[c,d,e]		
Intra-day % RSD	≤1.2	≤2.1
Intra-day % RE	≤ ± 14.1	≤ ± 11.5
Inter-day % RSD	–	–
Inter-day % RE	–	–

Validation Parameter	Rat	Mouse
Dilution Verification – Water Predigestion	up to 125,000 ng/g	up to 125,000 ng/g
% RSD	2.1	2.6
% RE	5.6	7.2
Dilution Verification – Acid Postdigestion	up to 125,000 ng/g	up to 125,000 ng/g
% RSD	1.7	1.8
% RE	−3.2	−3.2
Matrix Evaluation[d,e,f]		
Sprague Dawley rat % RSD	≤4.3	–
Sprague Dawley rat % RE	≤ ± 3.5	–
B6C3F1/N mouse % RSD	–	≤4.6
B6C3F1/N mouse % RE	–	≤ ± 9.9
Postpreparative Stability (Ambient Storage) (% RE)[e,g]	≤ ± 11.6 up to 28 days	≤ ± 10.8 up to 28 days
Matrix Stability (% RE)[d,e]		
Freeze-thaw (six cycles)	≤ ± 12.5	≤ ± 5.8
Frozen matrix (−70°C up to approximately 55 days)	≤ ± 10.3	≤ ± 11.9

LOQ = limit of quantitation; LOD = limit of detection; RSD = relative standard deviation; RE = relative error.
[a]Method was fully validated in rat urine and cross-validated in Sprague Dawley rat and B6C3F1 and mouse kidney homogenate using quality control (QC) samples prepared in rat and mouse kidney homogenates at three concentrations (375, 1,875, and 9,375 ng tungsten/g) and analyzed using solvent curve.
[b]Estimated by comparing response of matrix samples to solvent samples.
[c]Precision was estimated as % relative standard deviation (RSD). Accuracy was estimated as average % relative error (RE).
[d]Determined for six replicate QCs at three concentrations: 375, 18,75, and 9,375 ng tungsten/g for kidney homogenate.
[e]Corrected for endogenous tungsten in kidney.
[f]Study samples matrices were assessed using six replicates of QCs at three concentrations: 75, 375, and 1,875 ng tungsten/g.
[g]Determined for six replicate QCs at three concentrations: 75, 375, and 1,875 ng tungsten/mL for rat and mouse kidney evaluated using freshly extracted standards and stored standard extracts.

Table E-5. Analytical Method Validation and Stability Data for Xanthine and Methionine in Rat Urine

Parameter	Xanthine	Methionine
Matrix Concentration Range (µg/mL)	0.075–0.6	0.075–0.6
LOQ (µg/mL)	0.075	0.075
Correlation Coefficient (r)	≥0.994	≥0.999
Precision and Accuracy[a,b]		
Intra-day % RSD	≤27.2	≤12.5
Intra-day % RE	≤ ± 25.4	≤ ± 13.5
Inter-day % RSD	23.1	11.9
Inter-day % RE	−13.1	4.4
Stability (% RE)[b]		
Freeze-thaw (three cycles)	−29.7	−11.8
Frozen matrix (−20°C up to approximately190 days)	−15.0	2.2

LOQ = limit of quantitation; RSD = relative standard deviation; RE = relative error.
[a]Precision was estimated as % relative standard deviation (RSD). Accuracy was estimated as average % relative error (RE).
[b]Determined for six replicate quality control samples at 3 µg/mL for xanthine and 1.5 µg/mL for methionine.

Appendix F. Peer-review Report

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F.1. Attendees F-2
F.2. Peer Review of the Draft NTP Technical Reports on the Toxicology and Carcinogenesis Studies of Sodium Tungstate Dihydrate, Di-*n*-butyl Phthalate, and Di(2-ethylhexyl) Phthalate F-3

The National Toxicology Program (NTP) virtually convened the NTP Technical Reports Peer-Review Panel ("the Panel") on April 2, 2021, to peer review the *Draft NTP Technical Reports on the Toxicology and Carcinogenesis Studies of Sodium Tungstate Dihydrate, Di-n-butyl Phthalate, and Di(2-ethylhexyl) Phthalate*. Meeting information, including the draft reports, actions, and presentations is currently archived with NTP.

The panel peer reviewed the draft reports and provided its opinion on NTP's preliminary conclusions regarding the level of evidence of carcinogenic activity of sodium tungstate dihydrate, di-n-butyl phthalate, and di(2-ethylhexyl) phthalate. The panel's comments for the *Draft NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Tungstate Dihydrate* begin at Section F.2.3. The panel's recommendations do not necessarily represent NTP's opinion.

F.1. Attendees[1]

Peer-Review Panel
Chair: Gabriele Ludewig, University of Iowa
Tracie E. Bunton, Eicarte, LLC
Michael R. Elwell, Apex Toxpath, LLC
Charles R. Mahrt, Retired, formerly with Flagship Biosciences
Daniel J. Spade, Brown University
John Pierce Wise, University of Louisville

National Toxicology Program Board of Scientific Counselors Liaison
Susan Felter, Procter & Gamble

National Institute of Environmental Health Sciences Staff
Mamta Behl
Chad Blystone
Mark Cesta
Sheba Churchill
Helen Cunny
Susan Elmore
Dori Germolec
Michelle Hooth
Madelyn (Mimi) Huang
Angela King-Herbert
Barry McIntyre
Georgia Roberts
Sheena Scruggs, Designated Federal Official
Kelly Shipkowski
Keith Shockley
Robert Sills
Stephanie Smith-Roe
Suramya Waidyanatha
Nigel Walker

[1]The meeting was held via webcast. Individuals who viewed the webcast are not listed except as noted.

Mary Wolfe

Other Federal Agency Staff
Shirisha Chittiboyina, National Institute for Occupational Safety and Health
Gonçalo Gamboa da Costa, U.S. Food and Drug Administration

Contract Support Staff
Amy Brix, EPL, Inc.
Canden Byrd, ICF
Lindsey Green, ICF
Shawn Harris, Social & Scientific Systems, a DLH Company
Elizabeth Maull, Kelly Government Services
Megan Rooney, ICF
Alessandria Schumacher, ICF
Cynthia Willson, Integrated Laboratory Systems, LLC

F.2. Peer Review of the Draft NTP Technical Reports on the Toxicology and Carcinogenesis Studies of Sodium Tungstate Dihydrate, Di-*n*-butyl Phthalate, and Di(2-ethylhexyl) Phthalate

F.2.1. Introductions and Welcome

The National Toxicology Program (NTP) convened a peer-review panel for the *Draft NTP Technical Reports on the Toxicology and Carcinogenesis Studies of Sodium Tungstate Dihydrate, Di-n-butyl Phthalate, and Di(2-ethylhexyl) Phthalate* on April 2, 2021 via webcast.

- Dr. Gabriele Ludewig, panel chair, called the meeting to order at 10:00 a.m. and welcomed everyone to the meeting. She asked all attendees to introduce themselves, and reviewed the peer-review meeting format for the panel and audience.
- Dr. Mary Wolfe, Acting Deputy Director for Policy & Communication, welcomed all participants to the meeting.
- Dr. Sheena Scruggs read the conflict-of-interest policy statement and briefed the attendees on meeting logistics.
- Dr. Susan Felter attended as the liaison to the NTP Board of Scientific Counselors.
- Dr. Shirisha Chittiboyina attended as the liaison for the National Institute for Occupational Safety and Health.
- Dr. Gonçalo Gamboa da Costa attended as the liaison for the U.S. Food and Drug Administration.

F.2.2. Background and Charge to the Panel

Dr. Chad Blystone briefly presented the NTP draft technical report objectives, including a review of the levels of evidence for the potential carcinogenic activity and factors considered for tested chemicals. He also described how NTP collects historical control data[2] on neoplastic lesions and

[2]https://ntp.niehs.nih.gov/results/dbsearch/historical

how these are utilized to provide context to report findings. Dr. Blystone provided the charge for the individual peer reviews:

- Review and evaluate the scientific and technical elements of the study and its presentation.
- Determine whether the study's experimental design, conduct, and findings support NTP's conclusions under the conditions of this study.

The peer-review meeting materials can be found on the NTP website.

F.2.3. Toxicology and Carcinogenesis Studies of Sodium Tungstate Dihydrate

F.2.3.1. Presentation and Clarifying Questions

Dr. Mamta Behl summarized the studies and conclusions reported in the *Draft NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Tungstate Dihydrate (CASRN 10213-10-2) in Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice (Drinking Water Studies)*.

Tungsten occurs naturally in the environment and can enter waterways through the weathering of rocks and soil. It was nominated for study due to concerns about potential widespread human exposure via contaminated drinking water. Sodium tungstate dihydrate was selected because it is a naturally occurring, water-soluble form of tungsten. Drinking water was selected as the most likely route of exposure for the general population.

Dr. Behl first presented a summary of results from the perinatal and postweaning toxicity/carcinogenicity study in Hsd:Sprague Dawley® SD® rats. NTP exposed time-mated female rats to 0, 250, 500, or 1,000 mg/L sodium tungstate dihydrate in drinking water from gestational day (GD) 6 through postnatal day (PND) 21. NTP provided the F_1 generation rats with the same respective sodium tungstate dihydrate concentrations as their dam for 2 years (n = 50/sex/concentration). In addition, F_1 generation rats were provided dosed drinking water or the vehicle control for 3, 6, 12, or 18 months for interim evaluations (n = 40/sex/concentration).

Dr. Behl then presented a summary of results from the chronic toxicity/carcinogenicity study in B6C3F1/N mice. NTP exposed mice to 0, 500, 1,000, or 2,000 mg/L sodium tungstate dihydrate in drinking water for 2 years (n = 50/sex/concentration). An additional 40 mice/sex/concentration were included for interim evaluations at 3, 6, 12, and 18 months.

Under the conditions of these 2-year studies, NTP's draft conclusions were:

- ***No evidence of carcinogenic activity*** in male Hsd:Sprague Dawley® SD® rats at 250, 500, and 1,000 mg/L.
- Exposure to sodium tungstate dihydrate in drinking water caused increased incidences of nonneoplastic lesions in the kidney of male rats.
- ***Equivocal evidence of carcinogenic activity*** in female Hsd:Sprague Dawley® SD® rats based on increased incidences of C-cell adenoma or carcinoma (combined) of the thyroid gland.
- Exposure to sodium tungstate dihydrate in drinking water caused increased incidences of nonneoplastic lesions in the kidney and uterus of female rats.

- ***Equivocal evidence of carcinogenic activity*** in male B6C3F1/N mice based on the occurrences of renal tubule adenoma or carcinoma (combined) in exposed animals.
- Exposure to sodium tungstate dihydrate in drinking water caused increased incidences of nonneoplastic lesions in the kidney, testes, and bone marrow of male mice.
- ***No evidence of carcinogenic activity*** in female B6C3F1/N mice at 500, 1,000, and 2,000 mg/L sodium tungstate dihydrate.
- Exposure to sodium tungstate dihydrate in drinking water caused increased incidences of nonneoplastic lesions in the kidney and spleen of female mice.

There were no clarifying questions or comments about the presentation.

F.2.3.2. Public Comments

Dr. Ludewig acknowledged the receipt of one written public comment from Dr. Ranulfo Lemus on behalf of the International Tungsten Industry Association. Dr. Ludewig noted that the panel did not receive requests for oral public comments on the draft technical report.

F.2.3.3. Peer-Review Comments and Panel Discussion

F.2.3.3.1. First Reviewer – Dr. Michael Elwell

- Dr. Elwell indicated that the dose selection was appropriate, the studies were well-conducted, the results were discussed clearly, and the rationale was clearly presented for neoplastic findings of equivocal evidence and several nonneoplastic effects. The important sodium tungstate-related findings were well-described and represented by quality pathology images in the report.
- Dr. Elwell noted some inconsistencies across report sections on the relationship of atypical hyperplasia in the uterus of female rats to sodium tungstate dihydrate exposure. Text on page 104 of the draft report indicates that the relationship is unknown; however, text in the abstract, summary, and conclusions states that atypical hyperplasia in the uterus is a nonneoplastic effect caused by sodium tungstate dihydrate.
    - Dr. Amy Brix stated that the sentence in the discussion about the unknown relationship to atypical hyperplasia was a typo and NTP may consider removing it. She stated that NTP believes that the effects were related to exposure and would consider make those edits.
- Dr. Elwell noted that on page 69 under "other tissues" for rats, several nonneoplastic findings were mentioned and considered to be of unknown biological significance. However, a significant decrease in fibroadenomas in the mammary gland was mentioned with no comment regarding biological significance or relationship to sodium tungstate dihydrate exposure. The fibroadenomas occurred in female rats with decreased body weights. He recommended that the report discuss the potential relevance of decreased body weight in relation to the tumors given that the effect of lower body weight on occurrence of this tumor has been noted in earlier NTP reports and in published studies.
    - Dr. Brix said that information about the mammary gland would be considered by NTP along with citing Dr. Haseman's article that compares body weight

changes to certain tumor incidences. For the nonneoplastic findings in other tissues, NTP can make it clear that they do not consider them toxicologically significant, treatment-related, or biologically significant.

- Dr. Elwell questioned the rationale for including two of the effects listed in the abstract, specifically increased spleen hematopoiesis and bone marrow hyperplasia. For both findings, the increased incidences occurred in the low and mid-exposure groups with no significant effect in the high-exposure group, and the average severity was similar across exposure groups. Given that the histopathology section states that the biological significance for the bone marrow and spleen is unknown, Dr. Elwell asked if there were other effects (e.g., inflammatory, hematologic, or hematopoietic) that supported listing these as effects in the abstract? As a point of reference, Dr. Elwell noted that NTP concluded that the kidney pigment findings, which were significantly higher in the high-exposure group, were of questionable toxicologic importance and therefore not brought into the abstract.
    - Dr. Behl agreed with Dr. Elwell's comment on the spleen and explained that NTP noted an effect in bone marrow hyperplasia in males and hematopoietic cell proliferation in females at both low and mid doses. NTP is open to discussion about whether to bring these effects forward into the abstract. Dr. Behl asked if the panel recommended including spleen effects in the abstract.
        - Dr. Elwell commented that the other effects in the abstract were dose- or target organ-related; the spleen effects were weaker than what might be expected for a finding listed in the abstract.
        - Dr. Brix noted that for the spleen and bone marrow, the incidences were two to three times higher in the low and mid-dose groups, but agreed that it is a weak connection and that NTP may be open to removing them from the abstract based on the panel discussion.

F.2.3.3.2. Second Reviewer – Dr. John Pierce Wise

- Dr. Wise concurred with Dr. Elwell. The report was well-written, and the study was well-designed.
- Given the pressure to evaluate environmentally relevant concentrations, Dr. Wise recommended that NTP provide language on dose selection rationale to help readers unfamiliar with the NTP approach.
    - Dr. Behl noted that the comment on dose selection rationale was well-taken. She explained how NTP selected the concentrations and indicated that they have been criticized in the past for doses that failed to challenge the animals. The concentrations used in this study allowed NTP to state that tungstate at high levels does not result in overt toxicity. The effects observed in the kidney were common with this strain and species.
        - Dr. Wise clarified that it was not that NTP should use different doses. Rather, he recommended that NTP specify that their intention is to determine whether a substance causes cancer, not to define whether a substance causes cancer at the most environmentally relevant dose. It

would help to add that context for a reader who does not understand that approach.

- Dr. Behl said that NTP can add some clarifying language in the report.

- Dr. Wise recommended that NTP provide clarifying language to address the occurrence of pinworms in the rats for reader unfamiliar with rat use.
  - Dr. Behl explained that the rats were positive for pinworms for the duration of the study, and they did not receive medication for elimination. At study termination, the incidences of pinworms were similar between the exposed groups. Based on histological sections, the pinworms were not associated with morphological changes in the large intestine and no inflammatory response was noted. NTP can clarify that point.
  - Dr. Wise indicated it would be helpful to include that no medication was administered.
- Dr. Wise recommended that NTP add a second parameter in the table when presenting comet assay results, since it is standard to show two different measures such as tail length, olive moment, or tail moment.
  - Dr. Stephanie Smith-Roe noted that NTP uses percent tail DNA, which is the OECD guideline for comet assay. Some comet assays report more than one measure, with those measures usually based on tail length. As there is variability in electrophoretic conditions that can influence tail length, NTP has found that percent tail DNA is a more reliable measure.
  - Dr. Ludewig commented that she was unsure of how to interpret the significant comet effects even at the lowest concentration given the lack of pathology in the liver and other published positive comet assay results in the liver and other tissues.
    - Dr. Smith-Roe replied that the results suggest that sodium tungstate may be capable of damaging DNA, but the liver was not a target organ for neoplastic effects.

F.2.3.3.3. Third Reviewer – Dr. Charles Mahrt

- Dr. Mahrt commented that the studies were well-designed, well-conducted, and the report was clear. However, he suggested NTP clarify whether the progression in rats from uterine atypical hyperplasia to adenocarcinomas (noted in Table 31) also included an increase in uterine adenomas.
  - Dr. Brix agreed that it was unclear and indicated that NTP will consider adding language regarding the progression. From experience, adenomas are less common and NTP often sees a direct progression from atypical hyperplasia to adenocarcinomas.

F.2.3.3.4. Panel Discussion

Dr. Daniel Spade indicated he had no additional comments that were not already addressed by the other reviewers.

Dr. Ludewig noted that the comet assay should be brought forward into the abstract since DNA damage was observed in the liver, despite no associated pathology.

Dr. Tracie Bunton asked about the justification for the large number of interim sacrifices and questioned whether NTP could have obtained information on tungsten accumulation using fewer sacrifices.

- Dr. Behl explained that the study was started several years ago when there were no data in the literature on the accumulation of tungsten in tissues following repeat dosing. Because the kidney is a target, NTP included multiple time points to determine if accumulation continued over the course of the study or if saturation eventually occurred. In addition, a question of sex differences required that NTP use males and females.

Dr. Bunton agreed with Dr. Mahrt's comment about Table 31 and thought the discussion was sufficient. She asked why Table 31 did not include incidence and statistics.

- Dr. Brix clarified that the adenocarcinomas and adenomas were included in Table 31 to show that the incidences were not significantly increased and that there was no progression from atypical hyperplasia. There were no statistics included because they were all negative.

Dr. Bunton noted that for female mice, there was a significant increase in the incidence of hepatocellular adenomas and carcinomas in the 500 mg/L group and that they were included in the "other tissues" groups. She asked why that was lumped into the "other tissues" category rather than brought into the tumor category.

- Dr. Brix stated that hepatocellular tumors are a common background lesion in this strain of mice, so it is not uncommon to have a dose group with significant differences due to biological variation. However, there was no dose response or reason to consider these treatments related.

Dr. Bunton asked for additional language or reorganization to be added in the discussion to explain how NTP came to an equivocal conclusion for female rats. NTP stated that the conclusion was based on increased incidences of C-cell adenoma and carcinoma (combined) of the thyroid gland, but the statement is incomplete as written because that same rationale could apply for a carcinogenic conclusion.

- Dr. Brix said that NTP could look at the discussion and clarify.

Dr. Ludewig asked if NTP had any information about adipose tissue or lipid content in the liver, given that absolute liver weight decreased, there were positive comet assay results in the males, and the text mentions that sodium tungstate dihydrate is an antidiabetic agent.

- Dr. Nigel Walker said that NTP does not have these data.

F.2.3.4. Vote on NTP Conclusions

F.2.3.4.1. Male Hsd:Sprague Dawley® SD® rats

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Wise so moved, and Dr. Mahrt seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

F.2.3.4.2. Female Hsd:Sprague Dawley® SD® rats

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Wise so moved, and Dr. Elwell seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

F.2.3.4.3. Male B6C3F1/N mice

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Wise so moved, and Dr. Elwell seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

F.2.3.4.4. Female B6C3F1/N mice

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Wise so moved, and Dr. Mahrt seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

F.2.4. Toxicology and Carcinogenesis Studies of Di-*n*-butyl Phthalate

F.2.4.1. Presentation and Clarifying Questions

Dr. Madelyn (Mimi) Huang summarized the studies and conclusions reported in the *Draft NTP Technical Report on the Toxicology and Carcinogenesis Studies of Di-n-butyl Phthalate (CASRN 84-74-2) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice*.

Di-n-butyl phthalate (DBP) is commonly used as a plasticizer and is found in a variety of consumer products, such as vinyl fabrics and flooring, personal care products, pharmaceuticals, and food packaging. Human exposure primarily occurs through ingestion of food packaged in materials containing DBP; some inhalation and dermal exposure occurs as well, but to a lesser extent. In the gut, DBP is rapidly metabolized to mono-n-butyl phthalate (MBP) and undergoes broad distribution throughout the body.

Dr. Huang presented a summary of results from the perinatal and postweaning toxicity and carcinogenicity study in Hsd:Sprague Dawley® SD® rats. Time-mated female rats were fed diets containing 0, 300, 1,000, 3,000, or 10,000 ppm DBP from gestation day (GD) 6 through postnatal day (PND) 21. NTP provided F_1 generation rats with the same respective DBP concentrations in feed as their dam for 2 years (generally two/sex/litter). In addition, select dams and their litters were removed on GD 18 and lactation day (LD) 4 to quantify the internal concentration of MBP.

Dr. Huang then presented a summary of results from the chronic toxicity and carcinogenicity study in B6C3F1/N mice. Mice were fed diets containing 0, 1,000, 3,000, or 10,000 ppm DBP for 2 years (n = 50/sex/group).

Under the conditions of these 2-year studies, NTP's draft conclusions were:

- ***Equivocal evidence of carcinogenic activity*** in male Hsd:Sprague Dawley® SD® rats based on marginal increases in the incidence of pancreatic acinus adenomas.

- Exposure to DBP resulted in increased incidences of gross lesions of the male reproductive system and nonneoplastic lesions of the male reproductive system, liver, and pituitary gland pars distalis in male rats.
- ***No evidence of carcinogenic activity*** in female Hsd:Sprague Dawley® SD® rats at 300, 1,000, 3,000, or 10,000 ppm.
- Exposure to DBP resulted in increased incidences of nonneoplastic lesions of the liver in female rats.
- ***No evidence of carcinogenic activity*** in male B6C3F1/N mice at 1,000, 3,000, or 10,000 ppm.
- Exposure to DBP increased incidences of nonneoplastic lesions of the male reproductive system and liver in male mice.
- ***No evidence of carcinogenic activity*** in female B6C3F1/N mice at 1,000, 3,000, or 10,000 ppm.
- Exposure to DBP increased incidences of nonneoplastic lesions of the liver and kidney in female mice.

There were no clarifying questions or comments about the presentation.

F.2.4.2. Public Comments

Dr. Gabriele Ludewig acknowledged that there were no written public comments on the draft technical report. She also noted that the panel did not receive requests for oral public comments on the draft technical report.

F.2.4.3. Peer-Review Comments and Panel Discussion

F.2.4.3.1. First Reviewer – Dr. Tracie Bunton

- Dr. Bunton said the presentation of the rationale, methods, and results was clear and concise.
- She commented that she liked the introduction and found it helpful in getting up to speed on the properties, uses, and reduction in use of DBP. The rationale for the overall significance of the study is solid, especially given that perinatal exposure is a knowledge gap.
- She indicated that all the information about stability, homogeneity, dose selection, and number of animals per dose group is appropriate for the design of the study.
- Dr. Bunton suggested including the word “microscopic” or eliminate the word “gross” in the conclusion statement of the report as it currently specifies gross lesions in the male reproductive system but does not specify that the nonneoplastic lesions are not gross lesions.
- Dr. Huang indicated that NTP is open to adding in the word “microscopic” to differentiate from gross lesions.
- Dr. Bunton noted that the findings to support the “equivocal” decision included an increased incidence in pancreatic acinar adenomas compared to controls, without a concurrent increase in hyperplasia, and a significant positive trend.

- She stated the lesion in the liver was compatible with effects of other phthalates, namely increased cytoplasmic alteration. This was an important point that was noted in the pathology review and in the discussion.
- She also agreed that the findings fit into the "equivocal" category.

F.2.4.3.2. Second Reviewer – Dr. Charles Mahrt

- Dr. Mahrt agreed with Dr. Bunton's comments and noted that the report was well-designed, conducted, and written.
- He appreciated the references to the literature to put the findings in perspective, especially the possibility of lesions related to peroxisome proliferation.

F.2.4.3.3. Third Reviewer – Dr. Daniel Spade

- Dr. Spade indicated that the study was well-conducted, and the report was clearly written.
- He noted that in the abstract, lines 39 to 40 indicate that there were fewer and less severe reproductive lesions in mice than in rats. He agreed with that conclusion, though suggested qualifying it by acknowledging the limits of cross-species comparison given that rats had perinatal exposure and mice did not.
    - Dr. Huang agreed that it was important to qualify the rat versus mouse comparison and said NTP could clarify those statements.
    - Dr. Nigel Walker explained that when NTP started adding the perinatal component to rat studies, people were trying to understand why the study design changed. With rat studies as perinatal and mouse studies as adults only, NTP has started to address that in the mid-2000s. In addition, it is difficult to do perinatal exposure on F_1 generation B6C3F1/N mice.
- Dr. Spade built on Dr. Bunton's comment about the equivocal conclusion for pancreatic acinar adenomas and asked if there was a significant trend test but not pairwise test.
    - Dr. Huang stated the affirmative.
        - Dr. Spade appreciated the response and agreed with the conclusion.
- He also asked why images were not included in some cases.
    - Dr. Huang clarified that NTP does not generally include images for common lesions or when the conclusion is equivocal.
        - Dr. Spade appreciated the response and agreed with the approach.

F.2.4.3.4. Panel Discussion

Dr. Michael Elwell noted that the last line of the discussion mentions that 2,4-dichlorophenoxyacetic acid and DBP did not produce hepatic lesions typical of peroxisome proliferators. DBP did result in hepatic lesions but did not produce hepatic neoplasms.

- Dr. Huang agreed that the last line should reference neoplasms instead of lesions, as Dr. Elwell suggested.

Regarding voting, Dr. Elwell noted that the original draft report mentions hypertrophy in the pituitary and hyperplasia, but that it was not mentioned in the oral presentation. He asked if hypertrophy was included in the pituitary findings.

- Dr. Mark Cesta explained that NTP relooked at the data about the pituitary and hyperplasia. Hyperplasia often occurs with hypertrophy lesions, so they wanted to explain the observed hyperplasia. However, after a closer look, NTP could not make that conclusion and decided to remove it from the abstract.

Dr. Ludewig noted that page 78 of the draft report explains the concentrations found in the amniotic fluid. When humans and rats both take up 1 mg/kg/day, a historic report showed that there were 22 ng/mL of the metabolite in the amniotic fluid of humans. However, NTP only found 5 ng/mL in rats. Dr. Ludewig said she was initially surprised until she noticed that NTP did not measure the glucuronic acid conjugate. She wondered if that would also cross the placenta or if conjugation would protect the fetus. She suggested adding a statement that the gluconate conjugate was not measured.

- Dr. Huang agreed that there are differences in distribution and metabolism between rodents and humans. She was unsure if glucuronidation affects its ability to cross the placenta and said that was something NTP would look into.

F.2.4.4. Vote on NTP Conclusions

F.2.4.4.1. Male Hsd:Sprague Dawley® SD® rats

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Bunton so moved, and Dr. Mahrt seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

F.2.4.4.2. Female Hsd:Sprague Dawley® SD® rats

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. John Pierce Wise so moved, and Dr. Elwell seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

F.2.4.4.3. Male B6C3F1/N mice

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Bunton so moved, and Dr. Wise seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

F.2.4.4.4. Female B6C3F1/N mice

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Wise so moved, and Dr. Bunton seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

F.2.5. Toxicology and Carcinogenesis Studies of Di(2-ethylhexyl) Phthalate

F.2.5.1. Presentation and Clarifying Questions

Dr. Chad Blystone summarized the studies and conclusions reported in the *Draft NTP Technical Report on the Toxicology and Carcinogenesis Studies of Di(2-ethylhexyl) Phthalate (CASRN 117-81-7) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats*.

Di(2-ethylhexyl) phthalate (DEHP) is a phthalate ester that was widely used in manufacturing of PVC polymers and corresponding products, such as cosmetics and toys. Over the years DEHP use has declined due to toxicity concerns, but chronic exposure throughout multiple life stages still occurs. The literature suggests that exposure to DEHP during an early life stage may result in chronic or carcinogenic health outcomes. However, previous DEHP chronic rodent studies did not include exposure during the gestational period up to weaning in rodents. To address this, NTP conducted two comparative DEHP carcinogenesis studies in rats to determine if including early life exposure would alter chronic toxicity or carcinogenicity outcomes.

Dr. Blystone presented a summary of results from the perinatal and postweaning toxicity/carcinogenicity study in Hsd:Sprague Dawley® SD® rats. In the perinatal and postweaning study, time-mated female rats were fed diets containing 0, 300, 1,000, 3,000, or 10,000 ppm DEHP from gestational day (GD) 6 through postnatal day (PND) 21 (n = 45/group). Select dams were removed on GD 18 to quantify internal concentrations of a metabolite of DEHP, mono(2-ethylhexyl) phthalate, in plasma and tissue samples. NTP provided F_1 generation rats with the same respective DEHP concentration in feed as their dam for 2 years (n = 2/sex/litter; n = 50 total/sex/group).

Dr. Blystone then presented a summary of results from the postweaning toxicity/carcinogenicity study in Hsd:Sprague Dawley® SD® rats. In the postweaning-only study, rats were fed diets containing 0, 300, 1,000, 3,000, or 10,000 ppm DEHP for 2 years (n = 50/sex/group).

Under the conditions of these 2-year studies, NTP's draft conclusions were:

- Perinatal and Postweaning Feed Study:
    - ***Clear evidence of carcinogenic activity*** in male Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular adenoma or carcinoma (combined) and acinar adenoma or carcinoma (combined) neoplasms (predominantly adenomas) of the pancreas.
    - Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, heart, pituitary gland, testis, and epididymis and increased incidences of gross lesions of the reproductive tract, bone marrow, and kidney in male rats.
    - ***Clear evidence of carcinogenic activity*** in female Hsd:Sprague Dawley® SD® rats based on the increased incidence of hepatocellular adenoma or carcinoma (combined).
    - The occurrence of pancreatic acinar adenoma or carcinoma (combined) was considered to be related to exposure. (*Some evidence*)
    - The occurrence of uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined) in female rats may have been related to exposure. (*Equivocal evidence*)
    - Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver and increased incidences of gross lesions of the kidney in female rats.
- Postweaning-only Feed Study

- ***Clear evidence of carcinogenic activity*** in male Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular adenoma or carcinoma (combined) and increased incidences of acinar adenoma or carcinoma (combined) neoplasms (predominantly adenomas) of the pancreas.
- The occurrence of testicular interstitial cell adenoma in male rats may have been related to exposure. (*Equivocal evidence*)
- Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, pancreas, bone marrow, heart, pituitary gland, testis, and epididymis.
- ***Clear evidence of carcinogenic activity*** in female Hsd:Sprague Dawley® SD® rats based on the incidences of hepatocellular adenoma or carcinoma (combined) and uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined).
- The occurrence of pancreatic acinar adenoma or carcinoma (combined) in female rats was considered to be related to exposure. (*Some evidence*)
- Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, pancreas, bone marrow, and uterus in female rats.

- Comparative Carcinogenic Benchmark Dose Analyses
  - No consistent pattern indicating that perinatal and postweaning exposure was more sensitive compared to postweaning-only exposure and modeled responses were within threefold of each other.
  - However, there was a stronger carcinogenic response in the reproductive organs (uterus and testis) in the postweaning-only exposure study compared to the perinatal and postweaning exposure study.

Dr. Gabriele Ludewig asked a clarifying question about a shift in male to female fetus ratios that was not mentioned during Dr. Blystone's talk. Dr. Blystone responded that female fetuses were lost at the highest concentration. The reduction in litter size was due to this but was inconsistent and not considered related to DEHP exposure.

F.2.5.2. Public Comments

Dr. Ludewig acknowledged that there were no written public comments submitted on the draft technical report. She noted that the panel also did not receive requests for oral public comments on the draft technical report.

F.2.5.3. Peer-Review Comments and Panel Discussion

F.2.5.3.1. First Reviewer – Dr. John Pierce Wise

- Dr. Wise indicated that the study was well-designed, well-conducted, and clear in its data presentation.
- He suggested that NTP clarify that pinworm infections were not treated with medication.
  - Dr. Blystone said that NTP can add this clarification.

- Dr. Wise also reiterated his comment from sodium tungstate dihydrate that NTP should explain that the choice of dose was deliberate and not intended to test the most environmentally relevant level of exposure, but rather to test whether the substance is carcinogenic.
    - Dr. Blystone agreed that the point of these studies is hazard characterization and noted that NTP can clarify that in the report and include it in the lay summary.
    - Dr. Wise suggested that NTP could add this in a dose selection rationale section.
    - Dr. Blystone responded that the reports have a section covering the technical aspects of the exposure concentration selection and that a sentence can be added to highlight rationale for exposure concentrations.

F.2.5.3.2. Second Reviewer – Dr. Daniel Spade

- Dr. Spade stated that the report was thorough and clear; it makes a massive amount of work easily understandable.
- He asked for clarification in how the reduction in litter size was presented, and asked whether there was an effort to address post-implantation loss as part of the reduced litter size. Reduced litter size could not be due to exposure related pre-implantation loss since the dosing window did not begin before implantation. However, Dr. Spade argued that with exposures beginning on GD 6, this overlaps with organogenesis and poses a risk for post-implantation loss which has been reported in the phthalate literature.
    - Dr. Blystone said that NTP only evaluated post-implantation loss in females which did not deliver, so there are no more data available on post-implantation loss and litter size.
- Dr. Spade asked why pup survival data for PND 1–4 and PND 5–21 on page 29 of the draft report were analyzed separately. Dr. Spade thought that there could have been a trend in mortality if PND 1–21 were analyzed together.
    - Dr. Blystone explained that NTP typically standardizes the litter size on PND 4, so the analysis looks at early (PND 1–4) and later (PND 5–21) mortality. Dr. Blystone acknowledged that based on Dr. Spade's written comment NTP reanalyzed the data after combining the two periods and there was still no significant trend or pairwise comparison.
- Dr. Spade also noted that it was unclear why some lesions of unknown biological significance on page 61 of the draft report were classified as such. Of note were the adrenal gland lesions, because of the known antiandrogenic effect of DEHP. Also, he questioned the ovarian atrophy as classified as unknown biological significance, because published data from academic studies indicate that phthalates change the rates of follicle maturation which could be related.
    - Dr. Blystone indicated that NTP can clarify the language and focus more on toxicological significance.

- Dr. Spade commented that 24 months is reproductively aged, so for certain findings such as seminiferous epithelium degeneration, the control levels are very high which makes it less likely that there will be a significant pairwise test. This limits the ability to know with certainty what the dose response would look like for an endpoint such as epithelial degeneration. If 4-month-old males were tested, you might see a significant response at lower levels. The study supports the conclusions within the constraints of the study, but this is a limitation.
    - Dr. Blystone agreed that at this age, the model is not very sensitive. NTP can add a statement about that to the report.
- Dr. Spade noted that for gestational transfer, as discussed on page 33 of the draft report, one limitation is that DEHP has many secondary metabolites. He noted that without measuring these secondary metabolites it is difficult to determine the total transfer.
    - Dr. Blystone agreed and said that NTP can clarify that metabolites can be transferred at different rates.

Dr. Ludewig stated that it was interesting that mono(2-ethylhexyl) phthalate was found in the amniotic fluid and the fetus of the control animals, but nothing in the serum of dams.

- Dr. Blystone agreed that this was unusual and noted that sometimes sample preparations can lead to irregularities.
- Dr. Suramya Waidyanatha said NTP concluded it was probably due to contamination of samples during collection or preparation and that this is likely due to the small volume of these samples.

F.2.5.3.3. Third Reviewer – Dr. Michael Elwell

- Dr. Elwell agreed with previous reviewers that the results are clearly presented and discussed.
- He noted that at the highest dose, body weights were reduced by approximately 30% in the perinatal postweaning and approximately 20% in the postweaning study. Although this did not affect survival, the decreased incidence of several neoplastic and nonneoplastic findings (especially for the high dose group of each study) might be attributable to significantly lower body weights. In the report (pages 61 and 77) these are indicated to be of unknown biological significance. If these findings are due to lower body weight, they should be addressed as such rather than reported as unknown significance. For example, neoplasms including the c-cell, pituitary, and mammary gland tumors can be affected by body weight. In females, a single pituitary gland neoplasm reported at the high dose is unexpected and notable in comparison to the control group where 16 animals were reported to have this tumor. The decreased incidence of the nonneoplastic lesions of testis polyarteritis and parathyroid hyperplasia could also be related to the lowered severity of chronic progressive nephropathy (CPN). In both studies, CPN was decreased in severity at the high dose in both studies and indicated as the cause of death in 0 and 1 animal while 16–18 animals in the control groups list CPN as the cause of death. This report cites other DEHP studies that attribute an increase in CPN to DEHP while this report suggests the opposite effect on CPN. This may be because the highest doses in this study were

lower, the decreased body weight, or that the NTP 2000 diet was not used in earlier studies.

  - Dr. Blystone indicated that NTP can clarify biological significance versus toxicological significance. Dr. Blystone agreed that some of the effects could be related to decreased body weight and stated that NTP can clarify.

- Dr. Elwell asked if page 87 of the draft report should state that it is unclear if any difference corresponded to developmental mechanisms when comparing the two studies, or if it is a typo. It appeared the kidney was affected by a developmental mechanism and DEHP was presumed to interfere with proper development.
  - Dr. Blystone stated that NTP can fix the typo.
- Dr. Elwell noted that for the nonneoplastic conclusions, there are dozens of neoplastic and nonneoplastic findings clearly related to DEHP. He questioned why the acute inflammation in the uterus in the perinatal/postnatal study was considered an effect. He also asked why the bone marrow in females in the postweaning-only study was included as a finding and if it was possibly a false positive.
  - Dr. Blystone said that although the response was not strong, NTP considered the bone marrow lesion exposure related since it was observed in males and females. The acute inflammation in the uterus was considered exposure related. NTP can review this and clarify.
  - Dr. Susan Elmore stated that she can see Dr. Elwell's point about the acute inflammation of the uterus possibly not being related to exposure, but that this was something NTP would need to discuss further.

F.2.5.3.4. Panel Discussion

Dr. Tracie Bunton said she wanted to see the presentation of the benchmark dose analysis, but beyond that, just had minor edits submitted in writing.

Dr. Spade asked if Dr. Elwell's comments about bone marrow and uterus related to one of the conclusions on which the panel would vote. He wondered if it was about including it in the abstract rather than the conclusion that there was a finding.

- Dr. Elwell said while the other findings listed in the abstract were convincing effects, the rationale to include the non-dose-related difference of acute uterus inflammation in the perinatal-post weaning study was not clear although there may have been reason to include it as chronic uterus inflammation was increased in the postweaning-only study. The question on including the bone marrow finding in females from the postweaning-only study in the abstract was based on very small group differences in incidence with no apparent effect on severity.

Dr. Bunton noted that a number of genetic toxicity tests were conducted and asked if they were related to this particular report or conducted over time.

- Dr. Blystone said they were not related to this report itself. They were accumulated over time and not published previously, so they were published with this report.

F.2.5.4. Vote on NTP Conclusions

F.2.5.4.1. Male Hsd:Sprague Dawley® SD® rats (perinatal and postweaning feed study)

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Bunton so moved, and Dr. Wise seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

F.2.5.4.2. Female Hsd:Sprague Dawley® SD® rats (perinatal and postweaning feed study)

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Wise so moved, and Dr. Elwell seconded the motion. Dr. Elwell asked to amend the motion to vote and moved that NTP delete "and increased incidences of gross lesions of the" from the conclusion and add "and uterus." Dr. Wise motioned to accepted revisions to the conclusion and Dr. Spade seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the new conclusion.

In a second round of revisions, Dr. Blystone noted that the end of a sentence was cut off. He added "and gross observations in the female reproductive tract" to the end of the final conclusion. Dr. Ludewig called for a motion from the panel to approve the second round of revised conclusions. Dr. Wise so moved, and Dr. Elwell seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the new conclusion, below.

Revised Conclusion:

- Clear evidence of carcinogenic activity
    - Increased incidence of hepatocellular adenoma or carcinoma (combined).
- The occurrence of pancreatic acinar adenoma or carcinoma (combined) was considered to be related to exposure. (*Some evidence*)
- The occurrence of uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined) in female rats may have been related to exposure. (*Equivocal evidence*)
- Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, ~~and increased incidences of gross lesions of the~~ kidney, <u>and uterus</u> in female rats <u>and gross observations in the female reproductive tract</u>.

F.2.5.4.3. Male Hsd:Sprague Dawley® SD® rats (postweaning-only study)

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Wise so moved, and Dr. Elwell seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

F.2.5.4.4. Female Hsd:Sprague Dawley® SD® rats (postweaning-only study)

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. The panel did not offer a motion. Dr. Elwell moved that NTP delete the reference to increased incidences of nonneoplastic lesions in the bone marrow from the conclusion. Dr. Wise seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the new conclusion, below.

Revised Conclusion:

- Clear evidence of carcinogenic activity
    - Increased incidences of hepatocellular adenoma or carcinoma (combined) and uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined).
- The occurrence of pancreatic acinar adenoma or carcinoma (combined) in female rats was considered to be related to exposure. (*Some evidence*)
- Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, pancreas, ~~bone marrow,~~ and uterus in female rats.

F.2.5.4.5. Hsd:Sprague Dawley® SD® rats (comparative benchmark dose analyses)

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Wise so moved, and Dr. Elwell seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

F.2.5.5. Final Conclusions

Because revisions were proposed and approved during the meeting, the final approved conclusions are presented below:

- Perinatal and Postweaning Feed Study:
    - ***Clear evidence of carcinogenic activity*** in male Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular adenoma or carcinoma (combined) and acinar adenoma or carcinoma (combined) neoplasms (predominantly adenomas) of the pancreas.
    - Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, kidney, bone marrow, heart, pituitary gland, testis, and epididymis and increased incidences of gross lesions of the reproductive tract
    - ***Clear evidence of carcinogenic activity*** in female Hsd:Sprague Dawley® SD® rats based on the increased incidence of hepatocellular adenoma or carcinoma (combined).
    - The occurrence of pancreatic acinar adenoma or carcinoma (combined) was considered to be related to exposure. (*Some evidence*)
    - The occurrence of uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined) in female rats may have been related to exposure. (*Equivocal evidence*)
    - Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, kidney, and uterus in female rats and gross observations in the female reproductive tract.
- Postweaning-only Feed Study
    - ***Clear evidence of carcinogenic activity*** in male Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular adenoma or carcinoma (combined) and increased incidences of acinar adenoma or carcinoma (combined) neoplasms (predominantly adenomas) of the pancreas.

  - The occurrence of testicular interstitial cell adenoma in male rats may have been related to exposure. (*Equivocal evidence*)
  - Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, pancreas, bone marrow, heart, pituitary gland, testis, and epididymis.
  - ***Clear evidence of carcinogenic activity*** in female Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular adenoma or carcinoma (combined) and uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined).
  - The occurrence of pancreatic acinar adenoma or carcinoma (combined) in female rats was considered to be related to exposure. (*Some evidence*)
  - Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, pancreas, and uterus in female rats.
- Comparative Carcinogenic Benchmark Dose Analyses
  - No consistent pattern indicating that perinatal and postweaning exposure was more sensitive compared to postweaning-only exposure and modeled responses were within threefold of each other.
  - However, there was a stronger carcinogenic response in the reproductive organs (uterus and testis) in the postweaning-only exposure study compared to the perinatal and postweaning exposure study.

F.2.6. Closing Remarks on the Draft Reports

Dr. Gabriele Ludewig welcomed additional panel comments on the draft report. There were no additional comments.

Closing the meeting, Dr. Sheena Scruggs thanked all the peer-review panelists.

Dr. Ludewig added her thanks to the NTP staff and the panel members for their efforts.

Dr. Ludewig adjourned the meeting at 2:00 p.m. EDT on April 2, 2021.

Appendix G. Supplemental Data

Tables with supplemental data can be found here: https://doi.org/10.22427/NTP-DATA-TR-599.[88]

G.1. Perinatal and Three-month Study in Rats

E03 – Growth Curves
0303801_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
0303801_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
0303801_E05_Clinical_Observations_Summary.pdf

E07 – Mean Water Consumption by Treatment Group
0303801_E07__Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Water and Compound Consumption Table
0303801_E08_Water_and_Compound_Consumption_Table.pdf

Gestational Body Weights (grams)
0303801_Gestational_Body_Weights_(grams).pdf

Gestational Water Consumption (grams)
0303801_Gestational_Water_Consumption_(grams).pdf

Gestational and Lactational Chemical Consumption
0303801_Gestational_and_Lactational_Chemical_Consumption.pdf

Lactational Body Weights (grams)
0303801_Lactational_Body_Weights_(grams).pdf

Lactational Water Consumption (grams)
0303801_Lactational_Water_Consumption_(grams).pdf

Litter Data by Dam – PND 1
0303801_Litter_Data_By_Dam-PND1.pdf

Live Litter Size and Survival – PND 4 and 21
0303801_Live_Litter_Size_and_Survival-PND_4_and_21.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
0303801_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
0303801_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Site (Systemic Lesions Abridged)
0303801_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Site_(Systemic_Lesions_Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal
0303801_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
0303801_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
0303801_P14_Individual_Animal_Pathology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
0303801_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
0303801_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
0303801_PA06_Organ_Weights_Summary.pdf

PA41 – Clinical Chemistry Summary
0303801_PA41_Clinical_Chemistry_Summary.pdf

PA43 – Hematology Summary
0303801_PA43_Hematology_Summary.pdf

PA44 – Urinalysis Summary
0303801_PA44_Urinalysis_Summary.pdf

PA48 – Tissue Concentration Summary
0303801_PA48_Tissue_Concentration_Summary.pdf

PA48C – Tissue Concentration Curve
0303801_PA48C%20-%20Tissue%20Concentration%20Curve.pdf

Pup Body Weights (grams)
0303801_Pup_Body_Weights_(grams).pdf

R02 – Reproductive Performance Summary
0303801_R02_Reproductive_Performance_Summary.pdf

R06 – Andrology Summary
0303801_R06_Andrology_Summary.pdf

R07 – Hormone Summary
0303801_R07_Hormone_Summary.pdf

G.2. Perinatal and Three-month Study in Rats – Individual Animal Data

Female Individual Animal Body Weight Data
0303801_Female_Individual_Animal_Body_Weight_Data.xls

Female Individual Animal Clinical Observations
0303801_Female_Individual_Animal_Clinical_Observations.xls

Female Individual Animal Non-Neoplastic Pathology Data
0303801_Female_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
0303801_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
0303801_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data
0303801_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data
0303801_Male_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
0303801_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
0303801_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Andrology Data
0303801_Individual_Animal_Andrology_Data.xlsx

Individual Animal Clinical Chemistry Data
0303801_Individual_Animal_Clinical_Chemistry_Data.xlsx

Individual Animal DamID and PupID Data
0303801_Individual_Animal_DamID_and_PupID_Data.xlsx

Individual Animal Hematology Data
0303801_Individual_Animal_Hematology_Data.xlsx

Individual Animal Hormone Data
0303801_Individual_Animal_Hormone_Data.xlsx

Individual Animal Organ Weight Data
0303801_Individual_Animal_Organ_Weight_Data.xlsx

Individual Animal Reproductive Performance Data
0303801_Individual_Animal_Reproductive_Performance_Data.xlsx

Individual Animal Tissue Concentration Data
0303801_Individual_Animal_Tissue_Concentration_Data.xlsx

Individual Animal Urinalysis Data
0303801_Individual_Animal_Urinalysis_Data.xlsx

G.3. Three-month Study in Mice

E03 – Growth Curves
0303802_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
0303802_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
0303802_E05_Clinical_Observations_Summary.pdf

E07 – Mean Water Consumption by Treatment Group
0303802_E07__Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Water and Compound Consumption Table
0303802_E08_Water_and_Compound_Consumption_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
0303802_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
0303802_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Site (Systemic Lesions Abridged)
0303802_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Site_(Systemic_Lesions_Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal
0303802_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
0303802_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
0303802_P14_Individual_Animal_Pathology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
0303802_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
0303802_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
0303802_PA06_Organ_Weights_Summary.pdf

PA43 – Hematology Summary
0303802_PA43_Hematology_Summary.pdf

PA48 – Tissue Concentration Summary
0303802_PA48_Tissue_Concentration_Summary.pdf

PA48C – Tissue Concentration Curve
0303802_PA48C - Tissue Concentration Curve.pdf

R06 – Andrology Summary
0303802_R06_Andrology_Summary.pdf

G.4. Three-month Study in Mice – Individual Animal Data

Female Individual Animal Body Weight Data
0303802_Female_Individual_Animal_Body_Weight_Data.xls

Female Individual Animal Clinical Observations
0303802_Female_Individual_Animal_Clinical_Observations.xls

Female Individual Animal Non-Neoplastic Pathology Data
0303802_Female_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
0303802_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
0303802_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data
0303802_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data
0303802_Male_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
0303802_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
0303802_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Andrology Data
0303802_Individual_Animal_Andrology_Data.xlsx

Individual Animal Hematology Data
0303802_Individual_Animal_Hematology_Data.xlsx

Individual Animal Organ Weight Data
0303802_Individual_Animal_Organ_Weight_Data.xlsx

Individual Animal Tissue Concentration Data
0303802_Individual_Animal_Tissue_Concentration_Data.xlsx

G.5. Perinatal and Two-year Study in Rats

Analysis of PND 1 Litter Data
0303803_Analysis_of_PND_1_0303803_Litter_Data.pdf

Analysis of PND 4 Live Litter Size and Survival
0303803_Analysis_of_PND_4_Live_0303803_Litter_Size_and_Survival.pdf

E01 – Animal Removal Summary by Treatment Group
0303803_E01_Animal_Removal_Summary_By_Treatment_Group.pdf

E02 – Animals Removed from Experiment
0303803_E02_Animals_Removed_from_Experiment.pdf

E03 – Growth Curves (Litter based)
0303803_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival (Litter based)
0303803_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
0303803_E05_Clinical_Observations_Summary.pdf

E07 – Mean Water Consumption by Treatment Group
0303803_E07__Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Water and Compound Consumption Table
0303803_E08_Feed_Water_and_Compound_Consumption_Table.pdf

E12 – Animal History
0303803_E12_Animal_History.pdf

I05 – Clinical Observations Summary
0303803_I05_Clinical_Observations_Summary.pdf

Litter Data Analysis of Gestational Body Weight
0303803_Litter_Data_Analysis_of_Gestational_Body_Weight.pdf

Litter Data Analysis of Gestational Water Consumption
0303803_Litter_Data_Analysis_of_Gestational_Water_Consumption.pdf

Litter Data Analysis of Gestational/Lactational Chemical Consumption
0303803_Litter_Data_Analysis_of_Gestational_Lactational_Chemical_Consumption.pdf

Litter Data Analysis of Lactational Body Weight
0303803_Litter_Data_Analysis_of_Lactational_Body_Weight.pdf

Litter Data Analysis of Lactational Water Consumption
0303803_Litter_Data_Analysis_of_Lactational_Water_Consumption.pdf

Litter Data Analysis of Pup Body Weights
0303803_Litter_Data_Analysis_of_Pup_Body_Weights.pdf

Litter Data Analysis of Pup Body Weights (cont'd)
0303803_Litter_Data_Analysis_of_Pup_Body_Weights_(cont'd).pdf

P02 – Incidence Rates of Neoplasms by Anatomic Site
0303803_P02_Incidence_Rates_of_Neoplasms_by_Anatomic_Site.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
0303803_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
0303803_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Site (Systemic Lesions Abridged)
0303803_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Site_(Systemic_Lesions_Abridged).pdf

P08 – Litter Statistical Analysis of Primary Tumors
0303803_P08_Litter_Statistical_Analysis_of_Primary_Tumors.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
0303803_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
0303803_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P11 – Statistical Analysis of Survival Data
0303803_P11_Statistical_Analysis_of_Survival_Data.pdf

P14 – Individual Animal Pathology Data
0303803_P14_Individual_Animal_Pathology_Data.pdf

P17 – Neoplasms by Individual Animal (Systemic Lesions Abridged)
0303803_P17_Neoplasms_By_Individual_Animal_(Systemic_Lesions_Abridged).pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
0303803_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
0308303_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
0303803_PA06_Organ_Weights_Summary.pdf

PA48 – Summary of Tissue Concentration
0303803_PA48_Summary_of_Tissue_Concentration.pdf

PA48C – Tissue Concentration Curve
0303803_PA48C - Tissue Concentration Curve.pdf

R02 – Reproductive Performance Summary
0303803_R02_Reproductive_Performance_Summary.pdf

G.6. Perinatal and Two-year Study in Rats – Individual Animal Data

Female Individual Animal Body Weight Data
0303803_Female_Individual_Animal_Body_Weight_Data.xls

Female Individual Animal Clinical Observations
0303803_Female_Individual_Animal_Clinical_Observations.xls

Female Individual Animal Neoplastic Pathology Data
0303803_Female_Individual_Animal_Neoplastic_Pathology_Data.xls

Female Individual Animal Non-Neoplastic Pathology Data
0303803_Female_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
0303803_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
0303803_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Female Pup Individual Animal Body Weight Data
0303897_Female_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data
0303803_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Clinical Observations
0303803_Male_Individual_Animal_Clinical_Observations.xls

Male Individual Animal Neoplastic Pathology Data
0303803_Male_Individual_Animal_Neoplastic_Pathology_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data
0303803_Male_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
0303803_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
0303803_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Pup Individual Animal Body Weight Data
0303896_Male_Individual_Animal_Body_Weight_Data.xls

Individual Animal Clinical Observations Data
0303803_Individual_Animal_Clinical_Observations_Data.xlsx

Individual Animal DamID and PupID Data
0303803_Individual_Animal_DamID_and_PupID_Data.xlsx

Individual Animal Organ Weight Data
0303803_Individual_Animal_Organ_Weight_Data.xlsx

Individual Animal Reproductive Performance Data
0303803_Individual_Animal_Reproductive_Performance_Data.xlsx

Individual Animal Tissue Concentration Data
0303803_Individual_Animal_Tissue_Concentration_Data.xlsx

Individual Animal Urinalysis Data
0303803_Individual_Animal_Urinalysis_Data.xlsx

Individual Pup Census and Litter Weight by Sex Data
0303803_Individual_Pup_Census_and_Litter_Weight_by_Sex_Data.xlsx

G.7. Two-year Study in Mice

E01 – Animal Removal Summary by Treatment Group
0303804_E01_Animal_Removal_Summary_By_Treatment_Group.pdf

E02 – Animals Removed from Experiment
0303804_E02_Animals_Removed_from_Experiment.pdf

E03 – Growth Curves
0303804_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
0303804_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
0303804_E05_Clinical_Observations_Summary.pdf

E07 – Mean Water Consumption by Treatment Group
0303804_E07__Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Water and Compound Consumption Table
0303804_E08_Feed_Water_and_Compound_Consumption_Table.pdf

E12 – Animal History
0303804_E12_Animal_History.pdf

P02 – Incidence Rates of Neoplasms by Anatomic Site
0303804_P02_Incidence_Rates_of_Neoplasms_by_Anatomic_Site.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
0303804_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
0303804_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Site (Systemic Lesions Abridged)
0303804_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Site_(Systemic_Lesions_Abridged).pdf

P08 – Statistical Analysis of Primary Tumors
0303804_P08_Statistical_Analysis_of_Primary_Tumors.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
0303804_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
0303804_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P11 – Statistical Analysis of Survival Data
0303804_P11_Statistical_Analysis_of_Survival_Data.pdf

P14 – Individual Animal Pathology Data
0303804_P14_Individual_Animal_Pathology_Data.pdf

P17 – Neoplasms by Individual Animal (Systemic Lesions Abridged)
0303804_P17_Neoplasms_By_Individual_Animal_(Systemic_Lesions_Abridged).pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
0303804_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
0303804_P40_Survival_Curves.pdf

PA06 – Organ Weight Summary
0303804_PA06_Organ_Weight_Summary.pdf

PA48 – Summary of Tissue Concentration
0303804_PA48_Summary_of_Tissue_Concentration.pdf

PA48C – Tissue Concentration Curve
0303804_PA48C - Tissue Concentration Curve.pdf

G.8. Two-year Study in Mice – Individual Animal Data

Female Individual Animal Body Weight Data
0303804_Female_Individual_Animal_Body_Weight_Data.xls

Female Individual Animal Clinical Observations
0303804_Female_Individual_Animal_Clinical_Observations.xls

Female Individual Animal Neoplastic Pathology Data
0303804_Female_Individual_Animal_Neoplastic_Pathology_Data.xls

Female Individual Animal Non-Neoplastic Pathology Data
0303804_Female_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
0303804_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
0303804_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data
0303804_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Clinical Observations
0303804_Male_Individual_Animal_Clinical_Observations.xls

Male Individual Animal Neoplastic Pathology Data
0303804_Male_Individual_Animal_Neoplastic_Pathology_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data
0303804_Male_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
0303804_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
0303804_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Organ Weight Data
0303804_Individual_Animal_Organ_Weight_Data.xlsx

Individual Animal Tissue Concentration Data
0303804_Individual_Animal_Tissue_Concentration_Data.xlsx

Individual Animal Urinalysis Data
0303804_Individual_Animal_Urinalysis_Data.xlsx

G.9. Genetic Toxicology

G.9.1. In Vivo Peripheral Blood Micronucleus Study G03038B in Mice

G03038B G04 In Vivo Micronucleus Summary Data
G03038B_G04_In_Vivo_Micronucleus_Summary_Data.pdf

G03038B Individual Animal In Vivo Micronucleus Data
G03038B_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

G.9.2. In Vivo Peripheral Blood Micronucleus Study G03038C in Rats

G03038C G04 In Vivo Micronucleus Summary Data
G03038C_G04_In_Vivo_Micronucleus_Summary_Data.pdf

G03038C Individual Animal In Vivo Micronucleus Data
G03038C_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

G.9.3. *Salmonella/Escherichia coli* Mutagenicity Test or Ames Test Study G03038D

G03038D G06 Ames Summary Data
G03038D_G06_Ames_Summary_Data.pdf

G.9.4. DNA Damage Study G03038E in Mice

G01 – In Vivo Alkaline Comet Summary Data
G03038E_G01_In_Vivo_Alkaline_Comet_Summary_Data.pdf

Individual Animal In Vivo Alkaline Comet Data
G03038E_Individual_Animal_In_Vivo_Alkaline_Comet_Data.xlsx

G.9.5. DNA Damage Study G03038F in Rats

G01 – In Vivo Alkaline Comet Summary Data
G03038F_G01_-_In_Vivo_Alkaline_Comet_Summary_Data.pdf

Individual Animal In Vivo Alkaline Comet Data
G03038F_Individual_Animal_In_Vivo_Alkaline_Comet_Data.xlsx

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